

Mudasir Bashir Gugjoo
Amar Pal *Editors*

Mesenchymal Stem Cell in Veterinary Sciences



Springer

Mesenchymal Stem Cell in Veterinary Sciences

Mudasir Bashir Gugjoo • Amar Pal
Editors

Mesenchymal Stem Cell in Veterinary Sciences

 Springer

Editors

Mudasir Bashir Gugjoo
Division of Veterinary Clinical Complex
FVSc & AH, SKUAST-Kashmir
Jammu and Kashmir, India

Amar Pal
Division of Surgery
Indian Veterinary Research Institute
Izatnagar, India

ISBN 978-981-15-6036-1 ISBN 978-981-15-6037-8 (eBook)
<https://doi.org/10.1007/978-981-15-6037-8>

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2020

This work is subject to copyright. All rights are solely and exclusively licensed by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd. The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

Tissues and/or organs are constituted by building blocks, the cells. These cells are either undifferentiated stem cells or differentiated tissue-specific cells. The miniscule number of available undifferentiated stem cells act as a reserve to maintain normal cell turn-over and thereby, tissue homeostasis. The differentiated cells perform the tissue-specific function. For a substantial and effective cellular therapy, the cells have to be sufficient in quantity and compatible with the recipient immune system, besides expressing as per the tissue requirements. The differentiated cells taken from the particular tissue source often are limited in number, have little or no proliferation potential and elicit the immune response in the recipient upon allogeneic or xenogeneic transplantation. Contrarily, the stem cells especially mesenchymal stem cells (MSCs) can be harvested from almost all the body tissue sources including the foetal membranes and have good proliferation and trans-differentiation potential. MSCs tend to have little tendency to elicit an immune response in the recipient even upon allogeneic or xenogeneic applications. These cells have minimal teratogenic and ethical issues normally associated with the pluripotent stem cells. However, MSCs unlike that of pluripotent stem cells tend to have limited proliferation potential.

The advancement of stem cell biology is increasing by each passing day and has found its way into the clinical applications under regenerative medicine, a branch of tissue engineering. Due to the limited understanding in the cellular characteristics, *ipso facto* MSCs therapeutic applications currently come under the advanced therapy medicinal products (ATMPs). The role of microenvironment in cellular fate remains to be understood. Such an understanding shall aid in their definitive utilization in regenerative medicine.

There are many books that discuss stem cells and their features and have invariably focused on their applications in humans. However, the book on stem cells especially mesenchymal stem cells and their applications in veterinary species is very much lacking. The pressing need for a book that provides a comprehensive introduction to the field and summarizes its recent progress in veterinary sciences has given rise to this book. This current work discusses the mesenchymal stem cell features and their applications in veterinary species. The book starts with introduction to the stem cells. In the introductory chapters, the stem cell properties and types are discussed followed by the details of the mesenchymal stem cell sources, isolation, culturing and characterization, and preservation. This is followed by a chapter

on the effect of different cues on mesenchymal stem cells properties. Thereafter, the species-specific mesenchymal stem cell features including the complexities in their characteristics, besides, their potential applications have been discussed. Finally, the book is concluded with the summary and future research avenues of MSCs.

We thank all the contributors for their hard work and valuable contributions.

Jammu and Kashmir, India
Izatnagar, India

Mudasir Bashir Gugjoo
Amar Pal

Acknowledgement

With all the humility I thank almighty “Allah” the most Beneficent, the most Merciful, who blessed me with limitless strength and favourable circumstances, to face and pass through all odds successfully at this juncture.

I feel a great sense of elation and fulfilment to submit this tiny drop of work in the ocean of human knowledge to benefit the differently abled creatures surrounding and helping us in many ways. The completion of this work would not have been possible without the help and support of many people.

I would like to appreciate the contribution of all those teachers from childhood till now who played an important role in my life to reach this place without which the realization of the present work into practice would have been a dream. Apart from the teaching faculty, schools/institutes that I attended like Miranda Public High School, Srinagar, SP Higher Secondary School, Srinagar, SKUAST-Jammu and Indian Veterinary Research Institute, Izatnagar, during the course have been the ocean that taught me to swim in all the spheres of life and reach to the stage. A special thanks go to all the stem cell scientists who burn themselves and provide the literature that makes the material in the book.

I am highly thankful to Science and Engineering Research Board (SERB), Government of India that provided the financial support and infused zeal in me to conduct the stem cell research and come out with this piece. I really feel elated to convey my gratitude to the SKUAST-Kashmir under which I am currently working. This institute has provided me the platform to express my potential to the best of my capacities. The faculty members of the institute have provided great company through the journey.

Literary skills grope in the dark when it comes to express what I owe to my family. These mean achievements to which I lay claim today have all precipitated from the sincere prayers and silent sacrifices of my Pappa and Mummy. The blessings, love, guidance and affection provided by my sister and brother-in-law deserve a special mention. I admit myself being incapable to pay regards in words to the affection, blessings, encouragement and dedication of my wife that made many difficult tasks easier for me. In recent times, the happiest moment and encouragement of my life has probably been my daughter.

I am highly thankful to Dr Bhavik Sawhney, Editor-Biomedicine, Springer Nature for his guidance and timely help in giving final shape to this book. I admit the affections and encouragement provided to me by my extended family. This list

is obviously incomplete but allows me to submit that the omissions are inadvertent and I once again record my deep-felt gratitude to all those associated with me in this endeavour.

Finally, I supplicate to *Allah* (SWT), the One who listens and responds to supplications, “Oh *Allah*, guide me to the right path, the path of those on whom You have bestowed Your favors, not of those who have incurred Your wrath, nor of those who have gone astray and let not me die except in the state of pure submission to You alone”. *Aameen*.

About the Book

During the last few years, a large volume of information has been generated on stem cell types, their biological nature, *in vitro* and *in vivo* behaviour and potential application in research and therapy. This book makes a systematic collection of the scientific information scattered in the literature on different aspects of stem cell research. The focus of the book is on mesenchymal stem cells as they have been investigated most extensively for therapeutic application in animals. The contents of the book have been designed to include all the relevant information about the mesenchymal stem cells of animal origin. The initial chapter covers the historical aspect and chronology of developments in the field of stem cell research. The details about the basic properties and nature of the stem cells are provided. A comprehensive account of the materials and methods used for isolation of mesenchymal stem cells from different sources, culture expansion, characterization and long-term storage has been given in the subsequent chapters. The book also provides a logical appraisal of the mechanisms involved in therapeutic efficacy, immunomodulation and anti-inflammatory properties of mesenchymal stem cells. The book also highlights knowledge about the interaction between the stem cells and their environment and factors responsible for the maintenance of stemness. A chapter in the book has been dedicated to genetic engineering relevant to stem cell research for their better exploitation for research and therapy in animals. The major section of the book is devoted to the recount of the research efforts and their outcomes towards the potential application of mesenchymal stem cells in regenerative research and therapy in important species of domestic and pet animals including sheep, goat, cattle, buffalo, cat, dog and horse. The book also presents an abridgement of challenges and future prospects of stem cell research and application in medicine in general and veterinary sciences in particular.

Contents

1	Introduction to Stem Cells	1
	M. B. Gugjoo	
2	Mesenchymal Stem Cell and Its Properties	13
	M. B. Gugjoo, Amar Pal, and G. T. Sharma	
3	Mesenchymal Stem Cell Isolation, Culture, Characterization and Cryopreservation	27
	M. B. Gugjoo, Amar Pal, V. Chandra, and G. T. Sharma	
4	Mesenchymal Stem Cell Immuno-Modulatory and/Anti-Inflammatory Properties	47
	M. B. Gugjoo and Amar Pal	
5	Mesenchymal Stem Cell Differentiation Properties and Available Microenvironment	67
	M. B. Gugjoo and Amar Pal	
6	Mesenchymal Stem Cell Genetic Engineering and Regenerative Medicine	89
	M. B. Gugjoo, E. Rasool, and Amar Pal	
7	Sheep Mesenchymal Stem Cell Basic Research and Potential Applications	99
	M. B. Gugjoo and Amar Pal	
8	Goat Mesenchymal Stem Cell Basic Research and Potential Applications	153
	M. B. Gugjoo, Amar Pal, M. R. Fazili, R. A. Shah, M. S. Mir, and G. T. Sharma	
9	Cattle/Buffalo Mesenchymal Stem Cell Basic Research and Potential Applications	181
	M. B. Gugjoo, Amar Pal, M. R. Fazili, R. A. Shah, and G. T. Sharma	
10	Cat Mesenchymal Stem Cell Characteristics and Potential Applications	197
	M. B. Gugjoo and Amar Pal	

11	Dog Mesenchymal Stem Cell Basic Research and Potential Applications	213
	M. B. Gugjoo, Amar Pal, and G. T. Sharma	
12	Equine Mesenchymal Stem Cell Basic Research and Potential Applications	283
	M. B. Gugjoo, Amar Pal, D. M. Makhdoomi, and G. T. Sharma	
13	Future of Mesenchymal Stem Cell Research	333
	M. B. Gugjoo and Amar Pal	

Editors and Contributors

About the Editors

Mudasir Bashir Gugjoo is an Assistant Professor at the Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences & Technology, Kashmir, India. He has received his PhD in mesenchymal stem cell translational studies from the Indian Veterinary Research Institute, Izatnagar. He has received various awards including the Best Faculty Teacher Award. His lab focuses on the isolation and characterisation of mesenchymal stem cells and their therapeutic applications, currently being funded by Science and Engineering Research Board (SERB), Government of India. He has published more than 60 papers in various reputed peer-reviewed national and international journals, authored or co-authored a number of book chapters and is a newspaper columnist. His work has been cited in various standard books on Veterinary Medicine, Radiology and Tissue Engineering, and in numerous international research papers. He is a member of various scientific societies and serves on the editorial boards of several journals. He is also a referee for a number of peer-reviewed journals including the Journal of Cellular Physiology, Cellular Reprogramming and Biomedicine and Pharmacotherapy, among others.

Amar Pal is currently working as a Principal Scientist & Head of the Division of Surgery at Indian Veterinary Research Institute, Izatnagar, India. He is also in-charge of the Referral Veterinary Polyclinic and the Anatomy Section at the Indian Veterinary Research Institute, Izatnagar, India. He has done pioneering research work in the areas of veterinary anaesthesia, pain management, urolithiasis, orthopaedic surgery, and stem cell biology and therapy in animals. He has developed and registered the designs of six orthopaedic devices for animals and eight veterinary mobile apps. His work has been cited in standard works on veterinary surgery, anaesthesia and pharmacology, and in countless international research papers. He has received several awards, including the Chancellor's Gold Medal, Vice-Chancellor's Gold Medal, and Best Teacher Award. He is a fellow of the National Academy of Veterinary Sciences, Indian Society for Veterinary Surgery and Indian Society for Advancement of Canine Practice. Dr Amarpal has published more than 360 research and clinical papers in respected national and

international journals and more than 380 abstracts in seminar and conference proceedings. He has also authored two books and contributed several book chapters. Dr Amarpal is Editor-in-Chief of the Indian Journal of Veterinary Surgery and Associate Editor of the Indian Journal of Canine Practice, and is a referee for numerous international journals.

Contributors

V. Chandra Division of Physiology and Climatology, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

M.R. Fazili Division of Veterinary Clinical Complex, FVSc and AH, SKUAST-K, Srinagar, Jammu and Kashmir, India

Mudasir Bashir Gugjoo Division of Veterinary Clinical Complex, FVSc & AH, SKUAST-Kashmir, Jammu and Kashmir, India

D.M. Makhdoomi Division of Surgery and Radiology, FVSc and AH, SKUAST-K, Srinagar, Jammu and Kashmir, India

M.S. Mir Directorate of Extension Education, SKUAST-K, Srinagar, Jammu and Kashmir, India

Amar Pal Division of Surgery, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

E. Rasool Division of Surgery and Radiology, FVSc and AH, SKUAST-K, Srinagar, Jammu and Kashmir, India

R.A. Shah Division of Animal Biotechnology, FVSc and AH, SKUAST-K, Srinagar, Jammu and Kashmir, India

G.T. Sharma Division of Physiology and Climatology, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Abbreviations

ALCAM	Activated leukocyte cell adhesion molecule
ATMPs	Advanced therapy medicinal products
bFGF	Bovine fibroblast growth factor
CD	Cluster of differentiation
ConA	Concanavalin A
COX2	Cyclooxygenase 2
DAZL	Deleted in azoospermia like
DCs	Dendritic cells
DMSO	Dimethyl sulfoxide
EGF	Epidermal growth factor
ESCs	Embryonic stem cells
FASL	Fas ligand
FBS	Foetal bovine serum
FGF-2	Fibroblast growth factor-2
Fox	Forkhead box protein
HGF	Hepatocyte growth factor
HMOX1	Heme oxygenase-1
ICAM-1	Intracellular adhesion molecule-1
IDO	Indoleamine 2,3 dioxygenase
IFNG	Interferon, gamma
IFN γ	Interferon gamma
IGF-1	Insulin growth factor-1
IL	Interleukin
IL1F10	Interleukin 1, family member 10
IL6	Interleukin 6
IL-6R	Interleukin-6 receptor
INFAC	Interferon alpha C (INF-alpha C)
INFB1	Interferon, beta 1
iNOS	Inducible nitric oxide synthase
iPSCs	Induced pluripotent stem cells
JSP.1	Major histocompatibility complex class I
LFA-3	Lymphocyte functional antigen-3
LPS	Lipopolysaccharide
LTBR	Lymphotoxin beta receptor

MAPK	Mitogen-activated protein kinase
MHC-II (DSB)	Major histocompatibility complex class II (DS beta antigen)
NK	Natural killer cells
PDGF	Platelet-derived growth factor
PDL1	Programmed death ligand-1
PDX-1	Pancreatic and duodenal homeobox-1
PMA	Phorbol 12-myristate 13-acetate
PrP ^c	PRion protein cellular
PTGES	Prostaglandin E synthase
PTGES1	Prostaglandin E synthase 1
RNA	Ribonucleic acid
STC-1	Stanniocalcin 1
TGF- α	Transforming growth factor- α
TGF β 1	Transforming growth factor beta 1
TLRs	Toll-like receptors
TNF	Tumour necrosis factor
TNF α	Tumour necrosis factor alpha
TRKA	Tropomyosin receptor kinase A
TSG	Tumour suppressor gene
VCAM	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor



Introduction to Stem Cells

1

M. B. Gugjoo

Abstract

The stem cell concept is as old as the understanding of the origin of life. The concept of origin of life has developed from initial 'spontaneous generation' to the current concept of development through 'zygote formation'. Understanding the concept of the development of life has paved way for the 'stem cell' concept. There are various types of stem cells that develop right from early phase of life to the adult life though with variable potentialities. An overview of the development of stem cell concept has been attempted in the current chapter.

1.1 Introduction

The first and foremost attempt to solve mystery around the origin of life and of human development may be attributed to Aristotle (384–322 BC) (Arey 1974). His observation made him to believe that embryonic development occurs in the uterus and that the mother's menstrual blood is the basis, supporting then previous hypothesis of 'spontaneous generation'. Until 1800, the concept behind development of an individual was of a pre-formed individual who exists in the egg or sperm, known as homunculus. The homunculus (egg), however, requires an activation to form an individual, which occurs through sperm. The sperm, thus, stimulates homunculus in the uterus that ultimately leads to the formation of an individual. Leydig later in 1855 hypothesized that life originates from an already existing life, *omne vivum ex vivo*. This hypothesis was extended by Rudolf Virchow with a view that all new cells develop from existing cells, *omnis cellula e cellula* (Oberling 1944). Later on

M. B. Gugjoo (✉)

Division of Veterinary Clinical Complex, FVSc & AH, SKUAST-Kashmir,
Jammu and Kashmir, India

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2020

M. B. Gugjoo, A. Pal (eds.), *Mesenchymal Stem Cell in Veterinary Sciences*,
https://doi.org/10.1007/978-981-15-6037-8_1

1

the concept of fertilization was developed, wherein an egg after fusion with the sperm leads to the formation of zygote (Needham 1959). The zygote forms the actual basis of life as it gives rise to all the cells required together to make an individual (totipotency) (Sell 2004a, b). Thus, the existence of cell, now popularly known as 'stem cell', carries the potential to develop into the complete individual.

1.2 Overview of the Historical Chart of the Stem Cells

The term 'stamzelle or stem cell' was initially used by a German biologist Ernest Haeckel in 1868 for a fertilized egg that develops into a complete organism (Reimer 1868). The term was also used for the single-celled organism that is considered as an ancestor to all the living organisms. The stem cell research actually started with the process of learning the basis of development and regeneration. In 1930, nuclear transplantation was first carried out in Amoeba (Comandon and deFonbrune 1930). In 1950s Briggs and King applied the same technique to replace nucleus of a frog's egg with other cell (Briggs and King 1952). Gurdon later cloned a frog from an egg and the nucleus of small intestine (Gurdon 1974). The experiments confirmed that the developmental changes that occur are through the environmental factors rather than the permanent changes to the genetic code and in the present case factors were present in oocyte of the frog (Bio-Rad Lab Inc.n.d.). This somatic cell nuclear transfer (SCNT) technique, however, proved to be comparably harder in mammals, and for long time the mammalian cells were considered to be far specialized and, thus, cannot be reverted to totipotent state. But as the research in the field advanced, the findings were proved to be wrong and different mammalian clones were developed. The most noted one was in the form of Dolly, developed by fusion of mammary gland epithelial cell with the enucleated oocyte (Wilmut et al. 1997), besides, the others (Forsberg et al. 2002; Gao et al. 2003; Walker et al. 2002). However, the frequency of successful transplants appears to be very less, <1–2% (Sell 2004a, b), giving rise to the question, why? Thus, not all adult cells are capable to give rise to successful transplants and the cell that give rise could actually be the 'stem cell' or 'tissue progenitor cell' (Van der Kooy and Weiss 2000). In 1945 the initial experimental basis of the stem cell was recognized (Owen 1945). Since then stem cell properties are being discussed (Lajtha et al. 1964; Lajtha 1967) with in vivo stem cell governing factors yet to be fully comprehended. The historical aspect of various stem cell types is briefed below.

1.2.1 Haematopoietic Stem Cell

The blood stem cell concept was first introduced in Berlin Haematological Society by Russian academician Alexander Maximow on 1 June 1909. He delivered a theoretical lecture that all the blood cells come from same ancestral cell and, thus, introduced the concept of multipotent nature of blood stem cell (Kalra and Tomar 2014). The stem cell search in blood had begun after the bombings in Hiroshima and

Nagasaki in 1945 when radiation exposure led to the haematopoietic failure in residents. It was found that the shielding of the spleen with lead (Jacobson et al. 1950) and transplantation of bone marrow or spleen cells (Lorenz et al. 1951; Jacobson et al. 1951) prevent such a failure. However, it was confirmed later in 1963 when Ernest McCulloch and James Till (Canadian scientists) carried out experiments on mice bone marrow. It was demonstrated that various blood cells originate from a specific class of cells bearing self-renewal and differentiation properties (Till et al. 1964; Till and McCulloch 1961). Even before actual laboratory confirmation of blood stem cell, the human bone marrow transplant was first attempted by E. Donnall Thomas in 1957 that later fetched him Nobel prize in 1990. The first successful transplant of bone marrow, however, was conducted by Robert A. Good in 1968. He transplanted bone marrow of a sibling to an immune-deficient child who later developed into a healthy adult (Thomson et al. 1998a, b). Since then the blood transfusion has become a common procedure in case of leukaemia patients.

1.2.2 Embryonic Stem Cell

The embryonic stem cell understanding has started with the development of spontaneous testicular tumour in a terato-carcinoma study. In 1970s, it was observed that such spontaneous tumours, composed of differentiated cell types and undifferentiated stem cell, may be produced following ectopic grafting of these tumour cells in the adults (Solter et al. 1970; Stevens 1970; Stevens 1983; Papaioannou and Rossant 1983). Those undifferentiated stem cells (embryonal carcinoma cells) were correlated to the pluripotent stem cells of an early embryo. This paved the way to harvest stem cells directly from embryos. It was in 1981 when independent researchers, Marlin Evans and Kaufmann of University of Cambridge and Garl Martin of University of California, could successfully derive embryonic stem cells (ESCs) from mouse blastocysts (Evans and Kaufman 1981). Trophoectoderm, however, was demonstrated to harbour stem cells with limited differentiation potential called trophoblast stem cells (Tanaka et al. 1998). ESCs could grow on a feeder layer formed by mitotically inactivated mouse embryonic fibroblast cells. Later on ESCs were also derived from primates (Thomson et al. 1995, 1996). All these studies along with the research conducted on culturing human in vitro fertilized (IVF) embryos (Gardner et al. 1998) paved the way for successful cultivation and maintenance of ESCs into an undifferentiated state (Thomson et al. 1998a, b). It was demonstrated that feeder cell layer for mouse ESCs could be employed for human counterparts, but variability in biochemical pathways and humoral factors exist. In the same year, cultured human ESCs were differentiated into different tissues, viz. gut epithelium, cartilage, bone, muscle, neurons, etc. (Thomson et al. 1998a, b; Shambloott et al. 1998). Considering these factors ESC line maintenance and ability to trans-differentiate has led to the tremendous research in the field especially in relation to the development and repair of damaged or lost tissue (Donovan and Gearhart 2001). Attempts to create ESCs from veterinary species like cattle, sheep, dog and others too started immediately afterwards (Telugu et al. 2010). Due to the

limited availability, associated ethical issues, especially in humans and a tendency to give rise to teratoma, ESCs currently have limited in vivo applications (Gugjoo and Amarpal 2018; Gugjoo et al. 2019).

1.2.3 Induced Pluripotent Stem Cell

Recently developed transgenic techniques have opened ways to reprogram an adult tissue cell to pluripotency. An adult tissue cell is reprogrammed by insertion of the pluripotency induction genes like Oct4, Sox2, Klf4 and cMyc. These dedifferentiated cells are known as induced pluripotent stem cells (iPSCs) (Pittenger et al. 1999; Cavaleri and Scholer 2003; Chambers and Smith 2004; Takahashi and Yamanaka 2006). The work was seen with such an extensive potential that the scientists *John B. Gurdon* and *Shinya Yamanaka* were awarded 2012 Nobel Prize in medicine. Initially, the work was conducted in lab animals like mice, but now the cells have also been harvested in various animal species like goat (Song et al. 2013; Sandmaier et al. 2015; Chen et al. 2017), sheep (Li et al. 2011; Liu et al. 2012; Sartori et al. 2012; German et al. 2015), cattle (Zhao et al. 2017), dog (Nishimura et al. 2013; Baird et al. 2015) and equine (Nagy et al. 2011; Khodadadi et al. 2012; Breton et al. 2013; Whitworth et al. 2014). The current success rate in harvesting the dedifferentiated cell population is very limited. These cells like ESCs too have tendency to give rise to teratoma and, thus, fail to make a definitive way into the in vivo applications (Gugjoo et al. 2018a, b, 2019).

1.2.4 Mesenchymal Stem Cell

The presence of mesenchymal stem cells within the bone marrow may be documented from the work of Goujon (1869). An osteogenic potential of bone marrow was demonstrated after its heterotopic transplantation. The results were confirmed in the transplantation experiments by Biakow (1870). Later on the osteogenesis was shown to be due to the whole bone marrow and not by the chemo-attractants (Danis 1960). These experiments were also conducted by other researchers who demonstrated the same results and found that bone and cartilage formation does occur (Tavassoli and Crosby 1968; Friedenstein et al. 1966; Bruder and Caplan 1990). Friedenstein and co-workers observed that the heterotopic bone formation post-bone marrow cells transplantation was associated with minor subpopulation of such cells that can be differentiated from haematopoietic cells based on their specific features, viz. plastic adherence and fibroblast-like appearance. The fibroblast-like appearance indicated their origin from the stromal compartment of the bone marrow. The same authors also observed that seeding of bone marrow cell suspension had resulted in the establishment of discrete colonies. These colonies being initiated by single cell are known as colony-forming unit fibroblastic (CFU-Fs) (Friedenstein et al. 1970). The concept of stem cells in bone marrow was formally presented by Owen in 1978 (Owen 1978). It was hypothesized that bone marrow harbours a lineage that is

analogous to haematopoietic lineage (Till and McCulloch 1980). The *in vivo* experimental transplantation of such cells had resulted in the formation of numerous skeletal tissues, viz. bone and cartilage. The cells were named as osteogenic stem cell (Friedenstein et al. 1987) or bone marrow stromal stem cell (Owen and Friedenstein 1988). The non-haematopoietic stem cell hypothesis though well-established previously, its importance, however, was felt in 1999 when similar work was published by a commercial company, Osiris Therapeutics Inc. (Pittenger et al. 1999).

The term ‘mesenchymal stem cell’ has been coined by Caplan who showed that these cell types carry the potential to differentiate into other phenotypes including adipogenic, tenogenic, ligamentous, dermal or others (Caplan 1991, 1994). The term ‘mesenchymal stem cell’ although widely used has restricted acceptability (Sipp et al. 2018) and shall henceforth be called by that name in this chapter. The stem cell has ‘stemness’ characteristics depicted by ‘self-renewal and differentiation’ properties. Mesenchymal stem cell (MSC) most often undergoes culture-dependent senescence (Wagner et al. 2009), which also diminishes its ability to differentiate (Bonab et al. 2006; Lo Surdo et al. 2013). It is also unclear whether ectopically placed MSCs can actually differentiate *in vivo* or not. The stem cell label is, thus, questionable with respect to MSCs (Bianco 2014). As such these cells are also termed as stromal stem cells (Owen and Friedenstein 1988), mesenchymal progenitor cells (Dennis et al. 1999), skeletal stem cells (Bianco and Gehron 2000), multipotent adult progenitor cells and mesodermal progenitor cells (Jiang et al. 2002). In 2006, International Society for Cellular Therapy that gave specific guidelines for characterization of these cells recommended the name to be given as ‘Multipotent Mesenchymal Stromal Cells’ (Dominici et al. 2006). The latest work of Caplan, however, recommends these cells to be named as “Medicinal Signaling Cells” based on their ability to modulate host systems. It may, thus, be pointed out that more emphasis on the mechanism of their therapeutic effects may be laid (Caplan 2017).

A huge literature has been piled on MSCs including their properties, isolation protocols and their applications in diverse ailments both under preclinical experimental studies and in the clinical trials. In veterinary science, MSCs have been harvested from numerous adult tissue sources and foetal membranes (Gugjoo and Amarpal 2018; Gugjoo et al. 2018a, 2019). Mesenchymal stem cells (MSCs) have also been harvested from iPSCs (Chow et al. 2017; Xu et al. 2019). Currently, there are wide variations in their tri-lineage differentiation potential, especially in relation to a marked reduction in their chondrogenic and adipogenic lineages as compared to the bone marrow-derived (BM) MSCs (Xu et al. 2019). MSCs in veterinary sciences like human are characterized as per the criteria laid down by International Society for Cellular Therapy (ISCT) (Dominici et al. 2006). The early mesenchymal stem cell *in vivo* applications had employed non-specific cell fractions. A heterogeneous cell fraction containing tissue cells, leukocytes, endothelial cells, fibroblasts and a small subset of self-renewing multipotent cells had been utilized (Tiwary et al. 2014). However in recent time culture-expanded MSC preparations are being increasingly studied containing good number of MSCs. Further, advancement like specific cell selection methods need to be studied to make roadways to a more

purified cell preparation (Devireddy et al. 2017). The cells are even being cryopreserved for their future applications (Gugjoo and Amarpal 2018; Gugjoo et al. 2018a, 2019). Among various veterinary species, MSC clinical studies have been conducted mainly in dog, cat and horse (Quimby and Borjesson 2018; Gugjoo et al. 2019a, b). In horse, the therapeutic applications of stem cells have mainly been aimed at the issues of musculoskeletal system. An initial attempt was made to repair the superficial digital flexor tendon (Smith et al. 2003). In sheep and goat MSCs, in vivo experimental evaluations have been conducted mostly as human translational studies (Gugjoo and Amarpal 2018; Gugjoo et al. 2020). Cattle and buffalo MSCs have been evaluated in experimental studies mainly to address reproductive problems (Gugjoo et al. 2018b). It may be noted that all MSC studies have so far failed to provide any conclusive results.

1.2.5 Cancer Stem Cell

The cancer stem cell (CSC) concept was initially given by Cohnheim and Durante about one-and-a-half century back. The proliferation and differentiation potential of CSC was demonstrated to be comparable to that of the embryonic tissues and cancers. Thus, a concept was proposed that embryonic cells present in adults upon stimulation may lead to cancer. More curiosity in this concept was developed with the work of Furth and co-workers (Furth et al. 1937). It was demonstrated that acute myeloid leukaemia (AML) develops in a mouse after transplantation of single undifferentiated leukaemia cell (Furth et al. 1937; Al-Hajj et al. 2003). After a long gap of three decades, in vitro studies supported AML cells' ability to self-renew, proliferate and generate new tumours (Sell 2004a, b). Initially, such studies were performed on tumours in liquid (blood) phase. The first evidence of these stem cells in solid tumours came in 2003 and was reported from breast cancer (Al-Hajj et al. 2003). Thereafter, CSC concept was validated in a wide variety of solid tumours including lung cancer (Kim et al. 2005), melanoma (Fang et al. 2005), prostate cancer (Collins et al. 2005), brain cancer (Piccirillo et al. 2006), colon cancer (O'Brien et al. 2006) and ovarian cancer (Szotek et al. 2006). The oncogenic therapy on the basis of CSC concept is also currently being researched extensively (Gugjoo et al. 2015; Atashzar et al. 2020).

1.3 Conclusion

The stem cell concept dates back to the origin of life. The concept of development of life has changed from initial 'spontaneous generation' to the current concept of development through 'zygote formation'. Understanding the development of life has itself given rise to stem cell concept. Various stem cell types including MSCs have been identified with variable differentiation potential. Overall stem cell concept is still in its infancy, and there are lot of intricacies involved. Among these cell types, mostly MSCs are being evaluated in clinical trials. The stem cell research

though is being conducted at a very rapid pace; however, the understanding is yet to reach to the level that can harvest the fruitful results.

References

- Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci* 100:3983–3988
- Arey LB (1974) Developmental anatomy: a textbook and laboratory manual of embryology, 7th edn. W.B. Saunders, Philadelphia
- Atashzar MR, Baharlou R, Karami J, Abdollahi H, Rezaei R, Pourramezan F, Zoljalali Moghaddam SH (2020) Cancer stem cells: a review from origin to therapeutic implications. *J Cell Physiol* 235(2):790–803
- Baird AEG, Barsby T, Guest DJ (2015) Derivation of canine induced pluripotent stem cells. *Reprod Domest Anim* 50(4):669–676
- Biakow A (1870) Über transplantation Von Knochenmark. *Centralbl F DMed Wiss* 8:371–373
- Bianco P (2014) Mesenchymal stem cells. *Annu Rev Cell Dev Biol* 30:677
- Bianco P, Gehron RP (2000) Marrow stromal stem cells. *J Clin Invest* 105(12):1663–1668
- Bio-Rad Laboratories Inclusive. (n.d.) Stem cell basics for life science researchers. p. 2
- Bonab MM, Alimoghaddam K, Talebian F, Ghaffari SH, Ghavamzadeh A, Nikbin B (2006) Aging of mesenchymal stem cells in vitro. *BMC Cell Biol* 7:14
- Breton A, Sharma R, Diaz A et al (2013) Derivation and characterization of induced pluripotent stem cells from equine fibroblasts. *Stem Cells Dev* 22:611–621
- Briggs R, King TJ (1952) Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. *Proc Natl Acad Sci U S A* 38:455–463
- Bruder SP, Caplan AI (1990) Terminal differentiation of osteogenic cells in embryonic chick tibia is revealed by monoclonal antibody against osteocytes. *Bone* 11:189–198
- Caplan AI (1991) Mesenchymal stem cell. *J Orthop Res* 9:641–650
- Caplan AI (1994) The mesengenic process. *Clin Plast Surg* 21:429–435
- Caplan AI (2017) Mesenchymal stem cells: time to change the name! *Stem Cells Transl Med* 6:1445–1451
- Cavaleri F, Scholer HR (2003) Nanog: a new recruit to the embryonic stem cell orchestra. *Cell* 113:551–552
- Chambers I, Smith A (2004) Self-renewal of teratocarcinoma and embryonic stem cells. *Oncogene* 23:7150–7160
- Chen H, Zuo Q, Wang Y, Song J, Yang H, Zhang Y, Li B (2017) Inducing goat pluripotent stem cells with four transcription factor mRNAs that activate endogenous promoters. *BMC Biotechnol* 17:11
- Chow L, Johnson V, Regan D, Wheat W, Webb S, Koch P, Dow S (2017) Safety and immune regulatory properties of canine induced pluripotent stem cell derived mesenchymal stem cells. *Stem Cell Res* 25:221–232
- Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ (2005) Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 65:10946–10951
- Comandon J, deFonbrune P (1930) Greffe nucléaire totale, simple ou multiple, chez une Amibe. *C R Soc Biol Paris* 130:744–748
- Danis A (1960) A pres une greffe de tissu squelettique osteogene est a petir des cellulis transplan-tis que se constitue l'os denouvelle formation. *Bull Soc Int Chir* 6:647–652
- Dennis JE, Merriam A, Awadallah A, Yoo JU, Johnstone B, Caplan AI (1999) A quadri-poten-tial mesenchymal progenitor cell isolated from the marrow of an adult mouse. *J Bone Miner Res* 14:700
- Devireddy DR, Boxer L, Myers MJ, Skasko M, Screven R (2017) Questions and challenges in the development of mesenchymal stromal/stem cell-based therapies in veterinary medicine. *Tissue Eng* 23(4):462–470. <https://doi.org/10.1089/ten.teb.2016.0451>

- Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop DJ, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytherapy* 8(4):315–317
- Donovan PJ, Gearhart J (2001) The end of the beginning for pluripotent stem cells. *Nature* 414:92–97
- Evans MJ, Kaufman MH (1981) Establishment in culture of pluripotential cells from mouse embryos. *Nature* 292:154–156
- Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, Van Belle PA, Xu X, Elder DE, Herlyn M (2005) A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 65:9328–9337
- Forsberg EJ, Strelchenko NS, Augenstein ML et al (2002) Production of cloned cattle from in vitro systems. *Biol Reprod* 67:327–333
- Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV (1966) Osteogenesis of transplants of bone marrow cells. *J Embryol Exp Morphol* 16:381–390
- Friedenstein AJ, Chailakhyan RK, Lalykina KS (1970) The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet* 3:393–403
- Friedenstein AJ, Chailakhyan RK, Gerasimov UV (1987) Bone marrow osteogenic stem cells: in vitro cultivation and transplantation in diffusion chambers. *Cell Tissue Kinet* 20:263–272
- Furth J, Kahn MC, Breedis C (1937) The transmission of leukemia of mice with a single cell. *Am J Cancer* 32:276–282
- Gao S, McGarry M, Priddle H et al (2003) Effects of donor oocytes and culture conditions on development of cloned mice embryos. *Mol Reprod Dev* 66:126–133
- Gardner DK et al (1998) Culture and transfer of human blastocysts increases implantation rates and reduces the need for multiple embryo transfers. *Fertil Steril* 69:84–88
- German SD, Campbell KH, Thornton E, McLachlan G, Sweetman D, Alberio R (2015) Ovine induced pluripotent stem cells are resistant to reprogramming after nuclear transfer. *Cell Reprogram* 17(1):19–27
- Goujon E (1869) Recherché experimentales sur les propreties physiologiques de la moelle des os. *J L'Anat Physiol* 6:399–412
- Gugjoo MB, Amarpal (2018) Mesenchymal stem cell research in sheep: current status and future prospects. *Small Rumin Res* 169:46–56
- Gugjoo MB, Amarpal, Kinjavdekar P, Aithal HP, Pawde AM, Bodh D, Sharma GT (2015) Cancer stem cells: concepts and therapeutic applications. *Asian J Anim Vet Adv* 10(9):509–517
- Gugjoo MB, Amarpal, Chandra V, Wani MY, Dhama K, Sharma GT (2018a) Mesenchymal stem cell research in veterinary medicine. *Curr Stem Cell Res Ther* 13(8):645–657
- Gugjoo MB, Amarpal, Fazili MR, Shah RA, Sharma GT (2018b) Mesenchymal stem cell: basic research and potential applications in cattle and buffalo. *J Cell Physiol* 234(6):8618–8635
- Gugjoo MB, Amarpal, Makhdoomi DM, Sharma GT (2019a) Equine mesenchymal stem cells: properties, sources, characterization and potential therapeutic applications. *J Equine Veter Sci* 72:16–27
- Gugjoo MB, Amarpal, Sharma GT (2019b) Mesenchymal stem cell basic research and applications in dog medicine. *J Cell Physiol* 234(10):16779–16811
- Gugjoo MB, Amarpal, Fazili MR, Shah RA, Mir MS, Sharma GT (2020) Goat mesenchymal stem cell basic research and potential applications. *Small Rumin Res* 183:106045
- Gurdon JB (1974) The control of gene expression in animal development. Harvard University Press, Cambridge, MA, pp 3–36
- Jacobson LO, Simmons EL, Marks EK, Robson MJ, Bethard WF, Gaston EO (1950) The role of the spleen in radiation injury and recovery. *J Lab Clin Med* 35:746–770
- Jacobson LO, Simmons EL, Marks E, Eldredge JH (1951) Recovery from radiation injury. *Science* 113:510–511
- Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA,

- Verfaillie CM (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 418(6893):41–49
- Kalra K, Tomar PC (2014) Stem cell: basics, classification and applications. *Am Jf Phytomed Clin Therapeut* 2(7):919–930
- Khodadadi K, Sumer H, Pashaiasl M, Lim S, Williamson M, Verma PJ (2012) Induction of pluripotency in adult equine fibroblasts without c-MYC. *Stem Cells Int* 2012:429160
- Kim CFB, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, Jacks T (2005) Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 121:823–835
- Lajtha LG (1967) Stem cells and their properties. *Proc Can Cancer Conf* 7:31–39
- Lajtha LG, Gilbert CW, Porteous DD, Alexanian R (1964) Kinetics of a bone-marrow stem-cell population. *Ann N Y Acad Sci* 113:742–752
- Li Y, Cang M, Lee AS, Zhang K, Liu D (2011) Reprogramming of sheep fibroblasts into Pluripotency under a drug-inducible expression of mouse-derived defined factors. *PLoS One* 6(1):e15947
- Liu J, Balehosur D, Murray B, Kelly JM, Sumer H, Verma PJ (2012) Generation and characterization of reprogrammed sheep induced pluripotent stem cells. *Theriogenol* 77(2):338–346
- Lo Surdo JL, Millis BA, Bauer SR (2013) Automated microscopy as a quantitative method to measure differences in adipogenic differentiation in preparations of human mesenchymal stromal cell. *Cytotherapy* 15:1527
- Lorenz E, Uphoff D, Reid TR, Shelton E (1951) Modification of irradiation injury in mice and guinea pigs by bone marrow injections. *J Natl Cancer Inst* 12:197–201
- Nagy K, Sung HK, Zhang P et al (2011) Induced pluripotent stem cell lines derived from equine fibroblasts. *Stem Cell Rev* 7:693–702
- Needham A (1959) *History of embryology*. Cambridge University Press, New York, NY
- Nishimura T, Hatoya S, Kanegi R, Sugiura K, Wijewardana V, Kuwamura M, Tanaka M, Yamate J, Izawa T, Takahashi M, Kawate N, Tamada H, Imai H, Inaba T (2013) Generation of functional platelets from canine induced pluripotent stem cells. *Stem Cells Dev* 22:2026–2035
- O'Brien CA, Pollett A, Gallinger S, Dick JE (2006) A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 445:106–110
- Oberling C (1944) *The riddle of cancer*. Yale University Press, New Haven, CT
- Owen RD (1945) Immunogenetic consequences of vascular anastomoses between bovine twins. *Science* 102(2651):400–401
- Owen M (1978) Histogenesis of bone cells. *Calcif Tissue Cells* 25:205–207
- Owen M, Friedenstein AJ (1988) Stromal stem cells: marrow-derived osteogenic precursors. *Ciba Found Symp* 136:42–60
- Papaioannou VE, Rossant J (1983) Effects of the embryonic environment on proliferation and differentiation of embryonal carcinoma cells. *Cancer Surv* 2:165–183
- Piccirillo SGM, Reynolds BA, Zanetti N, Lamorte G, Binda E, Broggi G, Brem H, Olivi A, Dimeco F, Vescovi AL (2006) Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. *Nature* 444:761–765
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284:143–147
- Quimby JM, Borjesson DL (2018) Mesenchymal stem cell therapy in cats current knowledge and future potential. *J Feline Med Surg* 20:208–216
- Reimer G (1868) *Natürliche Schöpfungsgeschichte*. Springer, Berlin
- Sandmaier SE, Nandal A, Powell A, Garrett W, Blomberg L, Donovan DM, Talbot N, Telugu BP (2015) Generation of induced pluripotent stem cells from domestic goats. *Mol Reprod Dev* 82(9):709–721
- Sartori C, DiDomenico AI, Thomson AJ, Milne E, Lillico SG, Burdon TG, Whitelaw CB (2012) Ovine-induced pluripotent stem cells can contribute to chimeric lambs. *Cell Reprogram* 14(1):8–19
- Sell S (2004a) *Stem cells: a handbook of stem cells*. Humana Press, Totowa, NJ, p 1

- Sell S (2004b) Stem cell origin of cancer and differentiation therapy. *Crit Rev Oncol Hematol* 51:1–28
- Shamblott MJ, Axelman J, Littlefield JW, Donovan PJ, Blumenthal PD, Huggins GR, Gearhart JD (1998) Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc Natl Acad Sci U S A* 95(13):726–13,731
- Sipp D, Robey PG, Turner L (2018) Clear up this stem-cell mess. *Nature* 561:455–457
- Smith RKW, Korda M, Blunn GW, Goodship AE (2003) Isolation and implantation of autologous equine mesenchymal stem cells from bone marrow into the superficial digital flexor tendon as a potential novel treatment. *Equine Vet J* 35:99–102
- Solter D, Skreb N, Damjanov I (1970) Extrauterine growth of mouse egg-cylinders results in malignant teratoma. *Nat Lond* 227(503):504
- Song H, Li H, Huang M, Xu D, Gu C, Wang Z, Dong F, Wang F (2013) Induced pluripotent stem cells from goat fibroblasts. *Mol Reprod Dev* 80(12):1009–1017
- Stevens LC (1970) The development of transplantable teratocarcinomas from intratesticular grafts of pre- and postimplantation mouse embryos. *Dev Biol* 21:364–382
- Stevens LC (1983) The origin and development of testicular, ovarian and embryo-derived teratomas. In: Silver LM et al (eds) *Teratocarcinoma stem cells*, vol 10. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 23–36
- Szotek PP, Pieretti-Vanmarcke R, Masiakos PT, Dinulescu DM, Connolly D, Foster R, Dombkowski D, Preffer F, MacLaughlin DT, Donahoe PK (2006) Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian inhibiting substance responsiveness. *Proc Natl Acad Sci USA* 103:11154–11159
- Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126(4):663–676
- Tanaka S, Kunath T, Hadjantonakis A-K, Nagy A, Rossant J (1998) Promotion of trophoblast stem cell proliferation by FGF4. *Science* 282:2072–2075
- Tavassoli M, Crosby WH (1968) Transplantation of marrow to extramedullary sites. *Science* 161:54–56
- Telugu BP, Ezashi T, Roberts RM (2010) Porcine induced pluripotent stem cells analogous to naive and primed embryonic stem cells of the mouse. *Int J Dev Biol* 54:1703–1711
- Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Becker RA, Hearn JP (1995) Isolation of a primate embryonic stem cell line. *Proc Natl Acad Sci U S A* 92:7844–7848
- Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Hearn JP (1996) Pluripotent cell lines derived from common marmoset (*Callithrix jacchus*) blastocysts. *Biol Reprod* 55:254–259
- Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998a) Embryonic stem cell lines derived from human blastocysts. *Science* 282:1145–1147
- Thomson JA, Eldor EJ, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998b) Embryonic stem cell lines derived from human blastocysts. *Science* 282(5391):1145–1147
- Till JE, McCulloch EA (1961) A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 14:213–222
- Till JE, McCulloch EA (1980) Haematopoietic stem cell differentiation. *Biochem Biophys Acta* 605:431–459
- Till JE, McCulloch EA, Siminovitch L (1964) A stochastic model of stem cell proliferation, based on the growth of spleen Colony-forming cells. *Proc Natl Acad Sci U S A* 51:29–36
- Tiway R, Amarpal, Aithal HP, Kinjavdekar P, Pawde AM, Singh R (2014) Effect of IGF-1 and uncultured autologous bone-marrow-derived mononuclear cells on repair of osteochondral defect in rabbits. *Cartilage* 5:43–54
- Van der Kooy D, Weiss S (2000) Why stem cells? *Science* 287:1439–1441
- Wagner W, Bork S, Horn P, Kronic D, Walenda T, Diehlmann A, Benes V, Blake J, Huber FX, Eckstein V, Boukamp P, Ho D (2009) Aging and replicative senescence have related effects on human stem and progenitor cells. *PLoS One* 4:e5846
- Walker SC, Shin T, Zaunbrecher GM, Romano JE, Johnson GA, Bazer FW, Piedrahita JA (2002) A highly efficient method for porcine cloning by nuclear transfer using in vitro-matured oocytes. *Cloning Stem Cells* 4:105–112

- Whitworth DJ, Ovchinnikov DA, Sun J, Fortuna PR, Wolvetang EJ (2014) Generation and characterization of LIF-dependent equine induced pluripotent stem cells from adult dermal fibroblasts. *Stem Cells Dev* 23:1515–1523
- Wilmot I, Schnieke AE, McWhir J, Kind AJ, Campbell KH (1997) Viable offspring derived from fetal and adult mammalian cells. *Nature* 385:810–813
- Xu M, Shaw G, Murphy M, Barry F (2019) Induced pluripotent stem cell-derived Mesenchymal stromal cells are functionally and genetically different from bone marrow-derived Mesenchymal stromal cells. *Stem Cells* 37(6):754–765
- Zhao L, Wang Z, Zhang J, Yang J, Gao XF, Wu B, Zhao G, Bao S, Hu S, Liu P, Li X (2017) Characterization of the single-cell derived bovine induced pluripotent stem cells. *Tissue Cell* 49(5):521–527



Mesenchymal Stem Cell and Its Properties

2

M. B. Gugjoo, Amar Pal, and G. T. Sharma

Abstract

Stem cell (SC) concept is one of the important research areas seen to bring new vistas in the biological science and/or medicine. Various stem cell types have been identified including mesenchymal stem cells (MSCs). MSCs are present in almost all the body parts of an individual and carry the specialized stemness property, in addition to the immuno-modulation, plasticity and homing. These cells are studied for their therapeutic applications due to lack of any ethical issue together with a limited ability to develop tumour. However, there is dearth of understanding in their basic specialized physiological properties and as such definitive clinical applications are restricted. The current chapter details various stem cell types and discusses the characteristic properties of the stem cells with special focus on MSCs.

2.1 Introduction

The stem cell (SC) concept is one of the most thrilling scientific research areas in twenty-first century. Stem cell research is seen and hoped to bring new dawn in the field of biology and medicine. An individual in their lifespan undergoes through

M. B. Gugjoo

Division of Veterinary Clinical Complex, FVSc & AH, SKUAST-Kashmir,
Jammu and Kashmir, India

A. Pal

Division of Surgery, Indian Veterinary Research Institute, Izatnagar, UP, India

G. T. Sharma (✉)

Division of Physiology & Climatology, Indian Veterinary Research Institute,
Izatnagar, UP, India

© The Editor(s) (if applicable) and The Author(s), under exclusive license to
Springer Nature Singapore Pte Ltd. 2020

M. B. Gugjoo, A. Pal (eds.), *Mesenchymal Stem Cell in Veterinary Sciences*,
https://doi.org/10.1007/978-981-15-6037-8_2

13

various developmental stages each characterized by the availability of particular stem cell type. During embryonic stage, stem cells give rise to the complete individual and in post-natal life the cells serve a sort of an internal repair system to maintain homeostasis (Gugjoo and Amarpal 2018; Gugjoo et al. 2019). The inner cell mass of trophoblast during embryogenesis carries embryonic stem cells (ESCs) that give rise to the whole individual (pluripotency). Adult tissue stem cells, present in almost all the body tissues, have restricted differentiation potential (multipotent) (Fick et al. 2012; Ayala-Cuellar et al. 2018). The adult tissue stem cells like mesenchymal stem cells (MSCs) maintain tissue homeostasis through tissue cell and matrix turn-over (Gugjoo et al. 2018a, b, 2019). It may be inferred that developmental progression of an individual brings in reduced differentiation potential of the stem cells.

2.2 What Is Stem Cell?

Stem cell (SC) is an unspecialized cell carried by a multi-cellular organism that is able to produce an identical copy of itself through the process of ‘self-renewal’ and a more specialized cell through the process of differentiation (Till et al. 1964; Till and McCulloch 1961; Ab Kadir et al. 2012). Thus, the two main properties that contribute stemness feature to the stem cell are the ‘self-renewal’ and the ‘differentiation’.

2.3 Stem Cell Types

Stem cells are classified in many ways but most commonly based upon the degree of differentiation and source of isolation.

2.3.1 Based on the Degree of Differentiation

Totipotent Cell The cell differentiates into any cell type of the body including the foetal membrane like zygote up to morula stage of the embryogenesis.

Pluripotent Cell The cell differentiates into all the cell types of the body barring foetal membranes like embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs).

Multipotent Cell The cell gives rise to limited cell types of the body, e.g. mesenchymal stem cells (MSCs).

2.3.2 Based on the Source

Embryonic Stem Cell The cell is derived from an embryo and may be totipotent (cells up to the morula stage of embryogenesis) or pluripotent (inner cell mass of the blastocyst of embryo).

Induced Pluripotent Stem Cell The cell is developed by reprogramming of the adult somatic cell through “forced” expression of pluripotency-specific genes.

Adult Stem Cell It is an unspecialized cell present in all the tissues/organs of the body for internal repair or replacement of damaged cells. The self-renewal and the differentiation capacity are restricted in these cells as compared to pluripotent cells.

2.4 Mesenchymal Stem Cell

Among various stem cell types, MSCs currently form an important constituent of the regenerative medicine. This can be attributed to their diverse and numerous accessible sources, and the simplicity of isolation procedures with better success rates. These cells have minimal teratogenicity usually seen with the pluripotent stem cells and bear limited ethical issues commonly associated with human ESCs (Zuk et al. 2001; Cardoso et al. 2017). Additional features like ability to immunomodulate and provide anti-inflammatory response, thereby regeneration of tissue without scar formation, make it more suitable for regenerative medicine (Zuk et al. 2001; Stewart and Stewart 2011; Gugjoo et al. 2019). However, MSCs in contrary to ESCs have limited culture lifespan, and after certain culture passages these cells become senescent (Caplan 2012). Even upon in vivo implantation, MSCs (autologous/allogeneic) survive only for certain period (ranging from few hours to as long as 90 days) but not indefinitely (Guest et al. 2010; McDuffee et al. 2012). This questions their self-renewal property (Lopez and Jarazo 2015). In order to extend their lifespan, vector viruses like simian virus 40 (SV40-T) and human papillomavirus have been used to extend their culture lifespan (Klingelutz et al. 1994). The SV40-T retrovirus may immortalize dog adipose tissue-derived MSCs (Ayala-Cuellar et al. 2018) by reducing the growth inhibition on retinoblastoma protein (pRb) and tumour protein (p53) (DeCaprio et al. 1988; Pipas 2009). These are preliminary studies that need further extensive research in the area to confirm their clinical safety and efficacy.

2.4.1 Characteristic Features of MSCs

2.4.1.1 Stemness

The prime stemness property, represented by self-renewal and differentiation, of MSCs is poorly understood and has even been questioned. Only few genes support

MSCs stemness markers. There have to be deep insights into its molecular basis especially through the main transcription factors. So far, not a single key transcription factor to MSCs has been determined unlike pluripotent genes like Oct4, Nanog and Sox2 being attributed to the embryonic stem cells. Several highly expressed genes including nine transcription factors in undifferentiated MSCs have been detected after comparing undifferentiated and tri-lineage-differentiated MSCs. However, individual knockdown of such genes could only partially decrease the cell proliferation or their differentiation (Kubo et al. 2009). Several reasons that may be underlined to such poor understanding are as follows:

1. The cellular heterogeneity with respect to the species, age, sources, culture conditions and number of culture passages (Mareddy et al. 2010; Menicanin et al. 2010).
2. MSCs have finite culture lifespan characterized by ageing and senescence after certain passages. MSCs exhibit morphological abnormalities, attenuated expression of specific surface markers, reduced differentiation potential and proliferation arrest (Baxter et al. 2004; Wagner et al. 2008; Liu et al. 2013).
3. Poor understanding of microenvironment/niche that controls MSCs features. There is need to study the environmental effects. Among various niche cues studied include hypoxia (Drela et al. 2014; Hu et al. 2014; Fotia et al. 2015), available extra-cellular matrix (ECM) (Xiong et al. 2015; Wong et al. 2017), scaffolds (Cesarz and Tamama 2016; Obara et al. 2016), mechanical factors and/ humoral/ growth factors (Terraciano et al. 2007; Bottagisio et al. 2015; Hu et al. 2015). Such cues, however, have been studied in isolation while in vivo microenvironment/niche presents an intricate system.

Self-Renewal and Differentiation

The self-renewal property keeps the stem cell pool intact, which makes it possible to repair the damaged cells or to replace the non-functioning cells throughout an individual's life (Fig. 2.1). This feature of stem cell differentiates it from progenitor cell as the latter cell although able to differentiate but is unable to self-renew. Stem cell self-renewal occurs through either symmetric or asymmetric divisions. Through symmetric divisions, the parent cell is able to produce identical copies, that is, daughter cell copies entire genome including the epigenetic changes. In asymmetric divisions, two variable cells are produced either through divisional asymmetry or environmental asymmetry (Wilson and Trumpp 2006; He et al. 2009). In divisional asymmetry, cell fate determinants get reorganized prior to division and after division two cells are produced, one identical and other non-identical to the parent cell. In environmental asymmetry, the cell gets exposed to multiple signals from the local environment (micro-environment), which initially leads to symmetric division producing identical copies. One of the daughter stem cell then undergoes asymmetric cell division generating non-identical cells to perform tissue-specific function (Spradling et al. 2001; Ohlstein et al. 2004). This process is known as differentiation and is governed by available micro-environment. The growth factors and cytokines like IGF-1, TGF- β 1, BMP-1, etc. may facilitate stem cell proliferation or inhibition

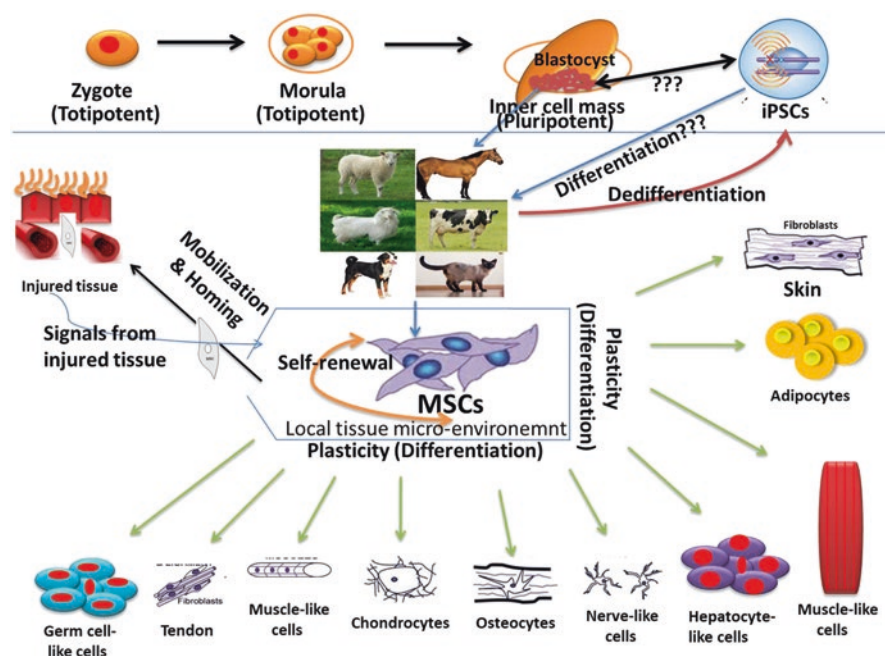


Fig. 2.1 Diagrammatic representation of development of mesenchymal stem cells (MSCs), their self-renewal, differentiation, and mobilization and homing properties

depending upon the tissue specific requirements (Fuchs et al. 2004; Potten et al. 2009; Spees et al. 2016). The microenvironment/niche tends to play an essential role in maintaining the stem cell self-renewal property. The cells lose their self-renewal potential upon loss of the niche (Xie and Spradling 2000; Mackenzie and Flake 2001; Li and Neaves 2006; Lilly et al. 2011).

The self-renewal potential varies among stem cell types. In culture environment, embryonic stem cells are able to self-renew almost indefinitely while MSCs self-renew only for few generations. The mechanism behind this wonderful property is still ambiguous though some information has been gained in recent times. One of the mechanisms reported is of telomerase, an enzyme required to maintain DNA regions at the chromosomal ends, telomeres, by preventing accumulation of DNA damage due to replication (Greenwood and Lansdorp 2003). It has been seen that telomerase absence decreases self-renewal capacity of haematopoietic stem cells (HSCs) while forced expression is insufficient to transplant such cells indefinitely (Allsopp et al. 2003a, b). The cells that are able to express elements of different signalling pathways like Wnt/ fzd/beta-catenin, sonic, Hedgehog, etc. usually undergo self-renewal divisions (Watt and Hogan 2000; Willert et al. 2003). Besides, over-expression of several proteins like homeobox 4 genes (Hoxb4) and Cdx4 (Kyba et al. 2002; Wang et al. 2005) may also favour self-renewal divisions. All these signalling pathways maintain pluripotency of the cells and may not be applicable to unipotent or oligopotent cells.

2.4.2 Plasticity

Differentiation capability of stem cell type varies. Embryonic stem cell is able to give rise to all the cell types of the three germinal layers while adult stem cells usually have restricted potential. Recent studies have shown an extended differentiation potential of adult stem cells. Mesenchymal stem cells (MSCs) differentiation potential earlier restricted to osteogenic, chondrogenic and adipogenic lineages (Gugjoo et al. 2015) has now been demonstrated to extend to other lineages like myocyte-/cardiomyocyte-like cells (Orlic et al. 2001; Ferrari et al. 1998; Vieira et al. 2010; Wang et al. 2018), neural-like cells (Oh et al. 2011; Ryu et al. 2012; Yan et al. 2015; Kriston-Pál et al. 2017; Li et al. 2017; Zhang et al. 2019) and germ cell-like cells (Ghasemzadeh-Hasankolaei et al. 2014, 2015), among others. This phenomenon is known as trans-differentiation. As such the stem cell fate is not fixed rather changeable or plastic (plasticity) in nature depending upon the available local microenvironment (Pelagalli et al. 2018).

2.4.2.1 Anti-Inflammatory and Immuno-Modulation

MSCs are considered as immune privileged or immune evasive cells, with an ability to immuno-modulate and provide anti-inflammatory activities (Caplan and Dennis 2006; Kang et al. 2008; Lee et al. 2011; Kim et al. 2010; Carrade et al. 2012; Prockop and Oh 2012). These cells avoid allogeneic reaction as they are hypo-immunogenic and often lack major histocompatibility complex (MHC)-II and costimulatory molecule expression, although variably. These cells prevent T-cell responses through the modulation of dendritic cells and disruption of natural killer (NK) cells (Wang et al. 2009). The cells may additionally modulate the function of CD8⁺ and CD4⁺ T-cells (Di Nicola et al. 2002). The cells interact with CD4 and CD8 lymphocytes and upon pro-inflammatory mediator's activation secrete factors that lead to down-regulation of the inflammation (Singer and Caplan 2011). MSCs hampering of T-cell activation and proliferation may also be induced by decreasing the expression of activation markers like CD25, CD38 and CD69 (Le Blanc et al. 2003; Groh et al. 2005). This property of MSCs makes their allogeneic application quite possible (Berner et al. 2013; Gugjoo et al. 2017, 2020). Considering the importance of these properties in regenerative medicine, a separate chapter on the immuno-modulatory and/anti-inflammatory properties of mesenchymal stem cells has been discussed later in the book.

2.4.2.2 Stem Cell Migration and Homing

MSCs have characteristic migratory and homing property (Mackenzie and Flake 2001). A significant amount of literature is available that tries to address the mechanism of stem cell migration and homing; properties that explain the basis of stem cell activity at sites beyond their site of implantation or original location. MSCs ability to migrate and home to the target site may be facilitated by humoral factors in addition to the involved unknown mechanisms (Kodeet al. 2009; Guest et al. 2010; McDuffee et al. 2012).

Mobilization of MSCs is activated by down-regulation of their adhesion molecules through the release of cytokines and/or chemokines (Liu et al. 2009). There are various factors relevant to MSCs mobilization including matrix metalloproteinase protein (MMP)-9 and stromal cell-derived factor (SDF)-1 α /chemokine (C-X-C motif) ligand (CXCL12) and its receptor CXCR4 (Hristov and Weber 2009). MSCs overexpression of insulin-like growth factor 1 (IGF-1) may induce their massive mobilization via SDF-1 α signalling (Haider et al. 2008). In addition to SDF-1 α , MSCs may release high mobility group box-1 (HMGB-1) extracellularly after their activation through inflammatory cytokine or by cell necrosis. This protein acts as a chemo-attractant for MSCs (Palumbo and Bianchi 2004; Chavakis et al. 2007). The injury-/ischemia-induced activation of fibroblasts cause an increased migration velocity and invasion of MSCs. Two important components like basic fibroblast growth factor (bFGF) and epithelial neutrophil-activating peptide-78 (ENA-78 or CXCL5) seem to be mainly involved in such activities (Neddeau et al. 2008). Surface receptors like chemokine receptors (CCR1, CCR4, CCR7, CCR9, CXCR5, CXCR6 and CCR10) expressed on human BM-MSCs may be involved in their migration (Wynn et al. 2004; Sordi et al. 2005; Von Luttichau et al. 2005).

MSCs homing to the target tissue sites follow cascade of events. This includes MSCs rolling into the blood vessels, endothelial cell surface adhesion, trans-endothelial migration, extravasation and ultimately their migration through the tissue matrix to the injured target area (Zhuge et al. 2010). Stem cells express numerous surface receptors and cell adhesion molecules, similar to that of leucocytes, that aide in migration and homing of the cells (Luster et al. 2005; Ley et al. 2007). The receptors include integrins, selectins, cell adhesion molecules (CAMs) and chemokine receptors. P-selectin, a cell adhesion molecule, is considered to be involved in MSCs rolling that slows their motion and allows them to adhere to endothelium (Ruster et al. 2006). Various other adhesion molecules that mediate MSC–endothelial cell interaction involve integrins such as α 1 down to α 5 and β 1 up to β 4 and CAMs such as VCAM-1 to ICAM-3, ALCAM and CD105 (Minguell et al. 2001; Krampera et al. 2006). These adhesion molecules and their counter-ligands are expressed on MSCs and/or endothelial cells. CD44 may act as a homing molecule for BM-MSCs as it traffics them to the bone through marrow vasculature supported E-selectin interaction (Sackstein et al. 2008). Equine MSCs (eMSCs) homing may be achieved through their expression of the stromal cell-derived factor 1 (SDF-1) and its receptor C-X-C chemokine receptor type 4 (CXCR4) (Angelone et al. 2017). Additionally, adhesion molecules like Galectin-1 and Galectin-3 might play an important role in equine MSCs homing to the inflamed and injured tissues (Reesink et al. 2017). The role of hypoxia in stem cell homing demands thorough investigation as hypoxia activates peripheral blood mononuclear cells to turn to the MSCs (Hopper et al. 2015).

All these studies demonstrate active inflammation to be prime factor involved in directing MSCs migration and homing, but such properties against chronic lesions that are devoid of inflammation currently remain questioned.

2.5 Significance of Stem Cell Research

Tissue-specific cells continuously undergo the process of wear and tear and ultimately die. The cells if not replaced may lead to malfunctioning of the tissue system and finally death. Such damaged cells are, thus, being replaced by an internal repair system formed by the stem cells. However, in extensive cases an intrinsic system fails to maintain such homeostasis. In an organ failure case (kidney, heart failure), donors are required who are hardly available that too at right time. Besides, some highly specialized tissues like nervous tissue have very limited internal repair system, which demands the medical support for damage repair. Currently, stem cells are seen to carry the potential to fill this gap in medical field. Further, stem cell differentiation if controlled can be used to produce specific tissue/organs. Such tissue/organs can be used as disease models to study the course of disease and the therapeutic options.

2.6 Conclusion

MSCs are important stem cell types that are considered to provide therapeutic effects in regenerative medicine through self-renewal, differentiation, immunomodulation, migration and homing. However, there are various queries that need to be addressed before stem cell application can be made successful.

2.6.1 Answers Required

1. How do stem cells proliferate without differentiation while non-stem cells do not?
2. What are the in vivo factors and how do they regulate stem cell self-renewal, proliferation and differentiation?
3. Why pluripotent stem cells are almost immortalized while adult stem cells like MSCs have limited lifespan?
4. How immuno-modulation, anti-inflammatory and homing properties of MSCs can be controlled?

The answers if discovered may pave the way for the following potential uses of stem cells:

1. Repair tissue/organ that is extensively damaged either by direct transplantation or through tissue engineering.
2. Stimulation of the intrinsic stem cell pool of an individual for effective repair.
3. Development of tissue/organ models for disease course study and their therapeutics.
4. Regulation of the developmental course of an individual.
5. Study inherited diseases like birth defects, Parkinson's disease, etc.
6. Understand cancer biology and, thus, control measures.

References

- Ab Kadir R, Hisham Zainal Ariffin S, Megat Abdul Wahab R, Senafi S (2012) Molecular characterisation of human peripheral blood stem cells. *S Afr J Sci* 108:1–7
- Allsopp RC, Morin GB, DePinho R, Harley CB, Weissman IL (2003a) Telomerase is required to slow telomere shortening and extend replicative lifespan of HSCs during serial transplantation. *Blood* 102:517–520
- Allsopp RC, Morin GB, Horner JW, DePinho R, Harley CB, Weissman IL (2003b) Effect of TERT over-expression on the long-term transplantation capacity of hematopoietic stem cells. *Nat Med* 9:369–371
- Angelone M, Conti V, Biacca C, Battaglia B, Pecorari L, Piana F, Gnudi G, Leonardi F, Ramoni R, Basini G, Dotti S, Renzi S, Ferrari M, Grolli S (2017) The contribution of adipose tissue-derived Mesenchymal stem cells and platelet-rich plasma to the treatment of chronic equine laminitis: a proof of concept. *Int J Mol Sci* 18:2122
- Ayala-Cuellar AP, Kim C-W, Hwang K-A, Kang J-H, Lee G, Cho J, Choi K-C (2018) Characterization of canine adipose tissue-derived mesenchymal stem cells immortalized by SV40-T retrovirus for therapeutic use. *J Cell Physiol* 234:9. <https://doi.org/10.1002/jcp.28338>
- Baxter MA, Wynn RF, Jowitt SN, Wraith JE, Fairbairn LJ, Bellantuono I (2004) Study of telomere length reveals rapid aging of human marrow stromal cells following in vitro expansion. *Stem Cells* 22:675–682
- Berner A, Reichert JC, Woodruff MA, Saifzadeh S, Morris AJ, Epari DR, Nerlich M, Schuetz MA, Hutmacher DW (2013) Autologous vs. allogenic mesenchymal progenitor cells for the reconstruction of critical sized segmental tibial bone defects in aged sheep. *Acta Biomater* 9(8):7874–7884
- Bottagisio M, Lovati AB, Lopa S, Moretti M (2015) Osteogenic differentiation of human and ovine bone marrow stromal cells in response to b-glycerophosphate and monosodium phosphate. *Cell Reprogram* 17(4):235–242
- Caplan AI (2012) MSCs as therapeutics. In: Hematti P, Keating A (eds) *Stem cell biology and Reg medicine: Mesenchymal stromal cells: biology and clinical applications*. Human Press, New York, pp 79–90
- Caplan AI, Dennis JE (2006) Mesenchymal stem cells as trophic mediators. *J Cell Biochem* 98:1076–1084
- Cardoso MT, Pinheiro AO, Vidane AS, Casals JB, de Oliveira VC, Gonçalves NJN, Martins DS, Ambrósio CE (2017) Characterization of teratogenic potential and gene expression in canine and feline amniotic membrane-derived stem cells. *Reprod Domest Anim* 52(s2):58–64
- Carrade DD, Lame MW, Kent MS, Clark KC, Walker NJ, Borjesson DL (2012) Comparative analysis of the immunomodulatory properties of equine adult-derived mesenchymal stem cells. *Cell Med* 4:1–11
- Cesarz Z, Tamama K (2016) Spheroid culture of Mesenchymal stem cells. *Stem Cells Int* 2016:9176357
- Chavakis E, Hain A, Vinci M et al (2007) High-mobility group box 1 activates integrin-dependent homing of endothelial progenitor cells. *Circ Res* 100(2):204–212
- DeCaprio JA, Ludlow JW, Figge J, Shew JY, Huang CM, Lee WH, Livingston DM (1988) SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. *Cell* 54(2):275–283
- Di Nicola M, Carlo-Stella C, Magni M, Milanese M, Longoni PD, Matteucci P, Grisanti S, Gianni AM (2002) Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or non-specific mitogenic stimuli. *Blood* 99(10):3838–3843
- Drela K, Sarnowska A, Siedlecka P, Szablowska-Gadomska I, Wielgos M, Jurga M, Lukomska B, Domanska-Janik K (2014) Low oxygen atmosphere facilitates proliferation and maintains undifferentiated state of umbilical cord mesenchymal stem cells in an hypoxia inducible factor-dependent manner. *Cytotherapy* 16:881–892

- Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 279:1528–1530
- Fick LJ, Fick GH, Li Z, Cao E, Bao B, Heffelfinger D, Riabowol K (2012) Telomere length correlates with life span of dog breeds. *Cell Rep* 2(6):1530–1536
- Fotia C, Massa A, Boriani F, Baldini N, Granchi D (2015) Hypoxia enhances proliferation and stemness of human adipose-derived mesenchymal stem cells. *Cytotechnology* 67:1073–1084
- Fuchs E, Tumber T, Guasch G (2004) Socializing with the neighbors: stem cells and their niche. *Cell* 116:769–778
- Ghasemzadeh-Hasankolaie M, Sedighi-Gilani MA, Eslaminejad MB (2014) Induction of ram bone marrow mesenchymal stem cells into germ cell lineage using transforming growth factor- β superfamily growth factors. *Reprod Domest Anim* 49:588–598
- Ghasemzadeh-Hasankolaie M, Eslaminejad BM, Sedighi-Gilani M (2015) Derivation of male germ cells from ram bone marrow mesenchymal stem cells by three different methods and evaluation of their fate after transplantation into the testis. *In Vitro Cell Dev Biol Anim* 52(1):49–61
- Greenwood MJ, Lansdorp PM (2003) Telomeres, telomerase, and hematopoietic stem cell biology. *Arch Med Res* 34:489–495
- Groh ME, Maitra B, Szekely E, Koc ON (2005) Human mesenchymal stem cells require monocyte-mediated activation to suppress alloreactive T cells. *Exp Hematol* 33:928–934
- Guest DJ, Smith MR, Allen WR (2010) Equine embryonic stem-like cells and mesenchymal stromal cells have different survival rates and migration patterns following their injection into damaged superficial digital flexor tendon. *Equine Vet J* 42(7):636–642
- Gugjoo MB, Amarpal (2018) Mesenchymal stem cell research in sheep: current status and future prospects. *Small Rumin Res* 169:46–56
- Gugjoo MB, Amarpal, Kinjavdekar P, Aithal HP, Ansari MM, Pawde AM, Sharma GT (2015) Isolation, culturing and characterization of New Zealand white rabbit mesenchymal stem cells derived from bone marrow. *Asian J Animal Vet Adv* 10(10):537–548
- Gugjoo MB, Amarpal, Abdelbaset-Ismael A, Aithal HP, Kinjavdekar P, Pawde AM, Kumar GS, Sharma GT (2017) Mesenchymal stem cells with IGF-1 and TGF- β 1 in laminin gel for osteochondral defects in rabbits. *Biomed Pharmacother* 93:1165–1174
- Gugjoo MB, Amarpal, Chandra V, Wani MY, Dhama K, Sharma GT (2018a) Mesenchymal stem cell research in veterinary medicine. *Curr Stem Cell Res Ther* 13(8):645–657
- Gugjoo MB, Amarpal, Fazili MR, Shah RA, Sharma GT (2018b) Mesenchymal stem cell: basic research and potential applications in cattle and buffalo. *J Cell Physiol* 234(6):8618–8635
- Gugjoo MB, Amarpal, Makhdoomi DM, Sharma GT (2019) Equine mesenchymal stem cells: properties, sources, characterization and potential therapeutic applications. *J Equine Veter Sci* 72:16–27
- Gugjoo MB, Hussain S, Amarpal, Shah RA, Dhama K (2020) Mesenchymal stem cell mediated Immuno-modulatory and anti-inflammatory mechanisms in immune and allergic disorders. *Recent Patents Inflamm Allergy Drug Discov* 14:1. <https://doi.org/10.2174/1872213X14666200130100236>
- Haider H, Jiang S, Idris NM et al (2008) IGF-1-overexpressing mesenchymal stem cells accelerate bone marrow stem cell mobilization via paracrine activation of SDF-1 α /CXCR4 signaling to promote myocardial repair. *Circ Res* 103(11):1300–1308
- He S, Nakada D, Morrison SJ (2009) Mechanisms of stem cell self-renewal. *Annu Rev Cell Dev Biol* 25:377–406
- Hopper N, Wardale J, Brooks R, Power J, Rushton N, Henson F (2015) Peripheral blood mononuclear cells enhance cartilage repair in in vivo Osteochondral defect model. *PLoS One* 10(8):e0133937
- Hristov M, Weber C (2009) Progenitor cell trafficking in vascular wall. *J Thromb Haemost* 7(suppl 1):31–34
- Hu X, Wu R, Shehadeh LA, Zhou Q, Jiang C et al (2014) Severe hypoxia exerts parallel and cell-specific regulation of gene expression and alternative splicing in human mesenchymal stem cells. *BMC Genom* 15:303

- Hu Y, Zhang Q, Zhang L, Tang X-X, He H-Y (2015) Basic fibroblast growth factor lentiviral vector-transfected sheep bone marrow mesenchymal stem cells and non-specific osteogenic gene expression. *Mol Med Rep* 12:267–272
- Kang JW, Kang K-S, Koo HC, Park JR, Choi EW, Park YH (2008) Soluble factors-mediated immunomodulatory effects of canine adipose tissue-derived mesenchymal stem cells. *Stem Cells Dev* 17(4):681–693
- Kim HS, Kim KH, Kim SH, Kim YS, Koo KT, Kim TI, Seol Y-J, Ku Y, Rhyu I-C, Chung C-P, Lee Y-M (2010) Immunomodulatory effect of canine periodontal ligament stem cells on allogenic and xenogenic peripheral blood mononuclear cells. *J Periodontal Implant Sci* 40:265–270
- Klingelhut AJ, Barber SA, Smith PP, Dyer K, McDougall JK (1994) Restoration of telomeres in human papillomavirus-immortalized human anogenital epithelial cells. *Mol Cell Biol* 14(2):961–969
- Kode JA, Mukherjee S, Joglekar MV, Hardikar AA (2009) Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration. *Cytotherapy* 11:377–791
- Krampera M, Pizzolo G, Aprili G et al (2006) Mesenchymal stem cells for bone, cartilage, tendon and skeletal muscle repair. *Bone* 39(4):678–683
- Kriston-Pál É, Czibula Á, Gyuris Z, Balka G, Seregi A, Sükösd F, Süth M, Kiss-Tóth E, Haracska L, Uher F, Monostori É (2017) Characterization and therapeutic application of canine adipose mesenchymal stem cells to treat elbow osteoarthritis. *Can J Vet Res* 81:73–78
- Kubo H, Shimizu M, Taya Y, Kawamoto T, Michida M, Kaneko E, Igarashi A, Nishimura M, Segoshi K, Shimazu Y, Tsuji K, Aoba T, Kato Y (2009) Identification of mesenchymal stem cell (MSC)-transcription factors by microarray and knockdown analyses, and signature molecule marked MSC in bone marrow by immuno histochemistry. *Genes Cells* 14:407–424
- Kyba M, Perlingeiro RC, Daley GQ (2002) HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. *Cell* 109:29–37
- Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringden O (2003) HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 31:890–896
- Lee WS, Suzuki Y, Graves SS, Iwata M, Venkataraman GM, Mielcarek M, Peterson LJ, Ikehara S, Torok-Storb B, Storb R (2011) Canine bone-marrow-derived mesenchymal stromal cells suppress alloreactive lymphocyte proliferation in vitro but fail to enhance engraftment in canine bone marrow transplantation. *Biol Blood Marrow Transplant* 17:465–475
- Ley K, Laudanna C, Cybulsky MI, Nourshargh S (2007) Getting to the site of inflammation: leukocyte adhesion cascade updated. *Nat Rev Immunol* 7(9):678–689
- Li L, Neaves WB (2006) Normal stem cells and cancer stem cells: the niche matters. *Cancer Res* 66:4553–4557
- Li PZ, Yan GY, Han L, Pang J, Zhong BS, Zhang GM, Wang F, Zhang YL (2017) Overexpression of STRA8, BOULE, and DAZL genes promotes goat bone marrow-derived Mesenchymal stem cells in vitro Transdifferentiation toward putative male germ cells. *Reprod Sci* 24(2):300–312
- Lilly AJ, Johnson WE, Bunce CM (2011) The haematopoietic stem cell niche: new insights into the mechanisms regulating haematopoietic stem cell behaviour. *Stem Cells Int* 2011:274564
- Liu ZJ, Zhuge Y, Velazquez OC (2009) Trafficking and differentiation of Mesenchymal stem cells. *Biochemist* 106(6):984–991
- Liu TM, Ng WM, Tan HS, Vinitha D, Yang Z, Fan JB, Zou Y, Hui JH, Lee EH, Lim B (2013) Molecular basis of immortalization of human mesenchymal stem cells by combination of p53 knockdown and human telomerase reverse transcriptase overexpression. *Stem Cells* 22:268–278
- Lopez MJ, Jarazo J (2015) State of the art: stem cells in equine regenerative medicine. *Equine Vet J* 47:145–154
- Luster AD, Alon R, von Adrian UH (2005) Immune cell migration in inflammation: present and future therapeutic targets. *Nat Immunol* 6(12):1182–1190
- Mackenzie TC, Flake AW (2001) Human mesenchymal stem cells persist, demonstrate site-specific multipotential differentiation, and are present in sites of wound healing and tissue regeneration after transplantation into fetal sheep. *Blood Cells Mol Dis* 27(3):601–604

- Mareddy S, Dhaliwal N, Crawford R, Xiao Y (2010) Stem cell-related gene expression in clonal populations of mesenchymal stromal cells from bone marrow. *Tissue Eng Part A* 16:749–758
- McDuffee LA, Pack L, Lores M, Wright GM, Esparza-Gonzalez B, Masaoud E (2012) Osteoprogenitor cell therapy in an equine fracture model. *Vet Surg* 41:773–783
- Menicanin D, Bartold PM, Zannettino AC, Gronthos S (2010) Identification of a common gene expression signature associated with immature clonal mesenchymal cell populations derived from bone marrow and dental tissues. *Stem Cells Dev* 19:1501–1510
- Minguell JJ, Erices A, Conget P (2001) Mesenchymal stem cells. *Exp Biol Med* (Maywood) 226(6):507–520
- Nedeau AE, Bauer RJ, Gallagher K et al (2008) A CXCL5- and bFGF-dependent effect of PDGF-B-activated fibroblasts in promoting trafficking and differentiation of bone marrow derived mesenchymal stem cells. *Exp Cell Res* 314(11–12):2176–2186
- Obara C, Tomiyama K, Takizawa K, Islam R, Yasuda T, Gotoh T, Tajima K (2016) Characteristics of three-dimensional prospectively isolated mouse bone marrow mesenchymal stem/stromal cell aggregates on nanoculture plates. *Cell Tissue Res* 366:113–127
- Oh HJ, Park JE, Kim MJ, Hong SG, Ra JC, Jo JY, Kang SK, Jang G, Lee BC (2011) Recloned dogs derived from adipose stem cells of a transgenic cloned beagle. *Theriogenology* 75:1221–1231
- Ohlstein B, Kai T, Decotto E, Spradling A (2004) The stem cell niche: theme and variations. *Curr Opin Cell Biol* 16:693–699
- Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A, Anversa P (2001) Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci U S A* 98:10344–10349
- Palumbo R, Bianchi ME (2004) High mobility group box 1 protein, a cue for stem cell recruitment. *Biochem Pharmacol* 68(6):1165–1170
- Pelagalli A, Nardelli A, Lucarelli E, Zannetti A, Brunetti A (2018) Autocrine signals increase ovine mesenchymal stem cells migration through Aquaporin-1 and CXCR4 overexpression. *J Cell Physiol* 233(8):6241–6249
- Pipas JM (2009) SV40: cell transformation and tumorigenesis. *Virology* 384(2):294–303
- Potten CS, Gandara R, Mahida YR, Loeffler M, Wright NA (2009) The stem cells of small intestinal crypts: where are they? *Cell Prolif* 42:731–750
- Prockop DJ, Oh JY (2012) Mesenchymal stem/stromal cells (MSCs): role as guardians of inflammation. *Mol Ther* 20:14–20
- Reesink HL, Sutton RM, Shurer CR, Peterson RP, Tan JS, Su J, Paszek MJ, Nixon AJ (2017) Galectin-1 and galectin-3 expression in equine mesenchymal stromal cells (MSCs), synovial fibroblasts and chondrocytes, and the effect of inflammation on MSC motility. *Stem Cell Res Ther* 8:243
- Ruster B, Gottig S, Ludwig RJ, Bistran R, Müller S, Seifried E, Gille J, Henschler R (2006) Mesenchymal stem cells display coordinated rolling and adhesion behavior on endothelial cells. *Blood* 108(12):3938–3944
- Ryu H-H, Kang B-J, Park S-S, Kim Y, Sung G-J, Woo H-M, Kim WH, Kweon O-K (2012) Comparison of Mesenchymal stem cells derived from fat, bone marrow, Wharton's jelly, and umbilical cord blood for treating spinal cord injuries in dogs. *J Vet Med Sci* 74(12):1617–1630
- Sackstein R, Merzaban JS, Cain DW, Dagia NM, Spencer JA, Lin CP, Wohlgenuth R (2008) Ex vivo glycan engineering of CD44 programs human multipotent mesenchymal stromal cell trafficking to bone. *Nat Med* 14(1):181–187
- Singer NG, Caplan AI (2011) Mesenchymal stem cells: mechanisms of inflammation. *Annu Rev Pathol* 6:457–478
- Sordi V, Malosio ML, Marchesi F, Mercalli A, Melzi R, Giordano T, Belmonte N, Ferrari G, Leone BE, Bertuzzi F, Zerbini G, Allavena P, Bonifacio E, Piemonti L (2005) Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets. *Blood* 106(2):419–427
- Spees JL, Lee RH, Gregory CA (2016) Mechanisms of mesenchymal stem/stromal cell function. *Stem Cell Res Ther* 7:125. <https://doi.org/10.1186/s13287-016-0363-7>

- Spradling A, Drummond-Barbosa D, Kai T (2001) Stem cells find their niche. *Nature* 414:98–104
- Stewart MC, Stewart AA (2011) Mesenchymal stem cells: characteristics, sources, mechanisms of action. *Vet Clin North Am Equine Pract* 27:243–261
- Terraciano V, Hwang N, Moroni L, Park HB, Zhang Z, Mizrahi J, Seliktar D, Elisseeff J (2007) Differential response of adult and embryonic Mesenchymal progenitor cells to mechanical compression in hydrogels. *Stem Cells* 25:2730–2738
- Till JE, McCulloch EA (1961) A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 14:213–222
- Till JE, McCulloch EA, Siminovitch L (1964) A stochastic model of stem cell proliferation, based on the growth of spleen colony-forming cells. *Proc Natl Acad Sci USA* 51:29–36
- Vieira NM, Brandalise V, Zucconi E, Secco M, Strauss BE, Zatz M (2010) Isolation, characterization, and differentiation potential of canine adipose-derived stem cells. *Cell Transplant* 19:279–289
- Von Luttichau I, Notohamiprodjo M, Wechselberger A, Peters C, Henger A, Seliger C et al (2005) Human adult CD34- progenitor cells functionally express the chemokine receptors CCR1, CCR4, CCR7, CXCR5, and CCR10 but not CXCR4. *Stem Cells Dev* 14(3):329–336
- Wagner W, Horn P, Castoldi M, Diehlmann A, Bork S, Saffrich R, Benes V, Blake J, Pfister S, Eckstein V, Ho AD (2008) Replicative senescence of mesenchymal stem cells: a continuous and organized process. *PLoS One* 3:2213
- Wang L, Menendez P, Shojaei F, Li L, Mazurier F, Dick JE, Cerdan C, Levac K, Bhatia M (2005) Generation of hematopoietic repopulating cells from human embryonic stem cells independent of ectopic HOXB4 expression. *J Exp Med* 201:1603–1614
- Wang M, Yang Y, Yang D, Luo F, Liang W, Guo S, Xu J (2009) The immunomodulatory activity of human umbilical cord blood-derived mesenchymal stem cells in vitro. *Immunology* 126(2):220–232
- Wang Q, Wang X, Lai D, Deng J, Hou Z, Liang H, Liu D (2018) BIX-01294 promotes the differentiation of adipose mesenchymal stem cells into adipocytes and neural cells in Arbas cashmere goats. *Res Vet Sci* 119:9–18
- Watt FM, Hogan BL (2000) Out of Eden: stem cells and their niches. *Science* 287:1427–1430
- Willert K, Brown JD, Danenberg E, Duncan AW, Weissman IL, Reyes T, Yates JR 3rd, Nusse R (2003) Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* 423:448–452
- Wilson A, Trumpp A (2006) Bone-marrow haematopoietic stem-cell niches. *Nat Rev Immunol* 6:93–106
- Wong TY, Chang CH, Yu CH, Huang LLH (2017) Hyaluronan keeps mesenchymal stem cells quiescent and maintains the differentiation potential over time. *Aging Cell* 16:451–460
- Wynn RF, Hart CA, Corradi-Perini C, O'Neill L, Evans CA, Wraith JE, Fairbairn LJ, Bellantuono I (2004) A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. *Blood* 104(9):2643–2645
- Xie T, Spradling AC (2000) A niche maintaining germ line stem cells in the *Drosophila* ovary. *Science* 290:328–330
- Xiong Y, He J, Zhang W, Zhou G, Cao Y, Liu W (2015) Retention of the stemness of mouse adipose-derived stem cells by their expansion on human bone marrow stromal cell-derived extracellular matrix. *Tissue Eng Part A* 21:1886–1894
- Yan G, Fan Y, Li P, Zhang Y, Wang F (2015) Ectopic expression of DAZL gene in goat bone marrow-derived mesenchymal stem cells enhances the trans-differentiation to putative germ cells compared to the exogenous treatment of retinoic acid or bone morphogenetic protein 4 signalling molecules. *Cell Biol Int* 39(1):74–83
- Zhang YL, Li PZ, Pang J, Wan YJ, Zhang GM, Fan YX, Wang ZY, Tao NH, Wang F (2019) Induction of goat bone marrow mesenchymal stem cells into putative male germ cells using mRNA for STRA8, BOULE and DAZL. *Cytotechnology* 71(2):563–572

- Zhuge Y, Liu Z-J, Velazquez OC (2010) Adult stem cell differentiation and trafficking and their implications in disease. In: Meshorer E, Plath K (eds) The cell biology of stem cells. Springer, New York, pp 169–183
- Zuk P, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 7:211–228



Mesenchymal Stem Cell Isolation, Culture, Characterization and Cryopreservation

3

M. B. Gugjoo, Amar Pal, V. Chandra, and G. T. Sharma

Abstract

The characteristic features of mesenchymal stem cells (MSCs) have made them an important subject of the regenerative medicine. These cells are being harvested, isolated and characterized from numerous adult body tissues/organs, in addition to the foetal membranes. The cells in donor tissues are present in a very limited and variable concentrations making it imperative to culture expand them for effective utilization. The variations in tissue types and/in culture techniques of the cells make it difficult to understand their features and clinical applications. For ready to use application, the cells are being banked, mostly through cryopreservation. The details of isolation, culture and characterization, in addition to cryopreservation, of MSCs are detailed in this topic.

3.1 Introduction

Initially, mesenchymal stem cells (MSCs) were identified by Friedenstein and co-workers from bone marrow. The typical fibroblast-like morphology and ability to adhere to the plastic were basis of their characterization (Friedenstein et al. 1976;

M. B. Gugjoo (✉)

Division of Veterinary Clinical Complex, FVSc & AH, SKUAST-Kashmir,
Jammu and Kashmir, India

A. Pal

Division of Surgery, Indian Veterinary Research Institute, Izatnagar, UP, India

V. Chandra · G. T. Sharma

Division of Physiology & Climatology, Indian Veterinary Research Institute,
Izatnagar, UP, India

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2020

M. B. Gugjoo, A. Pal (eds.), *Mesenchymal Stem Cell in Veterinary Sciences*,
https://doi.org/10.1007/978-981-15-6037-8_3

27

Phinney and Sensebe 2013). Subsequently, it had been shown that these cells are present in almost all the adult tissues of the body, in addition to the foetal membranes (Gugjoo and Amarpal 2018; Gugjoo et al. 2019). These cells represent very small population in a particular tissue and are mixed with other cell types. In bone marrow less than 0.1% of the mononuclear cells are MSCs and such a concentration decreases with ageing (Caplan 2007). The therapeutic applications of MSCs require much higher doses (Gugjoo et al. 2020a, b), making it imperative to harvest and culture expand them. The culture expansion of MSCs may change their phenotype making their regulations necessary. Bone marrow mononuclear cells and stromal vascular fraction that are minimally manipulated and applied directly are being used unrestrictedly (Gugjoo et al. 2018; Gugjoo et al. 2020a, b).

For development of any therapeutic agent comprehensive standardized procedures are mandatory in good manufacturing practices (GMPs). Likewise MSCs must be harvested, isolated, culture expanded and characterized as per the GMP norms for their successful utilization (Gugjoo et al. 2015; Akram et al. 2017). The techniques employed for MSCs processing, however, involve variable procedures. MSCs harvested from liquid source like bone marrow/peripheral blood are cultured from mononuclear cell (MNC) fraction being separated by density gradient method (Gugjoo et al. 2015). In contrast, MSCs harvested from solid tissue like adipose tissue or cord matrix, among others are harvested from stromal vascular fraction (SVF) or MNC fraction. MNC fraction conforms to the mixture of red blood cells, granulocytes, platelets and myeloid immature precursors, in addition to the MSCs obtained from peripheral blood or bone marrow. SVF refers to the nucleated cell fraction of heterogeneous mixture of endothelial, muscle, fibroblastic and mast cells, and pericytes, pre-adipocytes and MSCs (Mamidi et al. 2012; Salehinejad et al. 2012; Otsuru et al. 2013). The main difference in the procedure to harvest MSCs from these tissue types is that the solid tissues require an extra step of enzymatic digestion while such a step is not required for liquid sources (Fig. 3.1). For MSCs clinical applications, GMPs and good clinical practices (GCPs) must be ensured during the whole procedure of cell harvesting, isolation, culturing procedures and therapeutics (Gugjoo et al. 2019). Fortunately, GMP-compliant media and chemicals are available to process and culture expand MSCs (Bieback et al. 2008).

3.2 MSCs from Liquid Sources (Bone Marrow)

MSCs may be harvested from liquid sources by various methods involving classical and density gradient method. Density gradient solutions utilized are percoll (silica particle colloidal solution, density of 1.084–1.088) and ficoll (polysaccharide solution; density of 1.077) (Kadiyala et al. 1997; Smith et al. 2003; Hegewald et al. 2004; Vidal et al. 2006; Pacini et al. 2007; Wilke et al. 2007; Colleoni et al. 2009; Gugjoo et al. 2015). After bone marrow centrifugation, the supernatant is aspirated and pellet obtained therefrom is washed with phosphate buffer solution (PBS), followed by addition of growth medium in culture flask or plate. In case of density gradient methods, MNC fraction is obtained from bone marrow after addition of

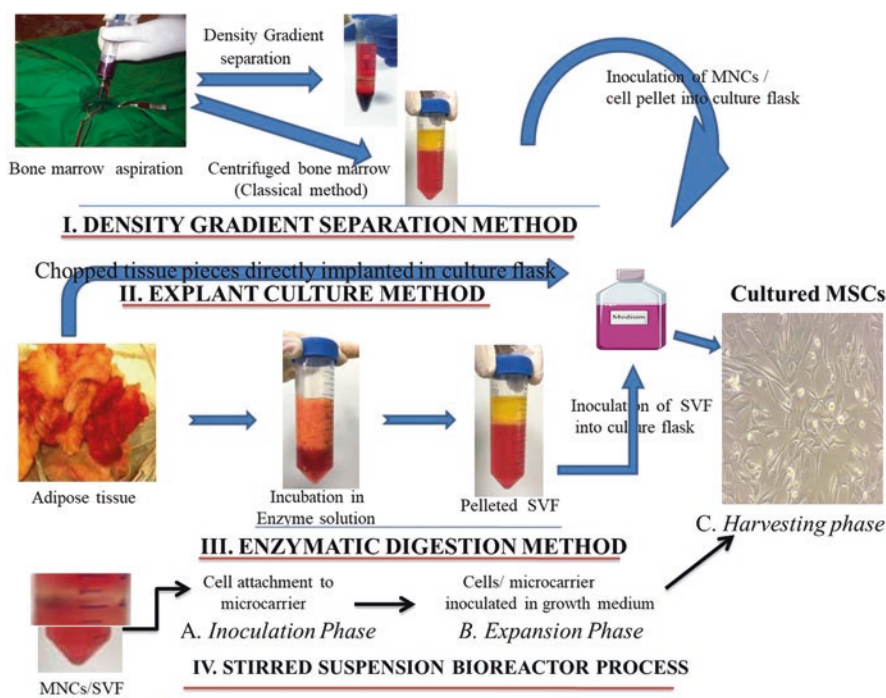


Fig. 3.1 Mesenchymal stem cell isolation protocols and their culture expansion

density gradient solutions and centrifugation. Classical method utilizes MSCs intrinsic property of plastic adherence (plastic culture dishes) without any separation of the MNCs (Fortier et al. 1998; Arnhold et al. 2007). MNCs seeding densities are regarded as the most critical parameter for expanding MSCs (Bieback et al. 2008). Equine BM-MSCs' better yield had been achieved at MNCs implantation concentration of 4×10^5 to 1×10^6 (Zahedi et al. 2018). Most of the studies currently follow the protocol of density gradient centrifugation (Bieback et al. 2008).

3.3 MSCs from Solid Tissues (Adipose Tissue)

MSCs from solid tissues are usually derived either by explant culture or enzymatic protocol. Explant culture method is one of the earliest protocol, in which well rinsed and mechanically split tissue (<few millimetres tissue pieces have better gas and nutrient diffusion towards the cells) is directly placed into the culture flask containing the growth medium (Atala 2002). The smaller fraction of MSCs comes out from these smaller tissue pieces and adheres to the culture dish surface. The residual tissue pieces are washed off after the visible attachment of the cells. In enzymatic protocol, an extra step of adding the enzymatic solution like collagenase to degrade the extracellular matrix (ECM) is performed and the assembly is kept in shaking

incubator for proper digestion. The resultant SVF or MNC fraction obtained after centrifugation is transferred to culture dishes containing growth media (detailed below). The culture flasks/plates are maintained at 37 °C incubation temperature with 5% carbon dioxide and under humid conditions. The cells are harvested after attaining 70–80% confluency in flasks (Gugjoo et al. 2015).

There is a dearth of extensive literature comparing the isolation protocols. However, few studies have compared different density gradient media, and classical/explants methods to the enzymatic protocol. In an equine study, bone marrow MSCs (BM-MSCs) isolated by various density gradient solutions had shown matching differentiation potential, but yield and properties like self-renewal were more desirable in MSCs isolated by percoll solution (Bourzac et al. 2010). Comparison of the enzymatic method to the explant method had shown that the latter method provided little heterogeneous cell population that exhibited higher proliferation rates and better viability (Salehinejad et al. 2012; Hilkens et al. 2013; Yoon et al. 2013). The explant methods keep tissue pieces intact and their extracellular matrix undissociated and thus, the cells might remain shielded from proteolytic action and mechanical stress (Hynes 2009; Hendijani 2017). The release of the cytokines and growth factors from the local tissue may additionally provide cell growth promoters (Hynes 2009; Jing et al. 2011; Shah et al. 2013). However, the explant method is more cumbersome. In enzymatic methods, type of enzyme and its concentration play an important role in cell yield and their viability (Can and Karahuseyinoglu 2007; Mitchell et al. 2003; Wang et al. 2004). Dissociation of extracellular matrix (ECM) tends to give lower cell yield (Karahuseyinoglu et al. 2007; Seshareddy et al. 2008) and also increases time of cell adherence (Han et al. 2013). To improve the cell yield to about 70% or more, a combination of enzymatic method and mechanical dissociation can be performed (Tong et al. 2011).

3.4 Growth Medium

Contrary to embryonic stem cells (ESCs), the cell feeder layer remains dispensable in MSCs culture. MSCs are plated directly into the plastic culture dishes/flasks supplemented with growth media. This is due to their characteristic property of plastic adherence. MSCs expand and proliferate in growth medium in the culture flasks. Growth medium includes basal medium, foetal bovine serum (FBS) and antibiotics and/antimycotics. The commonly used basal culture media for MSCs culture is Dulbecco's Modified Eagle's Medium (DMEM) and/or alpha minimal essential medium (Sampaio et al. 2015). Other media like TCM199/DMEM (1:1) (Colleoni et al. 2005), Aminomax-II complete medium (da Silva et al. 2016) and Mesencult medium (Hepsibha et al. 2011) have also been used. One of the studies had demonstrated that mesencult medium had favoured better growth and proliferation of buffalo adipose tissue derived MSCs (AD-MSCs) as compared to the DMEM. The results however, were obvious only at passage 1 as cells at passage 3 had better colony formation even in DMEM (Hepsibha et al. 2011). In general, the cells are allowed to attach for 4–8 days depending upon the culture ingredients, cell density

and species involved. Most of the cells in MNC or SVF fraction that lack plastic adherence properties (including haematopoietic cells) are removed after decanting the initial culture medium. After achieving 70–80% confluency in about 10–14 days, the cells are detached by various enzymes like trypsin, trypsin-ethylene diamine tetra acetic acid (EDTA), collagenase I or accutase (Fortier et al. 1998; Arnhold et al. 2007; Li et al. 2015a) and passaged for further proliferation. The buffalo amniotic membrane MSCs (bufAm-MSCs) extracted with the combined use of trypsin-EDTA and collagenase I had been reported to prove better than trypsin-EDTA alone (Deng et al. 2018). Between accutase and trypsin, immediate viability of human neural stem cells (NSCs) after trypsin disassociation had been lower as compared to that with accutase; however, subsequent apoptosis of NSCs caused by trypsin was demonstrated to be lower than that caused by accutase (Li et al. 2015a).

3.5 Foetal Bovine Serum (FBS) and Its Alternatives

FBS is one of the commonly used supplementation medium to culture MSCs (Vidal et al. 2008; Toupadakis et al. 2010; Gugjoo et al. 2015). FBS has been used at changeable concentration between 5 and 25%. Buffalo BM-MSCs had shown better growth at 15% FBS (Gade et al. 2013) and for cattle AD-MSCs and BM-MSCs 10% FBS concentration had been sufficient (Lu et al. 2014a, b). Although 20% FBS had also been used with good results (de Moraes et al. 2016; Deng et al. 2018). Goat- and equine-MSCs derived from adipose tissue, bone marrow and liver had accelerated cell confluency at higher FBS concentration (20%) than at its lower concentration (10%) (Heidari et al. 2013; Spaas et al. 2013; Martins et al. 2017). FBS furnishes nutrients, hormones/growth factors and cell adhesion plasma proteins for cellular growth and proliferation (Seo et al. 2013). GMP-compliant FBS is available and is being used to produce clinical-grade MSCs (Li et al. 2015a). However, there are associated limitations like potential threat of disease transmission, and xenogeneic protein induced change in cell behaviour and non-uniform cell preparations. FBS tends to hamper effective clinical applications of MSCs due to the potential immunogenic reactions that can arise from internalized FBS proteins (McIntosh et al. 2009).

To overcome these problems, FBS alternatives for culturing MSCs are being studied extensively. Among various FBS alternatives, autologous serum, allogeneic serum and platelet lysate (Del Bue et al. 2008; Toupadakis et al. 2010; Schwarz et al. 2012; Bieback 2013) and commercially available serum substitutes (Schwarz et al. 2012) have been evaluated. Overall, these alternatives have failed to provide consistent MSCs adhesion, morphology, growth patterns (Seo et al. 2013; Eydt et al. 2016; Schubert et al. 2018), proliferation rate (Toupadakis et al. 2010; Schwarz et al. 2012; Glynn et al. 2013) and immune-modulatory properties (Russell et al. 2015; Clark et al. 2016; Enciso et al. 2018) in animals. However, autologous serum had supported the replacement of foetal calf serum (FCS) to culture ovine BM-MSCs. The morphology, stem cell markers, osteogenic differentiation and protein expression of ovine BM-MSCs supplemented with autologous serum had been

comparable to that obtained with FBS supplementation (Weigand et al. 2017). Similarly, a dog study had shown that plasma rich in growth factors was able to improve adipose tissue MSCs (AD-MSCs) survival and had promoted their proliferation, migration but had induced their differentiation (Mellado-López et al. 2017).

Serum-free media had failed to maintain equine MSCs consistency in morphology and immunophenotype (Schubert et al. 2018). Contrarily, a study had demonstrated that bovine umbilical cord MSCs (UC-MSCs) can be cultured in serum-free medium for longer periods. The normal karyotype cells had good proliferation with an ability to differentiate into neuron-like cells, in addition to tri-lineages up to passage 60 (Cardoso et al. 2012). In absence of the serum, application of various growth/ humoral factors like bovine fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF), fibroblast growth factor-2 (FGF-2), insulin growth factor – 1 (IGF-1) and most significantly transforming growth factor- α (TGF- α) had supported MSCs proliferation especially of ovine and bovine sources (Mccarty et al. 2009; Lu et al. 2014a, b). Combined use of these growth factors may also affect MSCs proliferation, migration and invasion. Combined use of PDGF/TGF- β and PDGF/IGF-I had promoted proliferation; EGF/bFGF and EGF/bFGF/PDGF had promoted migration and EGF/bFGF/TGF- β had promoted BM-MSCs invasion (Somers et al. 2012). These growth factors however, tend to favour MSCs differentiation. However, further studies for better understanding in FBS alternatives are desired.

3.6 Modifications in Conventional MSCs Culture Protocol

The conventional culture expansion of MSCs follows static mechanism. This system is labour intensive, bears low reproducibility, poses contamination problem and above all is less economical for large-scale expansion. In order to make MSCs production economical and reduce chances of contamination various modifications are being tried. Among various modified techniques, the application of static magnetic field has proven to be useful to some extent. Application of static magnetic field to equine adipose tissue MSCs (eAD-MSCs) had accelerated their population doubling potential with high colony-forming potential. An enhanced secretory potential of growth factor rich microvesicles with no compromise on morphology was also demonstrated (Marędziak et al. 2015; Iacono et al. 2017).

3.6.1 Stirred Suspension Bioreactor

An alternative to the conventional system is the stirred suspension bioreactor for culture expansion of MSCs (Fig. 3.1), though it remains to be used extensively in veterinary practice. It can compensate the limitations normally associated with the conventional techniques. The cell population of clinical grade with effective numbers can be produced in shorter time at less input cost (Roberts et al. 2019). Among various factors selection of microcarrier (MC) is critical. Microcarrier (MC) acts as

attachment surface for cellular growth. Commercially manufactured microcarriers for MSCs expansion are either porous or non-porous types with variable materials (Schop et al. 2010; Goh and Reuveny 2013; Petry et al. 2016; Rafiq et al. 2016). Various features of MC like composition, porosity, topography and surface charge determine cell attachment. MCs may also be cell line specific (Martin et al. 2011). Thus, selection of microcarrier is an important factor for appropriate cell expansion (Rafiq et al. 2014).

In stirred suspension bioreactor process, various phases involving inoculation, expansion and harvesting phases are being accomplished to achieve cultured MSCs. The first 24 h are of inoculation phase. The main aim remains to achieve maximum possible cellular attachment to the microcarriers (Balint et al. 2015). Among various factors, cell to microcarrier (MC) ratios remain important. The appropriate ratio of initial cell to microcarrier density is determined by microcarrier surface area. Usually low initial cell to MC density provides greater cell yield (Sun et al. 2010; Li et al. 2015).

The second expansion phase continues till the desired MC cell density is obtained and is affected by culture medium composition and frequency of medium change. Replacement medium removes undesirable growth inhibitors and renews exhausted nutrients. For equine cord blood MSCs (CB-MSCs), growth medium consisting of DMEM, along with FBS (30%), L-glutamine (1%) and antibiotics (1%) has been used (Koch et al. 2007). However, lower FBS concentrations may also be used. Apart from these factors, rate of agitation needs to be set right. The appropriate agitation rate is determined by the geometry of bioreactor and its impeller, viscosity and density of media, type of cell line and MC type. Lower agitation rate favours cell-MC aggregate formation (Jossen et al. 2016). The higher agitation rates develop higher shear in the bioreactors favouring cells to detach from MCs (Yuan et al. 2014; Takahashi et al. 2016).

In the harvesting stage, the cells are detached from the MCs by enzymatic action involving trypsin. The cells are then filtered to obtain MSCs suspension. Trypsin (porcine origin) however, is usually replaced by bacteria derived recombinant substitute, TrypLE. The enzyme activity is usually maintained for 5–15 min, though exposures up to 1 h have also been made (Roberts et al. 2019).

In an equine study, cord blood derived MSCs in stirred suspension bioreactors could achieve very large cell densities of about 75,000 per sq. cm which corresponds to 40×10^6 cells in 100 ml bioreactor. The harvesting cell efficiency had remained 80% approximately and the cells had shown comparable surface marker expression and differentiation potential to that of the conventional culture system (Roberts et al. 2019).

3.7 MSCs Characterization

Characterization of MSCs is mandatory to confirm their presence. These cells are characterized on the recommendations of the International Society for Cellular Therapy (ISCT). MSCs are plastic adherent cells that express specific surface

markers, lack haematopoietic marker expression and are able to show tri-lineage (adipogenic, chondrogenic and osteogenic) differentiation. MSCs population must express $\geq 95\%$ of the CD73 (5'-Nucleotidase), CD90 (Thy1) and CD105 (Endoglin) as measured by flow cytometry. There should be $\leq 2\%$ of the CD11b (Integrin- α) or CD14, CD19, CD34, CD45, CD79 α and HLA Class II. Multipotent tri-lineage differentiation potential is confirmed by staining these in vitro differentiated cells (Dominici et al. 2006).

These cells from various animal species generally meet the norm of plastic adherence and multipotency (Guest et al. 2008; Ranera et al. 2011; De Schauwer et al. 2012) but consensus on surface marker expression is debatable (Kato et al. 2004; Knippenberg et al. 2005; Kang et al. 2008; Vieira et al. 2010; Lyahyai et al. 2012; Corradetti et al. 2013; Caminal et al. 2014; Mediano et al. 2015; Khan et al. 2016; De Cesaris et al. 2017; Ghaneialvar et al. 2018). In some species like rabbit markers including CD90 have been demonstrated to be transcribed by MSCs but their protein translation has failed (Kovac et al. 2017). Variable surface marker expression of MSCs from various sources may be explained by donor tissue differences, variability in enzyme harvesting methods and specific reactivity of antibodies used (Ranera et al. 2011; De Schauwer et al. 2012; Colleoni et al. 2009; Radcliffe et al. 2010). The cell detaching agents like trypsin and accutase may vitiate cell surface receptors (Ranera et al. 2011; De Schauwer et al. 2012). All antibodies are not reactive to interspecies molecules (Radcliffe et al. 2010). MSCs dynamic immune-phenotype may additionally promulgate such differences (Huss et al. 2000; Mosna et al. 2010; Strioga et al. 2012). The differences in surface markers expression especially of CD44 and CD90 may impact MSCs ability to migrate (Cabezas et al. 2018). Recent mice study had demonstrated that MSCs chromatin accessibility signatures and gene expression programs may be useful modes to provide insights into tissue specific cell differences, though chromatin signature molecules had been more accurate. This demands extensive MSCs epigenome analysis studies to characterize them on the basis of tissue source (Ho et al. 2018).

In order to avoid such donor tissue specific cell characteristics, a uniform source like induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs) are being evaluated. MSCs from these sources, although in preliminary studies, demand extensive studies so as to harvest sufficient and phenotypically stable cell number so as to utilize them clinically (Chan et al. 2018; Gugjoo et al. 2018; Steens and Klein 2018). Apart from basic characterization protocol, MSCs must be viable, free of microbes and/or endotoxin and pyrogen and finally dose titrated to conform to the standards of good-manufacturing and clinical practices (GMPs and GCPs) (Bieback et al. 2008).

3.8 Storage of MSCs

MSCs have the potential to be utilized as allogeneic therapy and as such may be stored for future use and for distant transport. Excipient medium (like PBS) can be retained along with the cells while ancillary medium (like bone marrow aspirate, plasma, serum and cryogenic medium) must be removed prior to the in vivo

implantation of the cells. For shorter duration (9–12 h) storage, ancillary biological media have no observable benefit over the excipient in maintaining MSCs viability (Bronzini et al. 2012). Ancillary biological media had been demonstrated to keep cells equally viable for about 24 h that had decreased to 25–78% by 72 h. Cryogenic medium had maintained cell viability better than the biological fluids (Garvican et al. 2016). Cryogenic storage of MSCs in different cryogenic media like serum, dimethyl sulphoxide (DMSO), FBS and media had comparable and satisfactory post-thaw cell viability (80–90%) and growth evaluated for shorter duration (Mitchell et al. 2015; Garvican et al. 2016).

3.8.1 Cryopreservation of MSCs

3.8.1.1 Cryopreservation

Cryopreservation of cells is achieved either by fast or slow freezing rate. During this process, various steps are ensured to avoid cell dehydration and damage. During fast cell freezing rate solute and electrolyte imbalances can do the damage while during slow freezing process intra- and extracellular ice crystal formation can have adverse effects on the cell viability (Mazur 1977). During these processes, incorporation of cryoprotectant is an important step. To prevent cell damage during such a delicate process cryoprotectant is being added to reduce overall freezing point of the system. The combined mixture of the cells, medium and the cryoprotectant makes a eutectic system that has lower freezing temperature as compared to the individual components. It is important to note that freeze-thaw method directly affects cell viability. One of the studies had reported an unacceptable post-thaw viability of cells and might have been due to the faulty thaw process (Espina et al. 2016).

Another way of cryopreservation that involves an extremely rapid (>-1000 °C/s) cooling process known as vitrification has also been used for cell preservation (Fowler and Toner 2005). In this process, the cells and potentially cytotoxic concentration of cryoprotectant are immersed in open storage vessels. However, requirement of higher concentration of cryoprotectant, continuous maintenance of cells at cryogenic temperature and need of open containers (that act as a potential source of contamination) makes vitrification least opted technique for MSCs cryopreservation (Taylor et al. 2004; Fowler and Toner 2005).

3.8.1.2 Why to Cryopreserve MSCs

- MSCs, unlike that of embryonic stem cells, cannot be cultured indefinitely as they undergo senescence after certain culture passages. At temperature below -120 °C, most of the metabolic processes are at stasis and thus, cells can be preserved for long (Colosimo et al. 2013; Ji et al. 2016; Tian et al. 2016).
- MSCs are very limited in number under natural sources and thus, in vitro expansion remains a pre-requisite process. Such an expansion takes a minimum of 2 weeks and thus a delay in autologous therapy is imminent and can be sufficed by preserved autologous/ allogeneic MSCs (Gugjoo et al. 2016; Perez-Silos et al. 2016).

- MSCs concentration and characteristics vary with the source and are affected by the feeding, age and health status of the donor (Han et al. 2010; Pillai et al. 2016). Allogeneic therapy can avoid such variations and assure uniform applications in clinical cases and/or trials. Besides, the studies can effectively be studied and compared for the results.
- MSCs viability during long distance can be safeguarded in cryopreservation state.

3.8.1.3 Cryoprotectant

MSCs cryopreservation is done in cryogenic media containing cryoprotectant that prevents cell damage during freezing and thawing (Fuller 2004; Thirumala et al. 2010). Variable concentration of cryoprotectant has been used. The concentration of cryoprotectant varied among species and sources, in addition to the freezing technique used (Fuller 2004; Thirumala et al. 2005). Cryoprotectants are of two types including cell membrane permeable and impermeable (Fuller 2004), with highly permeable cryoprotectants tend to be more toxic (Gao and Critser 2000). To prevent such a toxicity permeable cryoprotectants like DMSO, ethylene glycol, etc. are combined with less permeable ones like hydroxyethyl starch (HES), dextran and polyethylene glycol (Gao and Critser 2000; Fuller 2004). In veterinary practice cryoprotectants like DMSO and FBS are most commonly used (Martinello et al. 2010; Marx et al. 2015). The ancillary cryoprotectants like DMSO exhibit carcinogenic properties (Quimby et al. 2013). The xenogeneic proteins in FBS may alter cellular behaviour (Duan and Lopez 2016) and need to be removed before cell implantation.

Dimethyl Sulphoxide (DMSO)

It is one of the most commonly used cryoprotectant in veterinary practice that works by stabilizing the cell proteins and equilibrating intracellular and extracellular electrolytes. Hydrophobic interactions between DMSO and proteins and/cell membrane phospholipids stabilize cellular proteins (Anchordoguy et al. 1991). Electrolyte equilibration occurs by displacing the intracellular fluids (Liu et al. 2010). In addition, DMSO forms high energy bonds with the water molecules that prevent ice crystal formation (Weng et al. 2011). Despite such features, DMSO can lead to potential chemical and osmotic shock to the MSCs (Scheinkonig et al. 2004; Liu et al. 2010). DMSO concentration has a significant effect on cell survival. An increase in DMSO concentration from 5% to 20% in the freezing medium tends to decrease cell survivability and enhance apoptotic gene expression in pig BM-MSCs (Ock and Rho 2011). Hydrophobic interactions that stabilize the cell membrane proteins may also lead to their denaturation and deactivation (Fahy et al. 1990). DMSO has been associated with the side effects like neurotoxicity and can lead to hypotension and anaphylactic shock upon intravenous injection along with the cells (Junior et al. 2008). Such side effects can be waived off by washing the cells, though that may result in lower colony-forming units (CFU) potential of the cells (Fry et al. 2015). Furthermore, DMSO complete removal is a complex and time-consuming process (Thirumala et al. 2009), demanding its alternatives.

Foetal Bovine Serum (FBS)

FBS apart from its utility as an important ingredient of culture medium, it acts as cryoprotectant. It protects the cell proteins and stabilizes the osmotic pressure. FBS provides nutrients and humoral/growth factors for cell proliferation. The post-thaw viability of the MSCs may increase with an increase in FBS concentration. Buffalo amniotic fluid MSCs (bufAF-MSCs) post-thaw viability had been demonstrated to improve from 28 to 85% with an increase in serum concentration (Yadav et al. 2011). Even the post-cryopreserved ovine amniotic-MSCs had failed to show any deleterious effect on their cartilage healing potential as the healing results were comparable to that of fresh amniotic-MSCs (Garcia et al. 2015).

FBS has issues of variable composition and can pose xenogeneic and/zoonotic threats, in addition to the ethical issues (Jochems et al. 2002; Liu et al. 2010; Verdanova et al. 2014). Even FBS may affect surface markers expression as an increased CD44 expression was demonstrated in dog cryopreserved AD-MSCs as compared to fresh ones (Duan and Lopez 2016). Considering all these impediments, FBS alternatives are being studied. Recently serum-free cryopreservation medium in human MSCs has been demonstrated to give similar or superior post-cryopreservation outcomes as compared to FBS-containing media (Freimark et al. 2011; Verdanova et al. 2014). However, such FBS alternatives are desired to be tested in animals given that such alternatives have failed to give satisfactory culture characteristics of animal MSCs (Schwarz et al. 2012; Seo et al. 2013; Russell et al. 2015; Clark et al. 2016; Enciso et al. 2018; Schubert et al. 2018).

For shorter duration (24–72 h) cryopreservation of MSCs during transit storage, allogeneic and/ autologous serum may replace FBS. Cryopreserved horse MSCs stored in allogeneic and/autologous serum had shown unaltered post-thaw growth and morphology (Mitchell et al. 2015). Conditioned medium may further support growth and viability of the cryopreserved equine umbilical cord MSCs (eUC-MSCs) (Rhodes et al. 2004; Maia et al. 2017). In veterinary species, MSCs are cryopreserved using cryoprotectant and/serum in the same way as are followed in human MSCs cryopreservation (Martinello et al. 2010; Ock and Rho 2011; Duan and Lopez 2016). The cells in our lab are cooled to about -80°C in 1°C cooler (containing isopropyl alcohol). The cells in 1°C cooler are initially kept in -20°C for few hours and transferred to -80°C for over a night. Later the cells are transferred to the liquid nitrogen (Martinello et al. 2011). The cells are revitalized by thawing and then rinsed prior to culture or implantation (Gao and Critser 2000).

3.8.2 MSCs Characteristics After Cryopreservation

Post-cryopreservation viability of dog MSCs (dMSCs), equine MSCs (eMSCs), bovine MSCs (bMSCs), buffalo MSCs (bufMSCs) and sheep MSCs (sMSCs) had been demonstrated to remain about 60–70%, $64.0 \pm 6.0\%$, 86.5%, 80% and $>70\%$, respectively without any adverse effect upon their characteristics (Vidal et al. 2006; Laura et al. 2008; Martinello et al. 2011; de Moraes et al. 2016; Campos et al. 2017; Okamura et al. 2017; Korovina et al. 2019). One of the studies had an unacceptable

post-thaw cellular viability at -20 and -80 °C, citing importance of freeze-thaw method (Espina et al. 2016).

The cryopreserved MSCs viability remains same irrespective of their sources (Knippenberg et al. 2005). Goat foetal adnexa derived MSCs had revived successfully upon thawing. The revived cells could express surface antigens and pluripotency markers and had shown tri-lineage differentiation (Somal et al. 2017). The population doubling time (PDT) too had remained the same (Somal et al. 2016, 2017) as had also been reported for ovine BM-MSCs (Rhodes et al. 2004). Storage of dog MSCs for a year had not affected fibroblast-like morphology, alkaline phosphatase (AP) activity, surface marker expression and tri-lineage differentiation potential. The cryopreserved cells however, had reduced proliferation ratio and telomerase activity in comparison to the fresh cells (Martinello et al. 2011).

Apart from the cells, cryopreservation of tissue engineered (TE) constructs may provide an off-the-shelf TE constructs. In one such study cryopreserved tissue engineered scaffold and goat BM-MSCs (gBM-MSCs) had good viability (Costa et al. 2012). Although other factors like scaffold design and structural properties might be affected by cryopreservation and should be studied in detail.

3.9 Conclusion

Culture expansion of MSCs is imperative for their effective utilization. The isolation of MNCs from liquid tissues can be achieved with conventional or density gradient separation methods while SVF from solid tissues can be separated either by direct explant method or by prior enzymatic digestion. MNCs and SVF cells are culture expanded in growth media containing basal culture medium, FBS and antibiotic-antimycotic solution being kept under standard culture conditions. FBS poses various impediments for clinical applications and as such its alternatives are being studied. The plastic adhered cells are harvested by enzymatic digestion and characterized as per ISCT recommendations. The cells that are plastic adherent express specific surface markers while lacking haematopoietic markers and are able to differentiate into at least tri-lineages are termed as MSCs. For large-scale and cost-effective production of MSCs, stirred suspension bioreactor process is being tried and seems useful. The culture expanded cells are stored for longer period to be utilized as ready to use supply. The cells are preserved in cryopreservation media under liquid nitrogen.

References

- Akram T, Hakim M, Mir BA, Hussain SS, Shabir N, Andrabi M, Dar PA, Ganaia NA, Shabir N (2017) Comparative efficiency of goat mesenchymal stem cell isolation from bone marrow and bone chip. *Small Rum Res* 153:87–94
- Anchordoguy TJ, Cecchini CA, Crowe JH, Crowe LM (1991) Insights into the cryoprotective mechanism of dimethyl sulfoxide for phospholipid bilayers. *Cryobiology* 28:467–473

- Arnhold SJ, Goletz I, Klein H, Stumpf G, Beluche LA, Rohde C, Addicks K, Litzke LF (2007) Isolation and characterization of bone marrow-derived equine mesenchymal stem cells. *Am J Vet Res* 68:1095–1105
- Atala A (2002) *Methods of tissue engineering*. Gulf Professional Publishing, Houston
- Balint R, Richardson SM, Cartmell SH (2015) Low-density subculture: a technical note on the importance of avoiding cell-to-cell contact during mesenchymal stromal cell expansion. *J Tissue Eng Regen Med* 9:1200–1203
- Bieback K (2013) Platelet lysate as replacement for fetal bovine serum in mesenchymal stromal cell cultures. *Transf Med Hemother* 40:326–335
- Bieback K, Schallmoser K, Klüter H, Strunk D (2008) Clinical protocols for the isolation and expansion of mesenchymal stromal cells. *Transf Med Hemother* 35(4):286–294
- Bourzac C, Smith LC, Vincent P, Beauchamp G, Lavoie JP, Laverty S (2010) Isolation of equine bone marrow-derived mesenchymal stem cells: a comparison between three protocols. *Equine Vet J* 42(6):519–527
- Bronzini I, Patruno M, Iacopetti I, Martinello T (2012) Influence of temperature, time and different media on mesenchymal stromal cells shipped for clinical application. *Vet J* 194(1):121–123
- Cabezas J, Rojas D, Navarrete F et al (2018) Equine mesenchymal stem cells derived from endometrial or adipose tissue share significant biological properties, but have distinctive pattern of surface markers and migration. *Theriogenol* 106:93–102
- Caminal M, Fonseca C, Peris D, Moll X, Rabanal RM, Barrachina J, Codina D, García F, Cairó JJ, Gódia F, Pla A, Vives J (2014) Use of a chronic model of articular cartilage and meniscal injury for the assessment of long-term effects after autologous mesenchymal stromal cell treatment in sheep. *New Biotechnol* 31(5):492–498
- Campos LL, Landim-Alvarenga FC, Ikeda TL, Monteiro BA, Maia L, Freitas-Dell'Aqua CP, De Vita B (2017) Isolation, culture, characterization and cryopreservation of stem cells derived from amniotic mesenchymal layer and umbilical cord tissue of bovine fetuses. *Pesquisa Veterinária Brasileira* 37(3):278–286
- Can A, Karahuseyinoglu S (2007) Concise review: human umbilical cord stroma with regard to the source of fetus-derived stem cells. *Stem Cells* 25:2886–2895
- Caplan AI (2007) Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol* 213:341–347
- Cardoso TC, Ferrari HF, Garcia AF, Novais JB, Silva-Frade C, Ferrarezi MC, Andrade AL, Gamiero R (2012) Isolation and characterization of Whartons jelly-derived multipotent Mesenchymal stromal cells obtained from bovine umbilical cord and maintained in defined serum free three-dimensional system. *BMC Biotechnol* 12:18
- Chan, CKF, Gulati GS, Sinha R, Tompkins JV, Lopez M, Carter AC, Ransom RC, Reinisch A, Wearda T, Murphy M, et al. (2018). Identification of the Human Skeletal Stem Cell. *Cell* 175(1): 43–56.e21.
- Clark KC, Kol A, Shahbenderian S, Granick JL, Walker NJ, Borjesson DL (2016) Canine and equine mesenchymal stem cells grown in serum free media have altered immunophenotype. *Stem Cell Rev Reports* 12:245–256
- Colleoni S, Donofrio G, Lagutina I, Duchi R, Galli C, Lazzari G (2005) Establishment, differentiation, electroporation, viral transduction, and nuclear transfer of bovine and porcine mesenchymal stem cells. *Cloning Stem Cells* 7(3):154–166
- Colleoni S, Bottani E, Tessaro I et al (2009) Isolation, growth and differentiation of equine mesenchymal stem cells: effect of donor, source, amount of tissue and supplementation with basic fibroblast growth factor. *Vet Res Commun* 33:811–821
- Colosimo A, Russo V, Mauro A, Curini V, Marchisio M, Bernabò N, Alfonsi M, Mattioli M, Barboni B (2013) Prolonged in vitro expansion partially affects phenotypic features and osteogenic potential of ovine amniotic fluid-derived mesenchymal stromal cells. *Cytotherapy* 15:930–950
- Corradetti B, Meucci A, Bizzaro D, Cremonesi F, Lange Consiglio A (2013) Mesenchymal stem cells from amnion and amniotic fluid in the bovine. *Reproduction* 145:391–400
- Costa PF, Dias AF, Reis RL, Gomes ME (2012) Cryopreservation of cell/scaffold tissue-engineered constructs. *Tissue Engineering: Part C* 18(11):852–858

- da Silva CG, Martins CF, Cardoso TC, da Cunha ER, Bessler HC, MacManus CM, Pivato I, Bao SN (2016) Isolation and characterization of Mesenchymal stem cells derived from bovine Wharton's jelly and their potential use in cloning by nuclear transfer. *Ciencia Rural*, Santa Maria 46(10):1830–1837
- De Cesaris V, Grolli S, Bresciani C, Conti V, Basini G, Parmigiani E, Bigliardi E (2017) Isolation, proliferation and characterization of endometrial canine stem cells. *Reprod Domest Anim* 52(2):235–242
- de Moraes CN, Maia L, Dias MC, Dell'Aqua CP, da Mota LS, Chapwanya A, Landim-Alvarenga FD, Oba E (2016) Bovine endometrial cells: a source of Mesenchymal stem/progenitor cells. *Cell Biol Int* 40(12):1332–1339
- De Schauwer C, Piepers S, Van de Walle GR et al (2012) In search for cross-reactivity to immunophenotype equine mesenchymal stromal cells by multicolor flow cytometry. *Cytometry A* 81(4):312–323
- Del Bue M, Ricco S, Ramoni R, Conti V, Gnudi G, Grolli S (2008) Equine adipose-tissue derived mesenchymal stem cells and platelet concentrates: their association in vitro and in vivo. *Vet Res Commun* 32:S51–S55
- Deng Y, Huang G, Zou L, Nong T, Yang X, Cui J, Wei Y, Yang S, Shi D (2018) Isolation and characterization of buffalo (*Bubalus bubalis*) amniotic mesenchymal stem cells derived from amnion from the first trimester pregnancy. *Journal of Veterinary Medical Sciences* 80(4):710–719
- Dominici M, Le Blanc K, Mueller I et al (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8(4):315–317
- Duan W, Lopez MJ (2016) Effects of cryopreservation on canine multipotent stromal cells from subcutaneous and infrapatellar adipose tissue. *Stem Cell Rev Rep* 12:257–268
- Enciso N, Ostronoff LLK, Mejías G, León LG, Fermín ML, Merino E, Fragio C, Avedillo L, Tejero C (2018) Stem cell factor supports migration in canine mesenchymal stem cells. *Vet Res Commun* 42(1):29–38
- Espina M, Jülke H, Brehm W, Ribitsch I, Winter K, Delling U (2016) Evaluation of transport conditions for autologous bone marrow-derived mesenchymal stromal cells for therapeutic application in horses. *Peer J* 4:e1773
- Eydt C, Geburek F, Carmen S, Hambruch N, Rohn K, Pfarrer C, Staszky C (2016) Sternal bone marrow derived equine multipotent mesenchymal stromal cells (MSCs): investigations considering the sampling site and the use of different culture media. *Vet Med Sci* 2:200
- Fahy GM, Lilley TH, Linsdell H, Douglas MS, Meryman HT (1990) Cryoprotectant toxicity and cryoprotectant toxicity reduction: in search of molecular mechanisms. *Cryobiology* 27:247–268
- Fortier LA, Nixon AJ, Williams J, Cable CS (1998) Isolation and chondrocytic differentiation of equine bone marrow-derived mesenchymal stem cells. *Am J Vet Res* 59:1182–1187
- Fowler A, Toner M (2005) Cryo-injury and biopreservation. *Ann NY Acad Sci* 1066:119–135
- Freimark D, Sehl C, Weber C et al (2011) Systematic parameter optimization of a me 2 SO-and serum-free cryopreservation protocol for human mesenchymal stem cells. *Cryobiology* 63:67–75
- Friedenstein AJ, Gorskaja JF, Kulagina NN (1976) Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Exp Hematol* 4(5):267–274
- Fry L, Querol S, Gomez S, McArdle S, Rees R, Madrigal JA (2015) Assessing the toxic effects of DMSO on cord blood to determine exposure time limits and the optimum concentration for cryopreservation. *Vox Sang* 109:181–190
- Fuller BJ (2004) Cryoprotectants: the essential antifreezes to protect life in the frozen state. *Cryo Letters* 25:375–388
- Gade NE, Pratheesh MD, Nath A, Dubey PK, Amarpal, Sharma B, Saikumar G, Sharma GT (2013) Molecular and cellular characterization of buffalo bone marrow-derived mesenchymal stem cells. *Reprod Domest Anim* 48(3):358–367. <https://doi.org/10.1111/j.1439-0531.2012.02156.x>
- Gao D, Critser J (2000) Mechanisms of cryoinjury in living cells. *ILAR J* 41:187–196

- Garcia D, Longo UG, Vaquero J, Forriol F, Loppini M, Khan WS, Denaro V (2015) Amniotic membrane transplant for articular cartilage repair: an experimental study in sheep. *Curr Stem Cell Res Therapy* 10:77–83
- Garvican ER, Cree S, Bull L, Smith RKW, Dudhia J (2016) Viability of equine mesenchymal stem cells during transport and implantation. *Stem Cell Res Ther* 5:1
- Ghaneialvar H, Soltani L, Rahmani HR, Lotfi AS, Soleimani M (2018) Characterization and classification of mesenchymal stem cells in several species using surface markers for cell therapy purposes. *Indian J Clin Biochem* 33(1):46–52
- Glynn ERA, Londono AS, Zinn SA, Hoagland TA, Govoni KE (2013) Culture conditions for equine bone marrow mesenchymal stem cells and expression of key transcription factors during their differentiation into osteoblasts. *J Anim Sci Biotechnol* 24:40
- Goh TK, Reuveny S (2013) Microcarrier culture for efficient expansion and osteogenic differentiation of human fetal mesenchymal stem cells. *Biores Open Access* 2(2):84–97
- Guest DJ, Smith MR, Allen WR (2008) Monitoring the fate of autologous and allogeneic mesenchymal progenitor cells injected into the superficial digital flexor tendon of horses: preliminary study. *Equine Vet J* 40:178–181
- Gugjoo MB, Amarpal (2018) Mesenchymal stem cell research in sheep: current status and future prospects. *Small Rumin Res* 169:46–56
- Gugjoo MB, Amarpal, Kinjavdekar P, Aithal HP, Ansari MM, Pawde AM, Sharma GT (2015) Isolation, culturing and characterization of New Zealand white rabbit mesenchymal stem cells derived from bone marrow. *Asian J Anim Vet Adv* 10(10):537–548
- Gugjoo MB, Amarpal, Sharma GT, Aithal HP, Kinjavdekar P (2016) Cartilage tissue engineering: role of mesenchymal stem cells along with growth factors and scaffolds. *Indian J Med Res* 144:339–347
- Gugjoo MB, Amarpal, Chandra V, Wani MY, Dhama K, Sharma GT (2018) Mesenchymal stem cell research in veterinary medicine. *Current Stem Cell Research and Therapy* 13(8):645–657
- Gugjoo MB, Amarpal, Makhdoomi DM, Sharma GT (2019) Equine mesenchymal stem cells: properties, sources, characterization and potential therapeutic applications. *Journal of Equine Veterinary Sciences* 72:16–27
- Gugjoo MB, Amarpal, Abdelbaset-Ismail A, Aithal HP, Kinjavdekar P, Kumar GS, Sharma GT (2020a) Allogeneic mesenchymal stem cells and growth factors in gel scaffold repair osteochondral defect in rabbits. *Reg Med* 15(2):1261–1275
- Gugjoo MB, Amarpal, Fazili MR, Shah RA, Mir MS, Sharma GT (2020b) Goat mesenchymal stem cell basic research and potential applications. *Small Rum Res* 183:106045
- Han J, Liu JY, Swartz DD, Andreadis ST (2010) Molecular and functional effects of organismal ageing on smooth muscle cells derived from bone marrow mesenchymal stem cells. *Cardiovasc Res* 87:147–155
- Han YF, Tao R, Sun TJ, Chai JK, Xu G, Liu J (2013) Optimization of human umbilical cord mesenchymal stem cell isolation and culture methods. *Cytotechnology* 65:819–827
- Hegewald AA, Ringe J, Bartel J, Krüger I, Notter M, Barnewitz D, Kaps C, Sittlinger M (2004) Hyaluronic acid and autologous synovial fluid induce chondrogenic differentiation of equine mesenchymal stem cells: a preliminary study. *Tissue Cell* 36:431–438
- Heidari B, Shirazi A, Akhondi MM, Hassanpour H, Behzadi B, Naderi MM, Sarvari A, Borjian S (2013) Comparison of proliferative and multilineage differentiation potential of sheep mesenchymal stem cells derived from bone marrow, liver, and adipose tissue. *Avicenna J Med Biotechnol* 5(2):104–117
- Hendijani F (2017) Explant culture: an advantageous method for isolation of mesenchymal stem cells from human tissues. *Cell Prolif* 50:e12334
- Hepsibha P, Meenambigai TV, Mangalagowri A, Palanisamy A, Stalin A, Nithya S, Kumanan K (2011) Multipotent differentiation potential of buffalo adipose tissue derived mesenchymal stem cells. *Asian Journal of Animal Veterinary Advances* 6:772–788
- Hilkens P, Gervois P, Fanton Y, Vanormelingen J, Martens W, Struys T, Politis C, Lambrechts I, Bronckaers A (2013) Effect of isolation methodology on stem cell properties and multilineage differentiation potential of human dental pulp stem cells. *Cell Tissue Res* 353:65–78

- Ho Y-T, Shimbo T, Wijaya E, Ouchi Y, Eiichi T, Yamamoto R, Kikuchi Y, Kaneda Y, Tama K (2018) Chromatin accessibility identifies diversity in mesenchymal stem cells from different tissue origins. *Scientific Rep* 8:17765
- Huss R, Claudia L, Weissinger EM, Kolb HJ, Thalmeier K (2000) Evidence of peripheral blood-derived, plastic-adherent CD34⁺/low hematopoietic stem cell clones with mesenchymal stem cell characteristics. *Stem Cells* 18:252–260
- Hynes RO (2009) The extracellular matrix: not just pretty fibrils. *Science* 326:1216–1219
- Iacono E, Pascuccib L, Rossia B et al (2017) Ultrastructural characteristics and immune profile of equine MSCs from fetal adnexa. *Reprod* 154(4):509–519
- Ji M, Bai C, Li L, Fan Y, Ma C, Li X, Guan W (2016) Biological characterization of sheep kidney-derived mesenchymal stem cells. *Exp Therap Med* 12:3963–3971
- Jing W, Xiao J, Xiong Z, Yang X, Huang Y, Zhou M, Chen S, Lin Y, Tian W (2011) Explant culture: an efficient method to isolate adipose-derived stromal cells for tissue engineering. *Artif Organs* 35:105–112
- Jochems CE, van der Valk JB, Stafleu FR, Baumanns V (2002) The use of fetal bovine serum: ethical or scientific problem? *Altern Lab Anim* 30:219–227
- Jossen V, Schirmer C, Mostafa Sindi D et al (2016) Theoretical and practical issues that are relevant when scaling up hMSC microcarrier production processes. *Stem Cells Int* 2016:4760414. <https://doi.org/10.1155/2016/4760414>
- Junior A, Arrais C, Saboya R, Velasques RD, Junqueira PL, Dulley FL (2008) Neurotoxicity associated with dimethyl sulfoxide preserved hematopoietic progenitor cell infusion. *Bone Marrow Transplant* 41:95–97
- Kadiyala S, Young RG, Thiede MA, Bruder SP (1997) Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. *Cell Transplant* 6(2):125–134
- Kang JW, Kang K-S, Koo HC, Park JR, Choi EW, Park YH (2008) Soluble factors-mediated immunomodulatory effects of canine adipose tissue-derived mesenchymal stem cells. *Stem Cells Development* 17(4):681–693
- Karahuseynoglu S, Cinar O, Kilic E, Kara F, Akay GG, Demiralp DO, Tukun A, Uckan D, Can A (2007) Biology of stem cells in human umbilical cord stroma: in situ and in vitro surveys. *Stem Cells* 25:319–331
- Kato Y, Imabayashi H, Mori T, Tani T, Taniguchi M, Higashi M, Matsumoto M, Umezawa A, Tsunoda Y (2004) Nuclear transfer of adult bone marrow mesenchymal stem cells: developmental totipotency of tissue-specific stem cells from an adult mammal. *Biol Reprod* 70(2):415–418
- Khan MR, Chandrashekan A, Smith RKW, Dudhia J (2016) Immunophenotypic characterization of ovine mesenchymal stem cells. *Cytometry A* 89(5):443–450
- Knippenberg M, Helder M, Doulabi BZ, Semeins CM, Wuisman PIJM, Klein-Nulend J (2005) Adipose tissue-derived mesenchymal stem cells acquire bone cell-like responsiveness to fluid shear stress on osteogenic stimulation. *Tissue Eng* 11(11/12):1780–1787
- Koch TG, Heerkens T, Thomsen PD, Betts DH (2007) Isolation of mesenchymal stem cells from equine umbilical cord blood. *BMC Biotechnol* 7(1):26
- Korovina DG, Volkova IM, Vasilieva SA, Gulukin MI, Savchenkova IP (2019) Multipotent mesenchymal stem cells derived from sheep bone marrow: isolation and cryopreservation. *Cell and Tissue Biology* 13(3):161–169
- Kovac M, Vasicek J, Kulikova B, Bauer M, Curlej J, Balazi A, Chrenek P (2017) Different RNA and protein expression of surface markers in rabbit amniotic fluid derived mesenchymal stem cells. *Biotechnol Prog* 33(6):1601–1613
- Laura C, Groza I, Oana L, Pall E, Pestean C, Cătană R, Cătaru M (2008) Canine mesenchymal stem cells isolation from bone marrow aspirates. *Bulletin UASVM, Veterinary Medicine* 65(2):96–101
- Li P, Liu F, Wu C, Jiang W, Zhao G, Liu L, Bai T, Wang L, Jiang Y, Guo L, Qi X, Kou J, Fan R, Hao D, Lan S, Li Y, Liu JY (2015) Feasibility of human hair follicle-derived mesenchymal stem cells/CultiSpher®-G constructs in regenerative medicine. *Cell Tissue Res* 362(1):69–86
- Li T, Li C, Zhang CY, Zhao J (2015a) Effect of accutase or trypsin dissociation on the apoptosis of human striatum-derived neural stem cells. *Acta Academiae Medicinae Sinicae* 37(2):185–194

- Liu Y, Xu X, Ma X, Martin-Rendon E, Watt S, Cui Z (2010) Cryopreservation of human bone marrow-derived mesenchymal stem cells with reduced dimethylsulfoxide and well-defined freezing solutions. *Biotechnol Prog* 26:1635–1643
- Lu T, Huang Y, Wang H, Ma Y, Guan W (2014a) Multi-lineage potential research of bone marrow-derived stromal cells (BMSCs) from cattle. *Applied Biochemistry & Biotechnology* 172:21–35
- Lu T, Xiong H, Wang K, Wang S, Ma Y, Guan W (2014b) Isolation and characterization of adipose-derived mesenchymal stem cells (ADSCs) from cattle. *Applied Biochemistry & Biotechnology* 174(2):719–728
- Lyahyai J, Mediano DR, Ranera B, Sanz A, Remacha AR, Bolea R, Zaragoza P, Rodellar C, Martín-Burriel I (2012) Isolation and characterization of ovine mesenchymal stem cells derived from peripheral blood. *BMC Vet Res* 8:169
- Maia L, Dias MC, de Moraes CN et al (2017) Conditioned medium: a new alternative for cryopreservation of equine umbilical cord mesenchymal stem cells. *Cell Biol Int* 41(3):239–248
- Mamidi MK, Nathan KG, Singh G, Thrichelvam ST, Yusof M, Nasim NA, Fakharuzi NA, Zakaria Z, Bhonde R, Das AK (2012) Comparative cellular and molecular analyses of pooled bone marrow multipotent mesenchymal stromal cells during continuous passaging and after successive cryopreservation. *J Cell Biochem* 113:3153–3164
- Marędziać M, Marycz K, Lewandowski D, Siudzińska A, Śmieszek A (2015) Static magnetic field enhances synthesis and secretion of membrane-derived microvesicles (MVs) rich in VEGF and BMP-2 in equine adipose-derived stromal cells (EqASCs)—a new approach in veterinary regenerative medicine. *In Vitro Cell Dev Biol Anim* 51:230–240
- Martin Y, Eldardiri MB, Lawrence-Watt DJ, Sharpe JR (2011) Microcarriers and their potential in tissue regeneration. *Tissue Eng Part B Rev* 17(1):71–81
- Martinello T, Bronzini I, Maccatrozzo L et al (2010) Cryopreservation does not affect the stem characteristics of multipotent cells isolated from equine peripheral blood. *Tissue Eng Part C Methods* 16:771–781
- Martinello T, Bronzini I, Maccatrozzo L et al (2011) Canine adipose derived- mesenchymal stem cells do not lose stem features after a long-term cryopreservation. *Res Vet Sci* 91:18–24
- Martins GR, Marinho RC, Bezerra-Junior RQ, Câmara LMC, Albuquerque-Pinto LC, Teixeira MFS (2017) Isolation, culture and characterization of multipotent mesenchymal stem cells from goat umbilical cord blood. *Pesquisa Vet Bras* 37(6):643–649
- Marx C, Silveira MD, Beyer NN (2015) Adipose-derived stem cells in veterinary medicine: characterization and therapeutic applications. *Stem Cells Dev* 24:803–813
- Mazur P (1977) The role of intracellular freezing in the death of cells cooled at supraoptimal rates. *Cryobiology* 14:251–272
- Mccarty R, Gronthos S, Zannettino AC, Foster BK, Xian CJ (2009) Characterisation and developmental potential of ovine bone marrow derived mesenchymal stem cells. *J Cell Physiol* 219:324–333
- McIntosh KR, Lopez MJ, Borneman JN, Spencer ND, Anderson PA, Gimble JM (2009) Immunogenicity of allogeneic adipose-derived stem cells in a rat spinal fusion model. *Tissue Eng Part A* 15:2677–2686
- Mediano DR, Sanz-Rubio D, Bolea R, Marín B, Vázquez FJ, Remacha AR, Pez-Pérez O, Fernández-Borges N, Castilla J, Zaragoza P, Badiola JJ, Rodellar C, Martín-Burriel I (2015) Characterization of mesenchymal stem cells in sheep naturally infected with scrapie. *J Gen Virol* 96:3715–3726
- Mellado-López M, Griffith RJ, Meseguer-Ripolles J, Cugat R, García M, Moreno Manzano V (2017) Plasma rich in growth factors induces cell proliferation, migration, differentiation, and cell survival of adipose-derived stem cells. *Stem Cells International* 2017:5946527
- Mitchell KE, Weiss ML, Mitchell BM, Martin P, Davis D, Morales L, Helwig B, Beerenstrauch M, Abou-Easa K, Hildreth T et al (2003) Matrix cells from Wharton's jelly form neurons and glia. *Stem Cells* 21:50–60
- Mitchell A, Rivas KA, Smith R III, Watts AE (2015) Cryopreservation of equine mesenchymal stem cells in 95% autologous serum and 5% DMSO does not alter post-thaw growth or mor-

- phology in vitro compared to fetal bovine serum or allogeneic serum at 20 or 95% and DMSO at 10 or 5%. *Stem Cell Res Ther* 6:231
- Mosna F, Sensebé L, Krampera M (2010) Human bone marrow and adipose tissue mesenchymal stem cells: a user's guide. *Stem Cells Dev* 19:1449–1470
- Ock S-A, Rho G-J (2011) Effect of dimethyl sulfoxide (DMSO) on cryopreservation of porcine mesenchymal stem cells (pMSCs). *Cell Transplant* 20:1231–1239
- Okamura LH, Cordero P, Palomino J, Parraguez VH, Torres CG, Peralta OA (2017) Myogenic differentiation potential of mesenchymal stem cells derived from fetal bovine bone marrow. *Anim Biotechnol* 29:1–11. <https://doi.org/10.1080/10495398.2016.1276926>
- Otsuru S, Hofmann TJ, Olson TS, Dominici M, Horwitz EM (2013) Improved isolation and expansion of bone marrow mesenchymal stromal cells using a novel marrow filter device. *Cytotherapy* 15:146–153
- Pacini S, Spinabella S, Trombi L et al (2007) Suspension of bone marrow-derived undifferentiated mesenchymal stromal cells for repair of superficial digital flexor tendon in race horses. *Tissue Eng* 13(12):2949–2955
- Perez-Silos V, Camacho-Morales A, Fuentes-Mera L (2016) Mesenchymal stem cells subpopulations: application for orthopedic regenerative medicine. *Stem Cells Int* 2016:3187491
- Petry F, Smith JR, Leber J, Salzig D, Czermak P, Weiss ML (2016) Manufacturing of human umbilical cord mesenchymal stromal cells on microcarriers in a dynamic system for clinical use. *Stem Cells Int* 2016:12
- Phinney DG, Sensebe L (2013) Mesenchymal stromal cells: misconceptions and evolving concepts. *Cytotherapy* 15(2):140–145
- Pillai SM, Sereda NH, Hoffman ML, Valley EV, Crenshaw TD, Park Y-K, Lee J-Y, Zinn SA, Govoni KE (2016) Effects of poor maternal nutrition during gestation on bone development and mesenchymal stem cell activity in offspring. *PLoS One* 11(12):e0168382
- Quimby JM, Webb TL, Habenicht LM, Dow SW (2013) Safety and efficacy of intravenous infusion of allogeneic cryopreserved mesenchymal stem cells for treatment of chronic kidney disease in cats: results of three sequential pilot studies. *Stem Cell Res Ther* 4:48
- Radcliffe CH, Flaminio JBF, Fortier LA (2010) Temporal analysis of equine bone marrow aspirate during establishment of putative mesenchymal progenitor cell populations. *Stem Cells Dev* 19:269–282
- Rafiq QA, Coopman K, Hewitt CJ (2014) Scale-up of human mesenchymal stem cell culture: current technologies and future challenges. *Curr Opin Chem Eng* 2(1):8–16
- Rafiq QA, Coopman K, Nienow AW, Hewitt CJ (2016) Systematic microcarrier screening and agitated culture conditions improve human mesenchymal stem cell yield in bioreactors. *Biotechnol J* 11:473–486
- Ranera B, Lyahyai J, Romero A et al (2011) Immunophenotype and gene expression profiles of cell surface markers of mesenchymal stem cells derived from equine bone marrow and adipose tissue. *Vet Immunol Immunopathol* 144:147–154
- Rhodes NP, Srivastava JK, Smith RF, Longinotti C (2004) Heterogeneity in proliferative potential of ovine mesenchymal stem cell colonies. *J Mater Sci Mater Med* 15(4):397–402
- Roberts EL, Dang T, Lepage SIM, Alizadeh AH, Walsh T, Koch TG, Kallos MS (2019) Improved expansion of equine cord blood derived mesenchymal stromal cells by using microcarriers in stirred suspension bioreactors. *Journal of Biological Engineering* 13:25
- Russell KA, Gibson TWG, Chong A, Co C, Koch TG (2015) Canine platelet lysate is inferior to fetal bovine serum for the isolation and propagation of canine adipose tissue- and bone marrow-derived mesenchymal stromal cells. *Plos ONE* 10(9):e0136621
- Salehinejad P, Alitheen NB, Ali AM, Omar AR, Mohit M, Janzamin E, Samani FS, Torshizi Z, Nematollahi-Mahani SN (2012) Comparison of different methods for the isolation of mesenchymal stem cells from human umbilical cord Wharton's jelly. *In Vitro Cell Dev Biol Anim* 48:75–83
- Sampaio RV, Chiaratti MR, Santos DCN, Bressan FF, Sangalli JR, Sá ALA, Silva TVG, Costa NN, Cordeiro MS, Santos SSD, Ambrosio CE, Adona PR, Meirelles FV, Miranda MS, Ohashi OM (2015) Generation of bovine (*Bos indicus*) and buffalo (*Bubalus bubalis*) adipose tissue derived

- stem cells: isolation, characterization, and multipotentiality. *Genetics & Molecular Research* 14(1):53–62
- Scheinkonig C, Kappicht S, Kolb HJ, Schleuning M (2004) Adoption of long-term cultures to evaluate the cryoprotective potential of trehalose for freezing hematopoietic stem cells. *Bone Marrow Transplant.* 34:531–536
- Schop D, Dijkhuizen-Radersma R, Van Borgart E, Jansen FW, Rozemuller H, Prins H-J, de Bruijn JD (2010) Expansion of human mesenchymal stromal cells on microcarriers: growth and metabolism. *J Tissue Eng Regen Med* 4(7):131–140
- Schubert S, Brehm W, Hillmann A, Burk J (2018) Serum-free human MSC medium supports consistency in human but not in equine adipose-derived multipotent mesenchymal stromal cell culture. *Cytometry A* 93(1):60–72
- Schwarz C, Leicht U, Rothe C et al (2012) Effects of different media on proliferation and differentiation capacity of canine, equine and porcine adipose derived stem cells. *Res Vet Sci* 93:457–462
- Seo JP, Tsuzuki N, Haneda S et al (2013) Comparison of allogeneic platelet lysate and fetal bovine serum for in vitro expansion of equine bone marrow-derived mesenchymal stem cells. *Res Vet Sci* 95:693–698
- Seshareddy K, Troyer D, Weiss ML (2008) Method to isolate mesenchymal-like cells from Wharton's jelly of umbilical cord. *Methods Cell Biol* 86:101–119
- Shah FS, Wu X, Dietrich M, Rood J, Gimble JM (2013) A non-enzymatic method for isolating human adipose tissue-derived stromal stem cells. *Cytotherapy* 15:979–985
- Smith RKW, Korda M, Blunn GW, Goodship AE (2003) Isolation and implantation of autologous equine mesenchymal stem cells from bone marrow into the superficial digital flexor tendon as a potential novel treatment. *Equine Vet J* 35:99–102
- Somal A, Bhat IA, Indu B, Pandey S, Panda BSK, Thakur N, Sarkar M et al (2016) A comparative study of growth kinetics, in vitro differentiation potential and molecular characterization of fetal adnexa derived caprine mesenchymal stem cells. *PLoS ONE* 11(6):e0156821
- Somal A, Bhat IA, Baiju I, Singh AP, Panda BSK, Desingu PA, Pandey et al (2017) Impact of cryopreservation on caprine fetal adnexa derived stem cells and its evaluation for growth kinetics, phenotypic characterization and wound healing potential in xenogenic rat model. *J Cellular Physiol* 232:2186–2200
- Somers P, Cornelissen R, Thierens H, Van Nooten G (2012) An optimized growth factor cocktail for ovine mesenchymal stem cells. *Growth Factors* 30(1):37–48
- Spaas JH, Schauwer CD, Cornillie P, Meyer E, Soom AV, Van de Walle GR (2013) Culture and characterisation of equine peripheral blood mesenchymal stromal cells. *Vet J* 195(1):107–113
- Steens J, Klein D. (2018). Current Strategies to Generate Human Mesenchymal Stem Cells In Vitro. *Stem Cells International* Art ID 6726185.
- Strioga M, Viswanathan S, Darinskas A, Slaby O, Michalek J (2012) Same or not the same? Comparison of adipose tissue-derived versus bone marrow-derived mesenchymal stem and stromal cells. *Stem Cells Dev* 21(14):2724–2752
- Sun LY, Hsieh DK, Syu WS, Li YS, Chiu HT, Chiou TW (2010) Cell proliferation of human bone marrow mesenchymal stem cells on biodegradable microcarriers enhances in vitro differentiation potential. *Cell Prolif* 43(5):445–456
- Takahashi I, Sato K, Mera H, Wakitani S, Takagi M (2016) Effects of agitation rate on aggregation during beads-to-beads subcultivation of microcarrier culture of human mesenchymal stem cells. *Cytotechnology* 69(3):503–509
- Taylor M, Song Y, Brockbank K (2004) Vitrification in tissue preservation: new developments. In: Fuller BJ, Lane N, Benson EE (eds) *Life in the frozen state*. CRC Press, Boca Raton, pp 604–641
- Thirumala S, Zvonic S, Floyd E, Gimble JM, Devireddy RV (2005) Effect of various freezing parameters on the immediate post-thaw membrane integrity of adipose tissue derived adult stem cells. *Biotechnol Prog* 21:1511–1524
- Thirumala S, Goebel WS, Woods EJ (2009) Clinical grade adult stem cell banking. *Organogenesis* 5:143–154

- Thirumala S, Gimble JM, Devireddy RV (2010) Evaluation of methylcellulose and dimethyl sulfoxide as the cryoprotectants in a serum-free freezing media for cryopreservation of adipose derived adult stem cells. *Stem Cells Dev* 19:513–522
- Tian Y, Tao L, Zhao S, Tai D, Liu D, Liu P (2016) Isolation and morphological characterization of ovine amniotic fluid mesenchymal stem cells. *Exp Anim* 65(2):125–134
- Tong CK, Vellasamy S, Tan BC, Abdullah M, Vidyadaran S, Seow HF, Ramasamy R (2011) Generation of mesenchymal stem cell from human umbilical cord tissue using a combination enzymatic and mechanical disassociation method. *Cell Biol Int* 35:221–226
- Toupadakis CA, Wong A, Genetos DC et al (2010) Comparison of the osteogenic potential of equine mesenchymal stem cells from bone marrow, adipose tissue, umbilical cord blood, and umbilical cord tissue. *Am J Vet Res* 71:1237–1245
- Verdanova M, Pytlik R, Kalbacova MH (2014) Evaluation of sericin as a fetal bovine serum-replacing cryoprotectant during freezing of human mesenchymal stromal cells and human osteoblast-like cells. *Biopreserv Biobank* 12:99–105
- Vidal M, Kilroy G, Johnson J, Lopez M, MoorGae R, Gimble J (2006) Cell growth characteristics and differentiation frequency of adherent equine bone marrow-derived mesenchymal stromal cells: adipogenic and osteogenic capacity. *Vet Surg* 35:601–610
- Vidal MA, Robinson SO, Lopez MJ et al (2008) Comparison of chondrogenic potential in equine mesenchymal stromal cells derived from adipose tissue and bone marrow. *Vet Surg* 37:713–724
- Vieira NM, Brandalise V, Zucconi E, Secco M, Strauss BE, Zatz M (2010) Isolation, characterization, and differentiation potential of canine adipose-derived stem cells. *Cell Transplant* 19:279–289
- Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, Fu YS, Lai MC, Chen CC (2004) Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells* 22:1330–1337
- Weigand A, Beier JP, Schmid R, Knorr T, Kilian D, Götzl R, Gerber T, Horch RE, Boos AM (2017) Bone tissue engineering under xenogeneic-free conditions in a large animal model as a basis for early clinical applicability. *Tissue Eng Part A* 23(5–6):208–222
- Weng L, Li W, Zuo J, Chen C (2011) Osmolality and unfrozen water content of aqueous solution of dimethyl sulfoxide. *J Chem Eng Data* 56:3175–3182
- Wilke MM, Nydam DV, Nixon AJ (2007) Enhanced early chondrogenesis in articular defects following arthroscopic mesenchymal stem cell implantation in an equine model. *J Orthop Res* 25:913–925
- Yadav P, Mann A, Singh V, Yashveer S, Sharma R, Singh I (2011) Expression of pluripotency genes in buffalo (*Bubalus bubalis*) amniotic fluid cells. *Reprod Domest Anim* 46:705–711
- Yoon JH, Roh EY, Shin S, Jung NH, Song EY, Chang JY, Kim BJ, Jeon HW (2013) Comparison of explant-derived and enzymatic digestion-derived MSCs and the growth factors from Wharton's jelly. *Biomed Res Int* 2013:428726
- Yuan Y, Kallos MS, Hunter C, Sen A (2014) Improved expansion of human bone marrow-derived mesenchymal stem cells in microcarrier-based suspension culture. *J Tissue Eng Regen Med* 8(3):210–225
- Zahedi M, Parham A, Dehghani H, Mehrjerdi HK (2018) Equine bone marrow-derived mesenchymal stem cells: optimization of cell density in primary culture. *Stem Cell Investig* 5:31

Mesenchymal Stem Cell Immuno-Modulatory and/Anti-Inflammatory Properties

4

M. B. Gugjoo and Amar Pal

Abstract

Among various attributed features of mesenchymal stem cells (MSCs), immuno-compromise/immuno-evasive, and immuno-modulation and/anti-inflammatory properties have an important role to play in the therapeutics. The immuno-compromised feature makes them suitable for allogeneic and/xenogeneic application while immuno-modulation and/ anti-inflammatory properties facilitate the healing process. Immuno-compromised feature is attributed to their minimal ability to express MHC-II and co-stimulatory molecules. The immune regulation may be achieved through their direct contact to immune cells and/by paracrine effect. Currently, a very limited control can be exerted on these features of MSCs. An extensive available literature has demonstrated these properties of MSCs, although some studies have reported otherwise. The current chapter deliberates on the immuno-modulatory and/anti-inflammatory features of MSCs in veterinary species.

Abbreviations

ConA	Concanavalin A
COX2	Cyclooxygenase 2
FASL	Fas ligand
HGF	Hepatocyte growth factor
HMOX1	Heme oxygenase 1

M. B. Gugjoo (✉)

Division of Veterinary Clinical Complex, FVSc & AH, SKUAST-Kashmir,
Jammu and Kashmir, India

A. Pal

Division of Surgery, Indian Veterinary Research Institute, Izatnagar, UP, India

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2020

M. B. Gugjoo, A. Pal (eds.), *Mesenchymal Stem Cell in Veterinary Sciences*,
https://doi.org/10.1007/978-981-15-6037-8_4

IDOn	Indole-amine 2,3 dioxygenase
IL6	Interleukin 6
INFAC	Interferon alpha C (INF-alpha C)
INFB1	Interferon, beta 1, INFG: Interferon gamma
iNOS	Inducible nitric oxide synthase
LPS	Lipopolysaccharide
LTBR	Lymphotoxin beta receptor (TNF superfamily member 3)
PDL1	Programmed death ligand 1
PMA	Phorbol 12-myristate 13-acetate
PTGES	Prostaglandin E synthase
RNA	Ribonucleic acid
STC-1	Stanniocalcin 1
TGF β 1	Transforming growth factor beta 1
TNF	Tumour necrosis factor
TNF α	Tumour necrosis factor
TSG	Tumour suppressor gene
VEGF	Vascular endothelial growth factor

4.1 Introduction

MSCs arise from pericytes upon an injury/assault to a particular tissue. Activated MSCs prove doubly rewarding: they provide immuno-regulation on the blood vasculature end (preventing aggressive immune response and establishment of chronic autoimmune reaction) and on injured/damaged tissue side MSCs secrete trophic factors to help regenerate tissue (Caplan and Dennis 2006; Caplan 2012; Prockop and Oh 2012). These characteristic features of MSCs make them potential candidates for therapeutics. The trend of utilizing MSCs in medical and/veterinary therapeutics is increasing by each passing day and even appears prospective (Gugjoo and Amarpal 2018; Gugjoo et al. 2019).

MSCs may bring therapeutic effects either through differentiation into particular lineage, rescuing injured/dying cell via cell fusion or through secretome (Rehman et al. 2004). Current notion favours their therapeutic role mainly through the release of secretory factors. MSCs secretome contains pro-healing proteins/peptides, hormones, RNA and other chemicals. Such secretome is being mediated through extracellular vesicles like micro-vesicles and exosomes. Even organelle transfer is being ensued and may be mediated through tunnelling nanotubes (Spees et al. 2016; Phinney and Pittenger 2017; Gugjoo et al. 2018a, b). Based on these features MSCs conditioned medium (contains metabolites, growth factors and extracellular matrix proteins) is considered as potential therapeutic option (Bhang et al. 2014; Joseph et al. 2020). These factors show immune-modulation, prevent cell apoptosis and through chemotactic agents bring other pro-healing cells or factors to the area of injury (Koch et al. 2008; Fortier et al. 2010; Owens et al. 2016)

4.1.1 Restricted Therapeutic Applications of MSCs

MSCs definitive therapeutic applications are currently marred by limited comprehension in their cellular processes. Lack of infinite self-renewal properties under culture conditions, variability in properties with respect to donor tissue source, age and health condition and above all the control of gene expression by the available environment make their applications dubious. An in-depth understanding in stemness marker genes with a well control over their molecular mechanisms can make their definitive therapeutic applications a reality (Zheng 2018). Recent mice study could demonstrate that transcriptome and chromatin markers direct MSCs characteristics on the basis of their source (Ho et al. 2018). This demands extensive work over the epigenomic features that govern MSCs characteristics including their fate.

Apart from the limited understanding in the cellular features in vitro culture studies and in vivo experimental studies too have constraints. In vitro studies have frequently incorporated diverse culture ingredients like other cells, matrices and humoral or growth factors without any conclusion. In vivo studies conducted lack appropriate control groups, animal size and evaluation period (Park et al. 2012; Spencer et al. 2012; de Bakker et al. 2013; Saulnier et al. 2016; Kresic et al. 2017; Gugjoo et al. 2019). In veterinary practice, literature has frequently relied on study designs that have failed to conduct studies as evidence based medicine. Most studies lack blinded randomized control trials and the sample size has been too little to draw any definitive conclusion(s). It may be attributed to the machination and economical limitations leading to faulty/compromised study design (de Bakker et al. 2013; Gugjoo et al. 2019). The duration of studies has to be appropriate as per the study design and condition involved (Gugjoo et al. 2019). Additionally, MSC doses have been supplemented with other biological materials like bone marrow (BM) supernatant, autologous serum, platelet-rich plasma, and/or scaffolds and growth factors adding further misery to the understanding (Clegg and Pinchbeck 2011; De Schauwer et al. 2013; Gugjoo et al. 2017; Gugjoo et al. 2020a, b).

4.2 Immuno-Modulation and/Anti-Inflammatory Properties

Initially, autologous MSC applications were preferred but their immuno-compromised/ immuno-evasive nature has shifted the preference towards their allogeneic applications (Berner et al. 2015; Gugjoo et al. 2017). Allogeneic MSCs may be harvested at large scale with similar culture conditions. This makes their ready-to-use cell therapy quite possible as against their autologous therapy that has a lag period of 14–21 days for isolation and culture expansion. Most of the in vivo MSCs allogeneic studies have supported their safety and useful applicability (McDonald et al. 2015; Zorzi et al. 2015).

An extensive literature exists that support human and animal MSCs immuno-modulatory and anti-inflammatory activities. Various animal studies described in the current manuscript are of dog (Kang et al. 2008; Kim et al. 2010; Lee et al. 2011; Russell et al. 2016; Bearden et al. 2017; Chow et al. 2017; Wheat et al. 2017; Yang

et al. 2018; Taguchi et al. 2019; Kafarnik et al. 2020), horse (Carrade and Borjesson 2011; Deuse et al. 2011; Peroni and Borjesson 2011; Carrade et al. 2012; Carrade et al. 2014; Colbath et al. 2017; Cassano et al. 2018; Cortés-Araya et al. 2018; Lepage et al. 2019; Longhini et al. 2019), sheep (Mrugala et al. 2008), chickens (Khatri et al. 2009), pig (Poncelet et al. 2007; Cho et al. 2008), rabbit (Liu et al. 2006; Moreno et al. 2010), goats (Somal et al. 2016), cattle (Cardoso et al. 2012; Jacca et al. 2014; Cardoso et al. 2017; de Moraes et al. 2017; Lara et al. 2017) and cat (Mumaw et al. 2015; Zajic et al. 2016; Chae et al. 2017; Clark et al. 2017; Parys et al. 2017). In general, MSCs immuno-modulation and/or anti-inflammatory activities occur through their interaction with immune cells. These activities are mediated through secretome or through cell-cell contact. MSCs lack direct cytotoxic or humoral defence activities unlike that of the immune cells. These cells fail to secrete granzymes or perforins, lack antibody production ability and are devoid of phagocytic activity (Tso et al. 2010).

4.2.1 MSCs Are Immuno-Privileged

Autologous and allogeneic MSCs are non-immunogenic and xenogeneic MSCs lack leukocyte proliferation in the absence of activation (Mrugala et al. 2008; Carrade and Borjesson 2011). The cells lack expression of major histocompatibility complex II (MHC-II), although variably and weakly express MHC-I (Krampera et al. 2006a, b; Carrade et al. 2012; De Schauwer et al. 2011; Schnabel et al. 2013; Berglund and Schnabel 2017; Cardoso et al. 2017; Merlo et al. 2019). Additionally, majority of the classical co-stimulatory molecules on MSCs have remained unexpressed (Fig. 4.1) (Tse et al. 2003; Carrade et al. 2012). The pro-inflammatory factors (IL2, IL6R, INFAC, INFB1, INFG, TNF and LTBR) too had remained downregulated in bovine Wharton's jelly MSCs (bWJ-MSCs) (Cardoso et al. 2017). Apart from the single in vivo parenteral implantation (Carrade et al. 2011), the repeated intra-articular or intradermal implantation of allogeneic equine MSCs too had failed to develop any hypersensitivity reaction (Ardanaz et al. 2016).

4.2.2 MSCs Immuno-Modulation

An initial study that had demonstrated immune-modulatory properties of MSCs was conducted by Bartholomew and colleagues in 2002. It was shown that MSCs suppressed mixed lymphocyte response and also prevented rejection of allograft in a baboon skin model (Bartholomew et al. 2002). Afterwards an extensive work in the field has been conducted piling up the huge literature on such properties (Fig. 4.1). MSCs are now known to bear anti-inflammatory properties, modulate immune response (Kang et al. 2008; Kim et al. 2010; Lee et al. 2011; Carrade and Borjesson 2011) and stimulate angiogenesis (Wu et al. 2007; El-Menoufy et al. 2010). These actions are undertaken when MSCs interact with immune cells. The inhibition may occur either by MSCs contact with immune cells and/or by their secretome. In

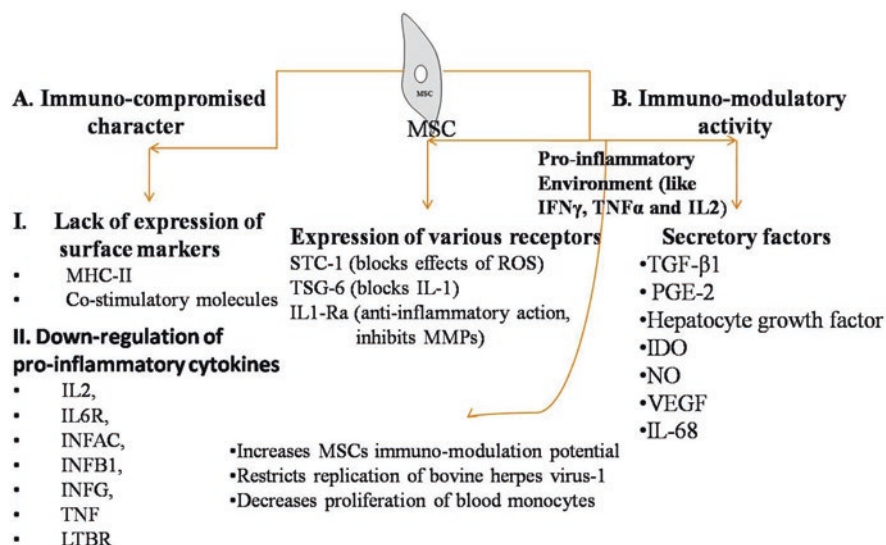


Fig. 4.1 MSCs immuno-compromised and/ or immuno-modulatory properties. MSCs increased immuno-modulation potential in presence of inflammatory environment

humans, contact based immune-modulation may be mediated through toll-like receptors (TLRs), intracellular adhesion molecule-1 (ICAM) and vascular cell adhesion molecule-1 (VCAM-1) present on the surface of MSC (Tomchuck et al. 2008; DelaRosa and Lombardo 2010; Akiyama et al. 2012). In canine transwell assays, wherein cells are separated physically from lymphocytes, certain soluble factors had been released that had brought about the inhibition of immune response (Kang et al. 2008; Lee et al. 2011). The former way of immuno-suppression however seems to be stronger than later (Di Nicola et al. 2002).

MSCs express various receptors and/factors that tend to decrease inflammation and free radical damage. Equine MSCs had inhibited adverse reactions of reactive oxygen species (ROS) (through *STC-1*), pro-inflammatory cytokine IL-1 (through *TSG-6*) and inhibited matrix metallo-proteinases and brought anti-inflammatory actions (through *IL1-Ra*) (Angelone et al. 2017). Even unstimulated feline AD-MSCs had expressed various immune-modulatory genes like *IDO1*, *COX2*, *PTGES1*, *PTGES2*, *PTGES3*, *HGF*, *TGF β 1*, *IL6*, *PDL1* and *HMOX1* barring *IL-10* (Parys et al. 2017).

In a recent advancement, MSCs are considered to secrete extracellular vesicles (exosomes) that carry modulatory paracrine actions (Katsuda et al. 2013; Yu et al. 2014), with some demonstrating immune-suppressive properties (Kordelas et al. 2014). MSCs derived exosomes are generated from plasma membrane lipid raft microdomains and after secretion accumulate in the culture medium (Tan et al. 2013; Perrini et al. 2016). Microvesicles secreted from equine MSCs had reduced apoptosis rate of equine endometrial cells, replenished their proliferation and prevented inflammation through inhibition of pro-inflammatory cytokines (Perrini

et al. 2016). MSCs microvesicle production varies with respect to their source. Among various equine foetal adnexal sources, Wharton's jelly derived MSCs (WJ-MSCs) had copious micro-vesicles as compared to the other adnexal sources (Iacono et al. 2017).

4.2.3 Role of Inflammatory Environment

The pro-inflammatory mediators especially interferon- γ (IFN γ), tumour necrosis factor- α (TNF α) and interleukin 2 (IL2) available in inflammatory environment tend to variably stimulate the immuno-modulatory action of MSCs (Liu et al. 2006; Poncelet et al. 2007; Russell et al. 2016). Co-culture of feline adipose tissue-MSCs (AD-MSCs) with peripheral blood derived mononuclear cells and macrophages had significantly reduced pro-inflammatory cytokine production (Chae et al. 2017). MSCs challenge against the prostaglandin E2 (PGE $_2$) had rearranged its transcriptome profile. The obvious effect was downregulated immune response, and an enhanced cell proliferation and vasculogenesis. Such a challenge had additionally downregulated transforming growth factor β (TGF- β) and Wnt- β -catenin signalling pathways (Lara et al. 2017). Lipopolysaccharide (LPS) activated bovine endometrium-MSCs (End-MSCs) secretome analysis had revealed presence of anti-inflammatory and antibacterial proteins. These proteins had led to protective anti-oxidant activity and were related to tissue remodelling and angiogenesis (de Moraes et al. 2017).

MSCs mediated immuno-modulation occur either constitutively and/or after activation with inflammatory mediators. The secretome includes TGF- β 1 and PGE2 (Liu et al. 2006; Poncelet et al. 2007; Kang et al. 2008; Berman et al. 2010; Lee et al. 2011; Carrade et al. 2012; Chow et al. 2017), hepatocyte growth factor (HGF) (Kang et al. 2008; Lee et al. 2011; Carrade et al. 2012) and indoleamine 2,3-dioxygenase (IDO) (Kang et al. 2008), nitric oxide (NO) (Khatri et al. 2009; Carrade et al. 2012), vascular endothelial growth factor (VEGF) (Berman et al. 2010; Lee et al. 2011), and interleukin-68 (IL-68) (Berman et al. 2010; Kang et al. 2008; Carrade et al. 2012; Bearden et al. 2017). The production of such factors, however, varies with species and tissue types though the functional basis of such differences are currently unknown (Carrade and Borjesson 2011).

The cell lines of various sources (adipose tissue-MSCs, Bone marrow-MSCs, Umbilical cord-MSCs, umbilical cord tissue-MSCs) may follow overlapping or varying pathways to exhibit immuno-modulatory effects. The source however, may not affect their comprehensive immune-modulatory effects under a particular inflammatory environment (Cassano et al. 2018). Dog bone marrow-MSCs (dbM-MSCs) and adipose tissue-MSCs (dAD-MSCs) (Russell et al. 2016; Chow et al. 2017) and horses cord blood MSCs (eCB-MSCs) and cord tissue-MSCs (eCT-MSCs) (Lepage et al. 2019) had shown comparable immuno-modulation, although variable pathways may be ensued (Fig. 4.2).

Apart from an immune-modulatory role, activated MSCs may restrict viral/bacterial growth. The antibacterial potential (against *Escherichia coli*) of conditioned

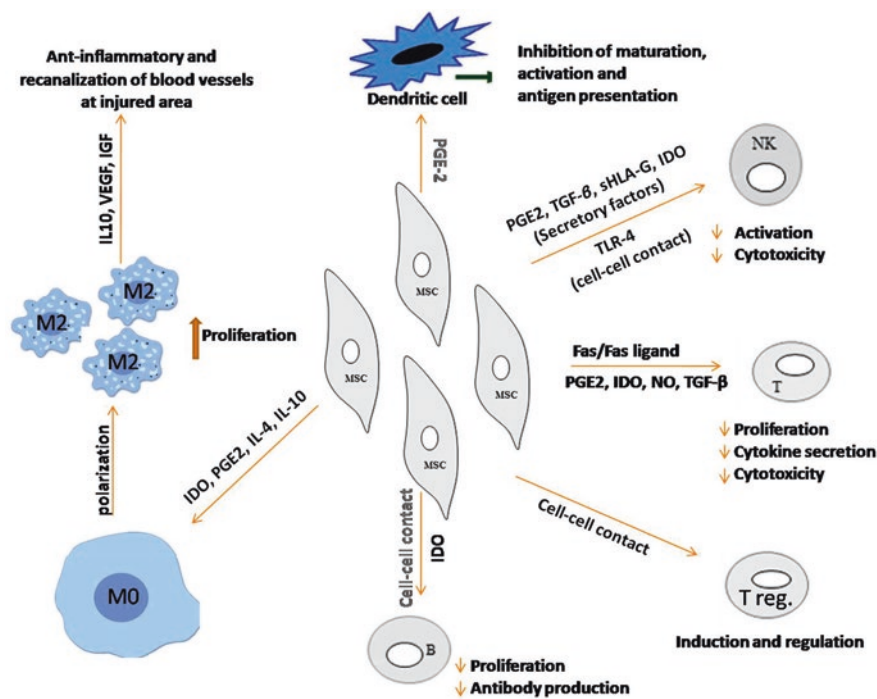


Fig. 4.2 MSCs immuno-modulation mechanism through paracrine secretion. $\downarrow$: reduction

media of equine Endometrium-MSCs (eEnd-MSCs) and AD-MSCs had been more pronounced as compared to that of equine BM-MSCs. Lipocalin-2 (antimicrobial agent) was highly expressed in eEnd-MSCs in comparison to the eAD-MSCs and eBM-MSCs. Contrarily, lipopolysaccharide (LPS) had enhanced its comparable production in these three source-MSCs (Cortés-Araya et al. 2018). Likewise dog MSCs had shown antibacterial effects upon interaction with host innate immune response and applied antibiotics (Johnson et al. 2017). IFN- γ activation of bovine End-MSCs had induced replication restriction in bovine herpes virus-1, depicting their antiviral activity. The followed pathway might have been other than the indoleamine 2,3 dioxygenase (IDO-1) (Cardoso et al. 2012). Various factors secreted after MSCs activation are detailed below:

4.2.3.1 Transforming Growth Factor- β 1 (TGF- β 1)

MSCs TGF- β 1 production varies with respect to species and tissue type. Activated lymphocytes had failed to stimulate MSCs expression of TGF- β 1 in dog BM-MSCs, horse BM-MSCs, AD-MSCs and cord tissue-MSCs (eCT-MSCs), and pig BM-MSCs (Poncelet et al. 2007; Lee et al. 2011; Carrade et al. 2012). Dog BM-MSC had failed to show improved production of TGF- β 1 after exposure to activated

lymphocytes (Lee et al. 2011) and even if produced levels were insufficient to inhibit lymphocyte proliferation. Contrarily, dog AD-MSC, and feline AD-MSCs and rabbit BM-MSCs had shown increased TGF- β 1 production after exposure to lymphocytes (Kang et al. 2008) and IFN- γ pre-treatment (Liu et al. 2006; Kang et al. 2008; Clark et al. 2017), respectively. TGF- β 1 production of feline AD-MSCs had also increased after stimulation with TNF α while combined stimulation (INF γ and TNF α) of feline AD-MSCs had resulted in decreased TGF- β 1 production (Parys et al. 2017).

4.2.3.2 Prostaglandin E2 and Indoleamine 2,3 Dioxygenase (IDO)

Dog MSCs (BM- and AD-), feline AD-MSCs secrete PGE2 (Kang et al. 2008; Lee et al. 2011; Chow et al. 2017; Clark et al. 2017; Parys et al. 2017; Taguchi et al. 2019) and IDO (Kang et al. 2008; Clark et al. 2017; Parys et al. 2017; Taguchi et al. 2019) and horse MSCs (AD-, BM-, CT- and CB-) secrete PGE2 (Carrade et al. 2012). The functional role of such factors has been shown to inhibit the lymphocyte proliferation as blocking of such factors restores the lymphocyte proliferation.

4.2.3.3 Nitrous Oxide (NO)

Secretory factors like NO show variation with respect to the tissue source. Horse BM-MSCs and CB-MSCs (hemic sources) produce NO while CT-MSCs and AD-MSCs (solid tissues) do not (Carrade et al. 2012). This is considered to be important with respect to the angiogenesis but not immune-modulatory function. Anecdotal evidence had shown increased healing time of equine tendon upon injection of solid tissue derived MSCs as compared to CB-MSCs, and may be attributed to the increased angiogenesis with CB-MSCs (Carrade and Borjesson 2011).

Apart from above factors, INF γ had been demonstrated to upregulate various other factors of human and feline AD-MSCs including programmed death ligand (PDL-1), interleukin 6 (IL-6), cyclooxygenase 2 (COX2) and hepatocyte growth factor (HGF) (Clark et al. 2017; Parys et al. 2017). TNF- α stimulation of these cells had led to the increased expression of IL-6, prostaglandin E synthase 1 (PTGES1) and COX2 (Parys et al. 2017).

4.3 Contrasting Reports

Some of the recent studies have been unsuccessful to demonstrate immuno-privileged character of MSCs (Nauta et al. 2006; Gugjoo et al. 2018a, b; Gugjoo et al. 2020a, b). Various studies could demonstrate MSCs receptor expression. These receptors have the ability to commune with T-cells. Among notable ones are MHC-I, integrins and other adhesion molecules that have their analogue ligands on T-cells. These analogues are cell adhesion molecules: VCAM, intercellular adhesion molecule 1 (ICAM-1), activated leukocyte cell adhesion molecule (CD166;

ALCAM) and lymphocyte functional antigen-3 (LFA-3; CD58) (Chamberlain et al. 2007). Bovine End-MSCs had expressed IFN- γ receptors 1 and 2 without their ligand whose production was stimulated with an exposure to the exogenous IFN γ (Jacca et al. 2014). MSCs additionally bear intracellular proteins and inflammatory mediator (INF γ) that may induce them to express MHC-II alloantigen (Le Blanc et al. 2003; Klyushnenkova et al. 2005; Le Blanc and Ringdén 2007; Cassano et al. 2018; Gale et al. 2019). Further, few recent studies have demonstrated that allo-MSC antibodies may generate against the allogeneic MSCs (Pezzanite et al. 2015; Owens et al. 2016). Even MHC mismatched MSCs had been able to activate complement dependent cytotoxicity (Owens et al. 2016).

Apart from ex vivo studies, in vivo equine studies that had implanted allogeneic equine BM-MSCs/umbilical cord blood MSCs (eUCB-MSCs) either once or multiple times intra-articularly had reported an undesirable clinical response (Joswig et al. 2017; Bertoni et al. 2019). Comparing cell induced synovial effusions, equine BM-MSCs injected joints had more significant effusions as compared to equine UCB-MSCs injected ones. The inflammatory signs developed have been more evident with fewer doses of cells (ten million) as compared to higher cell doses (20 million) (Bertoni et al. 2019). Thus, it is important to note that allogeneic MSCs implantation might incite some adverse reactions rather than useful outcomes (Berglund and Schnabel 2017). However, such undesirable responses may also have resulted from foetal bovine serum (FBS) particles being internalized by the cultured MSCs. Modification of cell culture conditions for 48-hour duration may help to deplete FBS particles (Joswig et al. 2017). MSCs adaptation to autologous serum-supplementation may be an effective way to maintain cell viability and prevent any adverse immunological reactions (Haque et al. 2015).

4.4 Effect of MSCs on Body's Immune System

MSCs in general exhibit properties of immunomodulation and is achieved by secreting certain factors that inhibit immune cells proliferation and/or their secretory factors (Fig. 4.2). Below are given the interactions of MSCs and immune cells.

4.4.1 Innate Immunity and MSCs

This type of immunity plays a pivotal role in elimination of pathogens by adaptive immune responses (Yamane and Paul 2013). MSCs interaction with innate immune cells like dendritic cells (DCs), natural killer (NK) cells, neutrophils and macrophages inhibits inflammatory processes and promotes regeneration (Le Blanc and Mougiakakos 2012). Inhibition of peripheral blood mononuclear cells may be variable with respect to the MSCs from various sources (Cardoso et al. 2012). Goat WJ-MSCs had higher inhibitory potential over peripheral blood mononuclear cells in comparison to the amniotic sac MSCs (AS-MSCs). Former cells achieved it

through indoleamine 2,3-dioxygenase (IDO) while later cell type had activated inducible nitric oxide synthase (iNOS) (Somal et al. 2016). The effect of MSCs on different immune cells is detailed below:

4.4.1.1 Myeloid Dendritic Cells (MDCs)

MSCs immunosuppressive effects on dendritic cells (DCs) may be achieved through restriction of their maturation (differentiation from monocyte) (Jiang et al. 2005; Wheat et al. 2017), surface protein expression like MHC-II, CD86, CD83, CD40 and CD1- α , capability to motivate lymphocytes (Gao et al. 2017), inhibiting their migration potential (Chiesa et al. 2011; Consentius et al. 2015) and by depressing their pro-inflammatory capacity (Aggarwal and Pittenger 2005). The maturation inhibition potential of MSCs increases in presence of inflammatory mediators like IFN γ and TNF- α (Wheat et al. 2017). The restricted DCs maturation may occur by PGE2 (Yañez et al. 2010). The decreased lymphocyte motivation of DCs occurs by downregulating IFN γ and TNF- α expression and accelerating release of IL10 (Gao et al. 2017). These responses may be mediated through Notch pathway that relies on IFN γ -secretase (Xu et al. 2017).

4.4.1.2 Natural Killer (NK) Cells

NK cells produce pro-inflammatory cytokines and carry cytolytic activity (Moretta 2002). MSCs tend to inhibit NK cell effects through secretory factors and/or direct cell interactions. MSCs secretome inhibiting NK cells are PGE2, TGF- β and sHLA-G (Sotiropoulou et al. 2006). MSCs suppress activated NK cell receptor expression being mediated by IDO and PGE2 (Cui et al. 2016). The direct cell-cell interaction may be achieved through MSCs Toll-like receptor-(TLR-) 4 (Michelo et al. 2016). These immune-suppressive effects of human occur in presence of high MSC:NK cell ratios (Sotiropoulou et al. 2006). Contrary to these findings, MSCs may be dissolved in presence of enough activating receptors on NK cells (Götherström et al. 2011). Thus, MSCs concentration and its surrounding environment has an important role to determine their immuno-modulatory properties.

4.4.1.3 Neutrophils

MSCs cultured with neutrophils tend to decrease their reactive oxygen species (ROS) potential. The inhibition potential of MSCs varies with respect to the source of cell. Feline BM-MSCs had quite significantly inhibited neutrophilic ROS potential in comparison to the feline AD-MSCs (Mumaw et al. 2015).

4.4.1.4 Macrophages

Macrophages based upon the available environment are polarized into two types: M1 possesses antimicrobial activity while M2 alleviates inflammation and expedites tissue repair. The anti-inflammatory activity and repair process of M2 may be ensured by secretion of IL10 and VEGF and IGF trophic factors (Mosser and Edwards 2008). MSCs induce production of M2 macrophages from M1. M2 increases the phagocytic activity and secretion of interleukin 10 and decreases inflammatory cytokines (Zhang et al. 2010; Selleri et al. 2016). Further, MSCs

decrease immune disorders and hasten tissue regeneration by enhancing macrophage concentration locally (Liu et al. 2015; Chaturvedi et al. 2014).

4.4.2 Adaptive Immunity and MSCs

T and B lymphocytes are mainly involved in adaptive immune responses. T lymphocytes comprise CD4 T helper and CD8 T cytotoxic lymphocytes. These cells show antigen specific immune response and have immunological memory (Yamane and Paul 2013). B lymphocytes act by producing specific antibodies (De Silva and Klein 2015). Dog AD-MSCs had decreased lymphocyte production of TNF- α while increasing their IFN γ production (Kang et al. 2008). Horse MSCs (AD-, BM-, CB- and CT-) had decreased TNF α and IFN γ production of lymphocytes (Peroni and Borjesson 2011). Pig BM-MSCs had decreased IFN γ and IL2 production of lymphocytes (Liu et al. 2004). Feline AD-MSCs lymphocyte inhibition tends to remain unaffected with donor age (Zajic et al. 2016). Below are the details of MSCs immune-modulation role against adaptive immunity.

4.4.2.1 T Cells

MSCs are considered to repress T-cell proliferation either through cell based or nonspecific mitogenic stimuli (Di Nicola et al. 2002). The action is mediated through secretome being responsible for effective T-cell suppression and apoptosis (Ren et al. 2008; Ren et al. 2010). The secretome involved in proliferation inhibition include PGE2, IDO, NO and TGF- β (Gao et al. 2016). The cells may prevent T-cell activation and proliferation by decreasing the expression of activation markers like CD25, CD38 and CD69 (Le Blanc et al. 2003; Groh et al. 2005). Furthermore, MSCs may promote apoptosis of activated T-cells via the Fas/Fas ligand pathway (Akiyama et al. 2012). Allogeneic and autologous cells may comparably suppress proliferation and IFN- γ production of T-cells (Colbath et al. 2017).

Despite all these reports, it is worth mentioning here that MSCs immunosuppressive potential may not be activated every time. Such a response is affected and determined by the inflammatory mediators and their strength (Renner et al. 2009). Besides, MSCs activities to inhibit T-cell proliferation may not be sufficient in presence of pathogen associated molecules. Toll-like receptors (TLR3 and TLR4) which damage Notch signalling may help in T-cells recuperation (Rashedi et al. 2017). Blocking activity of human BM-MSCs produced TGF- β 1 had failed to decrease T-regulatory cell production (English et al. 2009).

4.4.2.2 B Cells

MSCs suppress B cell proliferation, activation, differentiation, chemokine receptor expression either through cell-cell contact and/ or secretome (IDO) (Augello et al. 2005; O'Connor et al. 2006). However, MSCs ability to suppress B cell antibody production is dependent upon inflammatory stimulation strength, and MSCs:B cell ratio (Krampera et al. 2006a, b; Bernardo et al. 2009).

4.5 Conclusion(s)

Mesenchymal stem cells are immune-compromised/ immuno-evasive cells that achieve local immune-modulation and/anti-inflammatory role through secretion of paracrine factors and/or by cell-cell contact. The cells harvested from various sources may achieve comparable immune-modulatory role through different pathways. The cells act upon both the innate and acquired immunity without actually undertaking cytotoxic or humoral defence activities. The cells however, have failed to fully immune-suppress the in vivo immune responses and even some of the in vitro studies too have been unsuccessful to get the desired immuno-modulation. Thus, the mechanisms behind their immune-modulation and/anti-inflammatory action need further insights for their definitive clinical applications.

References

- Aggarwal S, Pittenger MF (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 105(4):1815–1822
- Akiyama K, Chen C, Wang DD, Xu X, Qu C, Yamaza T, Cai T, Chen W, Sun L, Shi S (2012) Mesenchymal stem- cell-induced immune-regulation involves FAS-ligand-/FAS-mediated T cell apoptosis. *Cell Stem Cell* 10(5):544–555
- Angelone M, Conti V, Biacca C, Battaglia B, Pecorari L, Piana F, Gnudi G, Leonardi F, Ramoni R, Basini G, Dotti S, Renzi S, Ferrari M, Grolli S (2017) The contribution of adipose tissue-derived Mesenchymal stem cells and platelet-rich plasma to the treatment of chronic equine laminitis: a proof of concept. *Int J Mol Sci* 18:2122
- Ardanaz N, Vázquez FJ, Romero A, Remacha AR, Barrachina L, Sanz A, Ranera B, Vitoria A, Albareda J, Prades M, Zaragoza P, Martín-Burriel I, Rodellar C (2016) Inflammatory response to the administration of mesenchymal stem cells in an equine experimental model: effect of autologous, and single and repeat doses of pooled allogeneic cells in healthy joints. *BMC Vet Res* 12:65
- Augello A, Tasso R, Negrini SM, Amateis A, Indiveri F, Cancedda R, Pennesi G (2005) Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. *Eur J Immunol* 35(5):1482–1490
- Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, Hardy W, Devine S, Ucker D, Deans R, Moseley A, Hoffman R (2002) Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol* 30(1):42–48
- Bearden RN, Huggins SS, Cummings KJ, Smith R, Gregory CA, Saunders WB (2017) In-vitro characterization of canine multipotent stromal cells isolated from synovium, bone marrow, and adipose tissue: a donor-matched comparative study. *Stem Cell Res Ther* 8:218
- Berglund AK, Schnabel LV (2017) Allogeneic major histocompatibility complex-mismatched equine bone marrow-derived mesenchymal stem cells are targeted for death by cytotoxic anti-major histocompatibility complex antibodies. *Equine Vet J* 49(4):539–544
- Berman DM, Willman MA, Han D, Kleiner G, Kenyon NM, Cabrera O, Karl JA, Wiseman RW, O'Connor DH, Bartholomew AM, Kenyon NS (2010) Mesenchymal stem cells enhance allogeneic islet engraftment in nonhuman primates. *Diabetes* 59:2558–2568
- Bernardo ME, Locatelli F, Fibbe WE (2009) Mesenchymal stromal cells. *Ann N Y Acad Sci* 1176(1):101–117
- Berner A, Henkel J, Woodruff MA, Steck R, Nerlich M, Schuetz MA, Hutmacher D, W. (2015) Delayed minimally invasive injection of Allogenic bone marrow stromal cell sheets regenerates large bone defects in an ovine preclinical animal model. *Stem Cells Transl Med* 4:503–512

- Bertoni L, Branly T, Jacquet S, Desancé M, Desquilbet L, Rivory P, Hartmann D-J, Denoix J-M, Audigié F, Galéra P, Demoor M (2019) Intra-articular injection of 2 different dosages of autologous and allogeneic bone marrow- and umbilical cord-derived Mesenchymal stem cells triggers a variable inflammatory response of the fetlock joint on 12 sound experimental horses. *Stem Cells Int* 2019:9431894
- Bhang SH, Lee S, Shin J-Y, Lee T-J, Jang H-K, Kim B-S (2014) Efficacious and clinically relevant conditioned medium of human adipose-derived stem cells for therapeutic angiogenesis. *Mol Ther* 22:862–872
- Caplan AI (2012) MSCs as therapeutics. In: Hematti P, Keating A (eds) *Stem cell biology and regenerative medicine: Mesenchymal stromal cells: biology and clinical applications*. Human Press, New York, pp 79–90
- Caplan AI, Dennis JE (2006) Mesenchymal stem cells as trophic mediators. *J Cell Biochem* 98:1076–1084
- Cardoso TC, Ferrari HF, Garcia AF, Novais JB, Silva-Frade C, Ferrarezi MC, Andrade AL, Gamiero R (2012) Isolation and characterization of Wharton's jelly-derived multipotent Mesenchymal stromal cells obtained from bovine umbilical cord and maintained in defined serum free three-dimensional system. *BMC Biotechnol* 12:18
- Cardoso TC, Okamura LH, Baptistella JC, Gameiro R, Ferreira HL, Marinho M, Flores EF (2017) Isolation, characterization and immunomodulatory-associated gene transcription of Wharton's jelly-derived multipotent mesenchymal stromal cells at different trimesters of cow pregnancy. *Cell Tissue Res* 367(2):243–256
- Carrade DD, Borjesson DL (2011) Immunomodulation by Mesenchymal stem cells in veterinary species. *Comp Med* 63(3):207–2017
- Carrade DD, Affolter VK, Outerbridge CA et al (2011) Intradermal injections of equine allogeneic umbilical cord-derived mesenchymal stem cells are well tolerated and do not elicit immediate or delayed hypersensitivity reactions. *Cytotherapy* 13(10):1180–1192
- Carrade DD, Lane MW, Kent MS, Clark KC, Walker NJ, Borjesson DL (2012) Comparative analysis of the immunomodulatory properties of equine adult-derived mesenchymal stem cells. *Cell Med* 4:1–11
- Carrade HDD, Wood JA, Granick JL, Walker NJ, Clark KC, Borjesson DL (2014) Equine Mesenchymal stem cells inhibit T cell proliferation through different mechanisms depending on tissue source. *Stem Cells Dev* 23(11):1258–1265
- Cassano JM, Fortier LA, Hicks RB, Harman RM, Van de Walle GR (2018) Equine mesenchymal stem cells from different sources display comparable immune related gene expression profiles in response to interferon gamma (IFN- γ). *Vet Immunol Immunopathol* 202:25–30
- Chae H-K, Song W-J, Ahn J-O, Li Q, Lee B-Y, Kwon K, Park S-C, Youn H-Y (2017) Immunomodulatory effects of soluble factors secreted by feline adipose tissue-derived mesenchymal stem cells. *Vet Immunol Immunopathol*. <https://doi.org/10.1016/j.vetimm.2017.07.013>
- Chamberlain G, Fox J, Ashton B, Middleton J (2007) Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem Cells* 25(11):2739–2749
- Chaturvedi P, Gilkes DM, Takano N, Semenza GL (2014) Hypoxia-inducible factor-dependent signaling between triple-negative breast cancer cells and mesenchymal stem cells promotes macrophage recruitment. *Proc Natl Acad Sci U S A* 111(20):E2120–E2129
- Chiesa S, Morbelli S, Morando S et al (2011) Mesenchymal stem cells impair in vivo T-cell priming by dendritic cells. *Proc Natl Acad Sci U S A* 108(42):17384–17389
- Cho PS, Messina DJ, Hirsh EL, Chi N, Goldman SN, Lo DP, Harris IR, Popma SH, Sachs DH, Huang CA (2008) Immunogenicity of umbilical cord tissue derived cells. *Blood* 111:430–438
- Chow L, Johnson V, Coy J, Regan D, Dow S (2017) Mechanisms of immune suppression utilized by canine adipose and bone marrow-derived Mesenchymal stem cells. *Stem Cells Develop* 26(5):374–389
- Clark KC, Fierro FA, Ko EM, Walker NJ, Arzi B, Tepper CG, Dahlenburg H, Cicchetto A, Kol A, Marsh L, Murphy WJ, Fazel N, Borjesson DL (2017) Human and feline adipose-derived

- mesenchymal stem cells have comparable phenotype, immunomodulatory functions, and transcriptome. *Stem Cell Res Ther* 8:69
- Clegg PD, Pinchbeck GL (2011) Evidence-based medicine and stem cell therapy: how do we know such technologies are safe and efficacious? *Vet Clin North Am Equine Pract* 27:373–382
- Colbath AC, Dow SW, Phillips JN, McIlwraith CW, Goodrich LR (2017) Autologous and allogeneic equine mesenchymal stem cells exhibit equivalent immunomodulatory properties in vitro. *Stem Cells Dev* 26(7):503–511
- Consentius C, Akyüz L, Schmidt-Lucke JA, Tschöpe C, Pinzur L, Ofir R, Reinke P, Volk H-D, Juelke K (2015) Mesenchymal stromal cells prevent allostimulation in vivo and control checkpoints of Th1 priming: migration of human DC to lymph nodes and NK cell activation. *Stem Cells* 33(10):3087–3099
- Cortés-Araya Y, Amilon K, Rink BE, Black G, Lisowski Z, Donadeu FX, Esteves CL (2018) Comparison of antibacterial and immunological properties of mesenchymal stem/stromal cells from equine bone marrow, endometrium and adipose tissue. *Stem Cells Dev* 27(21):1518–1525
- Cui R, Rekasi H, Hepner-Schefczyk M, Kai F, Petri RM, Bruderek K, Brandau S, Jäger M, Flohé SB (2016) Human mesenchymal stromal/stem cells acquire immunostimulatory capacity upon cross-talk with natural killer cells and might improve the NK cell function of immunocompromised patients. *Stem Cell Res Ther* 7(1):88
- de Bakker E, Van Ryssen B, De Schauwer C, Meyer E (2013) Canine mesenchymal stem cells: state of the art, perspectives as therapy for dogs and as a model for man. *Vet Q* 33(4):225–233
- de Moraes CN, Maia L, de Oliveira E, de Paula Freitas Dell'Aqua C, Chapwanya A, da Cruz Landim-Alvarenga F, Oba E (2017) Shotgun proteomic analysis of the secretome of bovine endometrial mesenchymal progenitor/stem cells challenged or not with bacterial lipopolysaccharide. *Vet Immunol Immunopathol* 187:42–47
- De Schauwer C, Meyer E, Van de Walle GR, Van Soom A (2011) Markers of stemness in equine mesenchymal stem cells: a plea for uniformity. *Theriogenology* 8(5):1431–1443
- De Schauwer C, Van de Walle GR, Soom AV, Meyer E (2013) Mesenchymal stem cell therapy in horses: useful beyond orthopedic injuries? *Vet Q* 33(4):234–241
- De Silva NS, Klein U (2015) Dynamics of B cells in germinal centres. *Nat Rev Immunol* 15(3):137–148
- DelaRosa O, Lombardo E (2010) Modulation of adult mesenchymal stem cells activity by toll-like receptors: implications on therapeutic potential. *Mediat Inflamm* 2010:865601
- Deuse T, Stubbendorff M, Tang-Quan K, Phillips N, Kay MA, Eiermann T (2011) Immunogenicity and immunomodulatory properties of umbilical cord lining mesenchymal stem cells. *Cell Transplant* 20(5):655–667
- Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM (2002) Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 99:3838–3843
- El-Menoufy H, Aly LA, Aziz MT, Atta HM, Roshdy NK, Rashed LA, Sabry D (2010) The role of bone marrow-derived mesenchymal stem cells in treating formocresol induced oral ulcers in dogs. *J Oral Pathol Med* 39:281–289
- English K, Ryan JM, Tobin L, Murphy MJ, Barry FP, Mahon BP (2009) Cell contact, prostaglandin E(2) and transforming growth factor β 1 play nonredundant roles in human mesenchymal stem cell induction of CD4+CD25highforkhead box P3+ regulatory T cells. *Clin Exp Immunol* 156:149–160
- Fortier LA, Potter HG, Rickey EJ et al (2010) Concentrated bone marrow aspirate improves full-thickness cartilage repair compared with microfracture in the quine model. *J Bone Joint Surg* 92:1927–1937
- Gale AL, Linardi RL, McClung G, Mammone RM, Ortved KF (2019) Comparison of the Chondrogenic differentiation potential of equine synovial membrane-derived and bone marrow-derived Mesenchymal stem cells. *Front Vet Sci* 6:178
- Gao F, Chiu SM, Motan DAL, Zhang Z, Chen L, Ji H-L, Tse H-F, Fu Q-L, Lian Q (2016) Mesenchymal stem cells and immunomodulation: current status and future prospects. *Cell Death Dis* 7:e2062

- Gao WX, Sun YQ, Shi J et al (2017) Effects of mesenchymal stem cells from human induced pluripotent stem cells on differentiation, maturation, and function of dendritic cells. *Stem Cell Res Ther* 8(1):48
- Götherström C, Lundqvist A, Duprez IR, Childs R, Berg L, le Blanc K (2011) Fetal and adult multipotent mesenchymal stromal cells are killed by different pathways. *Cytherapy* 13(3):269–278
- Groh ME, Maitra B, Szekely E, Koc ON (2005) Human mesenchymal stem cells require monocyte-mediated activation to suppress alloreactive T cells. *Exp Hematol* 33:928–934
- Gugjoo MB, Amarpal (2018) Mesenchymal stem cell research in sheep: current status and future prospects. *Small Rumin Res* 169:46–56
- Gugjoo MB, Amarpal, Abdelbaset-Ismail A, Aithal HP, Kinjavdekar P, Pawde AM, Kumar GS, Sharma GT (2017) Mesenchymal stem cells with IGF-1 and TGF- β 1 in laminin gel for osteochondral defects in rabbits. *Biomed Pharmacother* 93:1165–1174
- Gugjoo MB, Amarpal, Chandra V, Wani MY, Dhama K, Sharma GT (2018a) Mesenchymal stem cell research in veterinary medicine. *Curr Stem Cell Res Ther* 13(8):645–657
- Gugjoo MB, Amarpal, Fazili MR, Shah RA, Sharma GT (2018b) Mesenchymal stem cell: basic research and potential applications in cattle and buffalo. *J Cell Physiol* 234(6):1–18
- Gugjoo MB, Amarpal, Makhdoomi DM, Sharma GT (2019) Equine mesenchymal stem cells: properties, sources, characterization and potential therapeutic applications. *J Equine Vet Sci* 72:16–27
- Gugjoo MB, Hussain S, Amarpal SRA, Dhama K (2020a) Mesenchymal stem cell mediated Immuno-modulatory and anti-inflammatory mechanisms in immune and allergic disorders. *Recent Patents Inflamm Allergy Drug Discov* 14(1):3–14. <https://doi.org/10.2174/1872213X14666200130100236>
- Gugjoo MB, Amarpal, Ismail AA, Aithal HP, Kinjavdekar P, Saikumar GS, Sharma GT (2020b) Allogeneic mesenchymal stem cells and growth factors in gel scaffold repair osteochondral defect in rabbit. *Regen Med* 14:1261–1275. <https://doi.org/10.2217/rme-2018-0138>
- Haque N, Kasim NHA, Rahman MT (2015) Optimization of pre-transplantation conditions to enhance the efficacy of mesenchymal stem cells. *Int J Biol Sci* 11:324–334
- Ho Y-T, Shimbo T, Wijaya E, Ouchi Y, Takaki E, Yamamoto R, Kikuchi Y, Kaneda Y, Tamai K (2018) Chromatin accessibility identifies diversity in mesenchymal stem cells from different tissue origins. *Sci Rep* 8:1776
- Iacono E, Pascuccib L, Rossia B et al (2017) Ultrastructural characteristics and immune profile of equine MSCs from fetal adnexa. *Reproduction* 154(4):509–519
- Jacca S, Franceschi V, Agosti M, Cavarani S, Mistretta F, Donofrio G (2014) Interferon gamma-mediated BoHV-4 replication restriction in bovine endometrial stromal cells is host IDO1 gene expression independent and BoHV-4 IE2 gene expression dependent. *Biol Reprod* 91(5):112, 1–112,14
- Jiang XX, Zhang Y, Liu B et al (2005) Human mesenchymal stem cells inhibit differentiation and function of monocyte derived dendritic cells. *Blood* 105(10):4120–4126
- Johnson V, Webb T, Norman A, Coy J, Kurihara J, Regan D, Dow S (2017) Activated Mesenchymal stem cells interact with antibiotics and host innate immune responses to control chronic bacterial infections. *Sci Rep* 7(1):9575
- Joseph A, Baiju I, Bhat IA, Pandey S, Bharti M, Verma M, Pratap Singh A, Ansari MM, Chandra V, Saikumar G, Amarpal, Sharma GT (2020) Mesenchymal stem cell-conditioned media: a novel alternative of stem cell therapy for quality wound healing. *J Cell Physiol* 235(7–8):5555–5569
- Joswig A-J, Mitchell A, Cummings KJ et al (2017) Repeated intra-articular injection of allogeneic mesenchymal stem cells causes an adverse response compared to autologous cells in equine model. *Stem Cell Res Ther* 8:42
- Kafarnik C, McClellan A, Dziasko M, Daniels JT, Guest DJ (2020) Canine corneal stromal cells have multipotent mesenchymal stromal cell properties in vitro. *Stem Cells Dev* 29(7):429–435
- Kang JW, Kang K-S, Koo HC, Park JR, Choi EW, Park YH (2008) Soluble factors-mediated immunomodulatory effects of canine adipose tissue-derived mesenchymal stem cells. *Stem Cells Develop* 17(4):681–693

- Katsuda T, Tsuchiya R, Kosaka N, Yoshioka Y, Takagaki K, Oki K, Takeshita F, Sakai Y, Kuroda M, Ochiya T (2013) Human adipose tissue-derived mesenchymal stem cells secrete functional neprilysin-bound exosomes. *Sci Rep* 3:1197
- Khatri M, O'Brien TD, Sharma JM (2009) Isolation and differentiation of chicken mesenchymal stem cells from bone marrow. *Stem Cells Dev* 18:1485–1492
- Kim HS, Kim KH, Kim SH, Kim YS, Koo KT, Kim TI, Seol Y-J, Ku Y, Rhyu I-C, Chung C-P et al (2010) Immunomodulatory effect of canine periodontal ligament stem cells on allogenic and xenogenic peripheral blood mononuclear cells. *J Periodont Implant Sci* 40:265–270
- Klyushnenkova E, Mosca JD, Zernetkina V, Majumdar MK, Beggs KJ, Simonetti DW, Deans RJ, McIntosh KR (2005) T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. *J Biomed Sci* 12(1):47–57
- Koch TG, Berg LC, Betts DH (2008) Concepts for the clinical use of stem cells in equine medicine. *Canadian Vet J* 49:1009–1017
- Kordelas L, Rebmann V, Ludwig AK, Radtke S, Ruesing J, Doeppner TR, Eppler M, Horn PA, Beelen DW, Giebel B (2014) MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease. *Leukemia* 28:970–973
- Krampera M, Pasini A, Pizzolo G, Cosmi L, Romagnani S, Annunziato F (2006a) Regenerative and immunomodulatory potential of mesenchymal stem cells. *Curr Opin Pharmacol* 6(4):435–441
- Krampera M, Cosmi L, Angeli R et al (2006b) Role for interferon gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. *Stem Cells* 24(2):386–398
- Kresic N, Simic I, Lojic I, Bedekovic T (2017) Canine adipose derived mesenchymal stem cells transcriptome composition alterations: a step towards standardizing therapeutic. *Stem Cells Int* 2017:12. Art ID:4176292
- Lara E, Velásquez A, Cabezas J, Rivera N, Pacha P, Rodríguez-Alvarez L, Saravia F, Castro FO (2017) Endometritis and in vitro PGE2 challenge modify properties of cattle endometrial Mesenchymal stem cells and their Transcriptomic profile. *Stem Cells Int* 2017:4297639
- Le Blanc K, Mougiakakos D (2012) Multipotent mesenchymal stromal cells and the innate immune system. *Nat Rev Immunol* 12(5):383–396
- Le Blanc K, Ringden O (2007) Immunomodulation by mesenchymal stem cells and clinical experience. *J Intern Med* 262(5):509–525
- Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringden O (2003) HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 31:890–896
- Lee WS, Suzuki Y, Graves SS, Iwata M, Venkataraman GM, Mielcarek M, Peterson LJ, Ikehara S, Torok-Storb B, Storb R (2011) Canine bone-marrow-derived mesenchymal stromal cells suppress alloreactive lymphocyte proliferation in vitro but fail to enhance engraftment in canine bone marrow transplantation. *Biol Blood Marrow Transplant* 17:465–475
- Lepage SIM, Lee OJ, Koch TG (2019) Equine cord blood Mesenchymal stromal cells have greater differentiation and similar immunosuppressive potential to cord tissue Mesenchymal stromal cells. *Stem Cells Dev* 28(3):227–237
- Liu J, Lu XF, Wan L, Li YP, Li SF, Zeng LY, Zeng YZ, Cheng LH, Lu YR, Cheng JQ (2004) Suppression of human peripheral blood lymphocyte proliferation by immortalized mesenchymal stem cells derived from bone marrow of Banna minipig inbred line. *Transplant Proc* 36:3272–3275
- Liu H, Kemeny DM, Heng BC, Ouyang HW, Melendez AJ, Cao T (2006) The immunogenicity and immunomodulatory function of osteogenic cells differentiated from mesenchymal stem cells. *J Immunol* 176:2864–2871
- Liu W, Zhang S, Gu S, Sang L, Dai C (2015) Mesenchymal stem cells recruit macrophages to alleviate experimental colitis through TGF β 1. *Cell Physiol Biochem* 35(3):858–865
- Longhini ALF, Salazar TE, Vieira C, Trinh T, Duan Y, Pay LM, Li Calzi S, Losh M, Johnston NA, Xie H, Kim M, Hunt RJ, Yoder MC, Santoro D, McCarrel TM, Grant MB (2019) Peripheral blood-derived mesenchymal stem cells demonstrate immunomodulatory potential for therapeutic use in horses. *PLoS One* 14(3):e0212642

- McDonald CA, Oehme D, Pham Y, Kelly K, Itescu S, Gibbon A, Jenkin R (2015) Evaluation of the safety and tolerability of a high-dose intravenous infusion of allogeneic mesenchymal precursor cells. *Cytotherapy* 17(9):1178–1187
- Merlo B, Teti G, Lanci A, Burk J, Mazzotti E, Falconi M, Iacono E (2019) Comparison between adult and foetal adnexa derived equine post-natal mesenchymal stem cells. *BMC Vet Res* 15:277
- Michelo CM, Fasse E, van Cranenbroek B et al (2016) Added effects of dexamethasone and mesenchymal stem cells on early natural killer cell activation. *Transpl Immunol* 37:1–9
- Moreno R, Martinez-Gonzalez I, Rosal M, Farwati A, Gratacos E, Aran JM (2010) Characterization of mesenchymal stem cells isolated from the rabbit fetal liver. *Stem Cells Dev* 19:1579–1588
- Moretta A (2002) Natural killer cells and dendritic cells: rendezvous in abused tissues. *Nat Rev Immunol* 2(12):957–965
- Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 8(12):958–969
- Mrugala D, Bony C, Neves N, Caillot L, Fabre S, Moukoko D, Jorgensen C, Noel D (2008) Phenotypic and functional characterisation of ovine mesenchymal stem cells: application to a cartilage defect model. *Ann Rheum Dis* 67:288–295
- Mumaw JL, Schmiedt CW, Breidling S, Sigmund A, Norton NA, Thoreson M, Peroni JF, Hurley DJ (2015) Feline mesenchymal stem cells and supernatant inhibit reactive oxygen species production in cultured feline neutrophils. *Res Vet Sci* 103:60–69
- Nauta AJ, Westerhuis G, Kruisselbrink AB, Lurvink EG, Willemze R, Fibbe WE (2006) Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a non-myeloablative setting. *Blood* 108:2114–2120
- O'Connor BP, Vogel LA, Zhang W, Loo W, Shnider D, Lind FE, Ratliff M, Noelle RJ, Erickson LD (2006) Imprinting the fate of antigen-reactive B cells through the affinity of the B cell receptor. *J Immunol* 177(11):7723–7732
- Owens SD, Kol A, Walker NJ, Borjesson DL (2016) Allogeneic Mesenchymal stem cell treatment induces specific alloantibodies in horses. *Stem Cells Int* 2016:830103
- Park SS, Lee YJ, Lee SH, Lee D, Choi K, Kim WH, Kweon OK, Han HJ (2012) Functional recovery after spinal cord injury in dogs treated with a combination of Matrigel and neural-induced adipose-derived mesenchymal stem cells. *Cytotherapy* 14:584–597
- Parys M, Kruger JM, Yuzbasiyan-Gurkan V (2017) Evaluation of immunomodulatory properties of feline mesenchymal stem cells. *Stem Cells Dev* 26(10):776–785
- Peroni JF, Borjesson DL (2011) Anti-inflammatory and immunomodulatory activities of stem cells. *Vet Clin North Am Equine Pract* 27:351–362
- Perrini C, Strillacci MG, Bagnato A et al (2016) Microvesicles secreted from equine amniotic-derived cells and their potential role in reducing inflammation in endometrial cells in an in-vitro model. *Stem Cell Res Ther* 7:169
- Pezzanite LM, Fortier LA, Antczak DF, Cassano JM, Brosnahan MM, Miller D et al (2015) Equine allogeneic bone marrow-derived mesenchymal stromal cells elicit antibody responses in vivo. *Stem Cell Res Ther* 6:54
- Phinney DG, Pittenger MF (2017) Concise review: MSC-derived Exosomes for cell-free therapy. *Stem Cells* 35(4):851–858
- Poncelet AJ, Vercruysse J, Saliez A, Gianello P (2007) Although pig allogeneic mesenchymal stem cells are not immunogenic in vitro, intracardiac injection elicits an immune response in vivo. *Transplantation* 83:783–790
- Prockop DJ, Oh JY (2012) Mesenchymal stem/stromal cells (MSCs): role as guardians of inflammation. *Mol Ther* 20:14–20
- Rashedi I, Gómez-Aristizábal A, Wang XH, Viswanathan S, Keating A (2017) TLR3 or TLR4 activation enhances mesenchymal stromal cell-mediated Treg induction via notch signaling. *Stem Cells* 35(1):265–275
- Rehman J, Traktuev D, Li J, Merfeld-Clauss S et al (2004) Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* 109:1292–1298

- Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao ZR, Shi Y (2008) Mesenchymal stem cell mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2(2):141–150
- Ren G, Zhao X, Zhang L, Zhang J, L'Huillier A, Ling W, Roberts AI, Le AD, Shi S, Shao C, Shi Y (2010) Inflammatory cytokine-induced intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in mesenchymal stem cells are critical for immunosuppression. *J Immunol* 184(5):2321–2328
- Renner P, Eggenhofer E, Rosenauer A, Popp FC, Steinmann JF, Slowik P, Geissler EK, Piso P, Schlitt HJ, Dahlke MH (2009) Mesenchymal stem cells require a sufficient, ongoing immune response to exert their immunosuppressive function. *Transplant Proc* 41(6):2607–2611
- Russell KA, Chow NHC, Dukoff D, Gibson TWG, LaMarre J, Betts DH, Koch TG (2016) Characterization and Immunomodulatory effects of canine adipose tissue- and bone marrow-derived Mesenchymal stromal cells. *PLoS One* 11(12):e0167442
- Saulnier N, Loriau J, Febre M, Robert C, Rakic R, Bonte T, Buff S, Maddens S (2016) Canine placenta: a promising potential source of highly proliferative and immunomodulatory mesenchymal stromal cells? *Vet Immunol Immunopathol* 171:47–55
- Schnabel LV, Fortier LA, McIlwraith CW, Nobert KM (2013) Therapeutic use of stem cells in horses: which type, how, and when? *Vet J* 197:570–577
- Selleri S, Bifsha P, Civini S et al (2016) Human mesenchymal stromal cell-secreted lactate induces M2-macrophage differentiation by metabolic reprogramming. *Oncotarget* 7(21):30193–30210
- Somal A, Bhat IA, Indu B, Pandey S, BSK P, Bharti MK et al (2016) Comparative immunomodulatory analysis of caprine fetal adnexa derived mesenchymal stem cells. *Am J Reprod Immunol* 76(S1):1–65
- Sotiropoulou PA, Perez SA, Gritzapis AD, Baxeavanis CN, Papamichail M (2006) Interactions between human mesenchymal stem cells and natural killer cells. *Stem Cells* 24(1):74–85
- Spees JL, Lee RH, Gregory CA (2016) Mechanisms of mesenchymal stem/stromal cell function. *Stem Cell Res Ther* 7:125. <https://doi.org/10.1186/s13287-016-0363-7>
- Spencer ND, Chun R, Vidal MA, Gimble JM, Lopez MJ (2012) In vitro expansion and differentiation of fresh and revitalized adult canine bone marrow-derived and adipose tissue-derived stromal cells. *Vet J* 191(2):231–239
- Taguchi T, Borjesson DL, Osmond C, Griffon DJ (2019) Influence of Donor's age on Immunomodulatory properties of canine adipose tissue-derived Mesenchymal stem cells. *Stem Cells Dev* 28(23):1562–1571
- Tan SS, Yin Y, Lee T, Lai RC, Yeo RW, Zhang B, Choo A, Lim SK (2013) Therapeutic MSC exosomes are derived from lipid raft microdomains in the plasma membrane. *J Extracell Vesicles* 2:22614. <https://doi.org/10.3402/jev.v2i0.22614>
- Tomchuck SL, Zvezdaryk KJ, Coffelt SB, Waterman RS, Danka ES, Scandurro AB (2008) Toll-like receptors on human mesenchymal stem cells drive their migration and immunomodulating responses. *Stem Cells* 26:99–107
- Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC (2003) Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation* 75:389–397
- Tso GH, Law HK, Tu W, Chan GC, Lau YL (2010) Phagocytosis of apoptotic cells modulates mesenchymal stem cells osteogenic differentiation to enhance IL-17 and RANKL expression on CD4+ T cells. *Stem Cells* 28:939–954
- Wheat WH, Chow L, Kurihara JN, Regan DP, Coy JW, Webb TL, Dow SW (2017) Suppression of canine dendritic cell activation/maturation and inflammatory cytokine release by Mesenchymal stem cells occurs via multiple distinct biochemical pathways. *Stem Cells Development* 26(4):249–262
- Wu Y, Chen L, Scott PG, Tredget EE (2007) Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells* 25:2648–2659
- Xu LL, Fu HX, Zhang JM et al (2017) Impaired function of bone marrow mesenchymal stem cells from immune thrombocytopenia patients in inducing regulatory dendritic cell differentiation through the Notch-1/Jagged-1 signaling pathway. *Stem Cells Dev* 26(22):1648–1661

- Yamane H, Paul WE (2013) Early signaling events that underlie fate decisions of naive CD4+ T cells toward distinct T-helper cell subsets. *Immunol Rev* 252(1):12–23
- Yañez R, Oviedo A, Aldea M, Bueren JA, Lamana ML (2010) Prostaglandin E2 plays a key role in the immunosuppressive properties of adipose and bone marrow tissue derived mesenchymal stromal cells. *Exp Cell Res* 316(19):3109–3123
- Yang H-M, Song W-J, Qiang L, Kim S-Y, Kim H-J, Ryu M-O, Ahn J-O, Youn H-Y (2018) Canine mesenchymal stem cells treated with TNF- α and IFN- γ enhance anti-inflammatory effects through the COX-2/PGE2 pathway. *Res Vet Sci* 119:19–26
- Yu B, Zhang X, Li X (2014) Exosomes derived from mesenchymal stem cells. *Int J Mol Sci* 15:4142–4157
- Zajic LB, Webb TL, Webb P, Coy JW, Dow SW, Quimby JM (2016) Comparison of proliferative and immune-modulatory potential of adipose-derived mesenchymal stem cells from young and geriatric cats. *J Feline Med Surg*. <https://doi.org/10.1177/1098612X16680703>
- Zhang QZ, Su WR, Shi SH et al (2010) Human gingiva derived mesenchymal stem cells elicit polarization of m2 macrophages and enhance cutaneous wound healing. *Stem Cells* 28(10):1856–1868
- Zheng L (2018) Epigenetic regulation on stem cell fate and regeneration. *Curr Stem Cell Res Ther* 13(1):3
- Zorzi AR, Amstalden EMI, Plepis AMG, Martins VCA, Ferretti M, Antonioli E, Duarte ASS, Luzo ACM, Miranda JB (2015) Effect of human adipose tissue Mesenchymal stem cells on the regeneration of ovine articular cartilage. *Int J Mol Sci* 16:26813–26831



Mesenchymal Stem Cell Differentiation Properties and Available Microenvironment

5

M. B. Gugjoo and Amar Pal

Abstract

Mesenchymal stem cells (MSCs) have characteristic properties, the expression of which is being determined by the available microenvironment/niche. Extensive ex vivo studies have been conducted, aimed at understanding the effects of various niche based signals on MSCs. Numerous microenvironment cues studied include the cell, chemical and mechanical, and of the topography. The available conducted studies have mostly focused on limited and isolated cues. An ex vivo system is less extensive with more controllability. In contrast, in vivo system exposes them to an uncontrolled environment of numerous simultaneous cues. The ex vivo results of MSCs therefore may not be recapitulated under in vivo environment unless many simultaneous cues aping in vivo environment are studied. The current chapter focuses on the MSCs fate under various cues in ex vivo system.

5.1 Introduction

Mesenchymal stem cells (MSCs) due to their characteristic features are considered to have an all-in-one solution for diverse ailments (Gugjoo and Amarpal 2018; Gugjoo et al. 2019a). The characteristics of MSCs are believed to be controlled by the available microenvironment/or niche. The “microenvironment or niche” comprises cells, various chemical/mechanical and topographical cues. Such an

M. B. Gugjoo (✉)

Division of Veterinary Clinical Complex, FVSc & AH, SKUAST-Kashmir,
Jammu and Kashmir, India

A. Pal

Division of Surgery, Indian Veterinary Research Institute, Izatnagar, UP, India

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2020

M. B. Gugjoo, A. Pal (eds.), *Mesenchymal Stem Cell in Veterinary Sciences*,
https://doi.org/10.1007/978-981-15-6037-8_5

67

environment serves as source of cues that determine the cell migration and homing, local proliferation, secretome, and ultimately their fate towards particular lineage (Place et al. 2009). Understanding the mechanisms involved in MSCs fate based on these signals or cues becomes imperative for their fruitful applications. To confirm proof of principle, in vitro studies are initially being conducted which allows a wide range of permutations and combinations without any kind of involved risk. Such a system, however, has limitations of being less extensive and is more controlled. In vivo system in contrast presents an uncontrolled system subjected to numerous simultaneous cues from various quarters (Hwang et al. 2011).

The scientific field that studies such cell–microenvironment/niche interactions is tissue engineering. The *tissue engineering* (TE) concept has been introduced by Professor Robert Nerem in 1988 and was defined as “*the application of life sciences and engineering to develop a basic understanding of the functional and structural relationships of natural and pathologic mammalian tissues and the development of bio-substitutes that can be utilized to restore, maintain, or improve tissues damaged or lost by various disease conditions*” (Meyer et al. 2009; Lanza et al. 2011). To utilize tissue engineering and stem cells to the service of medicine, William Haseltine in 1999 has coined the term *regenerative medicine* (Atala and Lanza 2001). It is a translational research branch of tissue engineering and molecular biology and is aimed to develop technologies/therapeutics that can remove/provide relief in sufferings of diseased/malfunctional/non-functional tissues/organs (Gregor et al. 2017; Gugjoo et al. 2019a).

TE employs various ingredients like cells, signaling factors, and the bio-fabricated scaffold to develop the tissue graft (Gugjoo et al. 2018). In vivo implantation exposes cells to the unfavorable environment. To prevent such adverse effects and keep the cells viable and effective for a prolonged period, the biocompatible scaffold is being incorporated. To promote the cell specific expression various signaling factors and scaffolds play an important role. The role played by these critical factors in relation to the MSCs characteristics is of utmost importance for latter’s effective clinical applications. The current chapter provides an overview of the ex vivo effects of such cues on the MSCs activities including their differentiation (Fig. 5.1).

5.2 Effect of In Vitro Culture Environment on MSCs Viability and Activity

MSCs availability in donor tissues is very limited and as such their culture expansion becomes imperative. These cells are cultured in a specific medium being renewed after certain intervals. An extensive culturing exhausts the culture ingredients and needs to be replenished. Accumulation of higher lactate and ammonia concentration affects goat BM-MSCs growth and proliferation under in vitro conditions. To prevent the adverse effects of these accumulated noxious ingredients, the culture medium is being replaced after some intervals. Prolonged proliferation of goat MSCs had been maintained by replacing about 30% of medium after every 3 days.

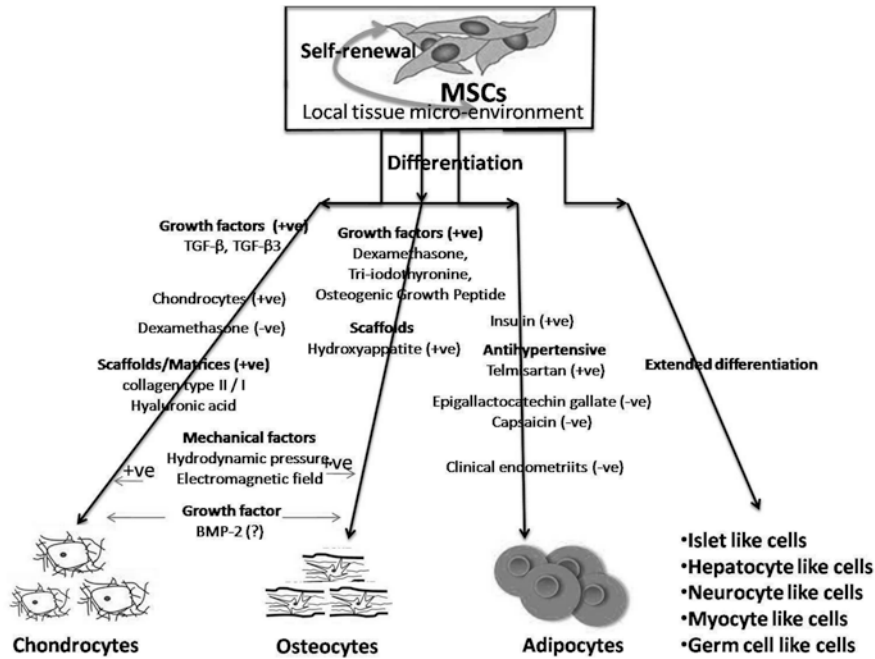


Fig. 5.1 Pictorial representation of role of different in vitro cues in determining MSCs fate

This helps to dilute noxious metabolites (ammonia), provides energy metabolites, and increases surface area to the cells (Schop et al. 2008). As such it becomes necessary to offer safe in vivo heaven to the implanted cells.

To aid MSCs proliferation autologous cellular therapy products like stromal vascular fraction (SVF), bone marrow (BM)- and cord blood (CB)-mononuclear cells (MNCs), and platelet lysate (PL) have been added to the culture systems. These products have promoted equine MSCs (eMSCs) proliferation, migration, and interleukin secretion, although variably. The proliferation and chemotaxis of eMSCs had been demonstrated to be promoted by all these products. The effect of stromal vascular fraction (SVF) had been more pronounced. Equine BM-MNCs in the presence of SVF had more potential of chemo-invasion. Equine BM-MSCs (eBM-MSCs) under the influence of CB-MNCs had secreted interleukin 6 (IL-6), transforming growth factor beta 1 (TGF- β 1) and prostaglandin E2 (PGE₂) while such effects were not demonstrated with SVF and PL (Kol et al. 2012). These findings may be cited as the prime reasons to supplement MSCs with autologous therapy products for in vivo applications. It is worth mentioning here that MSCs cultured under ex vivo system may undergo significant alterations in their phenotype and plasticity (Colosimo et al. 2013) and result in undesirable effects. Due to the associated risk factors, innovative MSCs have been put under the list of “Advanced Therapy Medicinal Products (ATMPs)” by European Medicines Agency (Gugjoo et al. 2019a).

MSCs may release various secretory factors and differentiate or express genes of particular lineages based on the availability of microenvironment/niche cues. The

paracrine secretion of MSCs especially in relation to the immune-modulation and/or anti-inflammatory properties has been evaluated in a separate chapter. This chapter focuses on the MSCs in vitro differentiation potential with respect to the available environment and is summarized in Fig. 5.1. Animal MSCs have been studied in detail for their tri-lineage differentiation with respect to the growth factors, scaffolds, tissue specific cells, mechanical cues, and other miscellaneous factors. The extended differentiation potential of animal MSCs has also been evaluated, although not extensively. The mechanisms involved in such trans-differentiation of MSCs are not fully understood as the cells from different sources undergo variable differentiation pathways towards particular cell lineage.

5.3 Tri-Lineage Differentiation of MSCs

5.3.1 Chondrogenesis of MSCs

MSCs chondrogenesis has generally been studied under pellet or micromass system, although 3D culture system is now increasingly being studied. MSCs in pellet culture system had undergone spontaneous chondrogenesis without the addition of any chondrogenic factor. At the center of bovine BM-MSCs pellet, cartilage specific matrix (collagen type II) had accumulated with its progressive increase as culturing continued (Bosnakovski et al. 2004). To achieve desired and specific cartilaginous matrix formation, chondrogenic factors like growth factors, scaffolds, cells, or mechanical factors are being evaluated (Guggoo et al. 2019b, c, d). There are various types of commercially available chondrogenic differentiation media that contains basic ingredients required for chondrogenesis.

5.3.1.1 Growth Factors and MSCs Chondrogenesis

Culture addition of growth factors like TGF- β 1, bone morphogenetic protein 2 (BMP-2), and BMP-7 promote MSCs chondrogenesis (Bosnakovski et al. 2005; Park et al. 2005; Jäger et al. 2006; Knippenberg et al. 2006; Zeiter et al. 2009; Kalaszczynska et al. 2013; Bottagisio et al. 2015; Hu et al. 2015a, b; Goldman and Barabino 2016; Desancé et al. 2018). Cattle BM-MSCs (cBM-MSCs) had expressed chondrogenic genes upon culture exposure to transforming growth factor β 1 (TGF- β 1) (10 ng/mL) (Zeiter et al. 2009) or TGF- β 3 (1, 10, and 100 ng/mL) (Goldman and Barabino 2016) after 14 days. TGF- β had increased aggrecan expression but the collagen II expression had remained lower than the non-treated cells (Park et al. 2005). Bone morphogenetic protein 2 (BMP-2) has variably supported MSCs chondrogenesis. BMP-2 had promoted chondro-specific MSCs expression when loaded into the alginate beads. Its concentration, however, provided a ceiling effect on MSCs chondrogenic potential. At 50 ng/mL BMP-2 concentration a plateau phase of MSCs chondrogenesis was developed (Park et al. 2005). Contrarily, one of the studies had demonstrated that BMP-2 (50 ng/mL) had no effect on MSCs (Zeiter et al. 2009), while other studies had shown that culture addition of BMP-2 (1, 10, and 100 ng/mL) yielded osteogenic lineage rather than the chondrogenic

(Knippenberg et al. 2006; Zhang and Jiang 2006; Goldman and Barabino 2016). This shows that combined utilization of growth factor (BMP-2) and scaffold (alginate beads) may promote MSCs chondrogenesis, while BMP-2 alone may favor their osteogenesis, though further studies are desired. The role of BMP-7 in MSCs differentiation is again controversial. BMP-7 may promote chondrogenic (Knippenberg et al. 2006), osteogenic (Elkhenany et al. 2016) differentiation of goat adipose tissue derived MSCs (gAD-MSCs) and adipogenic differentiation of human BM-MSCs (Katja et al. 2007). gAD-MSCs short (15 min) incubation under BMP-7 (10 ng/mL) had been sufficient to induce their chondrogenic differentiation while long term incubation had no additional effect on such differentiation (Knippenberg et al. 2006). Thus, MSCs may or may not undergo chondrogenesis under the influence of these above mentioned humoral factors. It is worth mentioning here that biologic activities of these recombinant growth factors are much lower than their analogues being synthesized endogenously and as such ectopic incorporation of these factors may be required in higher concentrations under in vivo conditions (Wang et al. 2010; Gugjoo et al. 2017; Gugjoo et al. 2020).

5.3.1.2 Scaffolds and MSCs Chondrogenesis

MSCs supplementation with specific matrices predisposes them towards specific lineage. Equine and bovine MSCs chondrogenic potential enhances in the presence of natural and synthetic joint and cartilage ingredients like synovial fluid, collagen type II, I, hyaluronic acid hydrogel, polyethylene glycol hydrogels, alginate hydrogel microcapsules, and chondroitin sulfate (Hegewald et al. 2004; Varghese et al. 2008; Hwang et al. 2011; Toh et al. 2012; Toh et al. 2013; Yin et al. 2016; Gugjoo et al. 2016; Santos et al. 2018). The proteoglycan expression in the presence of these tissue matrices had remained quite lower as compared to that stimulated by TGF- β 1 (Hegewald et al. 2004). However, addition of TGF- β 1 had further promoted such a differentiation potential of bovine MSCs (Bosnakovski et al. 2006). The hypertrophic chondrocyte formation, usually seen under in vivo MSCs studies, had been prevented by the addition of polyethylene glycol (PEG) to the chondroitin sulfate (Varghese et al. 2008). Similarly, incorporation of collagen type I in sheep BM-MSCs 2D-cultures ($5\text{--}10 \times 10^6/\text{cm}^3$ collagen type I) and 3D cultures ($50 \times 10^6/\text{cm}^3$ collagen I) had improved their potential to synthesize hyaline cartilage (Bornes et al. 2016). In dog study, pentosan polysulfate (PPS) in micromass culture had significantly enhanced dog MSCs (dMSCs) chondrogenesis. This was unlike the polysulfated glycosaminoglycans (PSGAG) that had inhibited dMSCs chondrogenesis while promoting their fibrocartilage-like phenotype. In alginate culture, MSCs differentiation potential had remained unaffected with these scaffolds (PPS and PSGAG) (Bwalya et al. 2017). Thus, culture technique in addition to the scaffold influences MSCs chondrogenesis.

The scaffold matrix is composed of intricate design with its nature and composition determining the spatial distribution and secretory action of the cells (Prins et al. 2016). As such impact of scaffold matrix design (comprising fiber alignment and number of layers) on MSCs secretory properties must be evaluated. Cattle bone marrow MSCs (cBM-MSCs) had been grown on a single layer scaffold

(poly(ϵ -caprolactone)) for 3–6 weeks. Cell/scaffold assembly bearing fiber alignment of 0° had better stiffness as compared to that of 90° aligned scaffold fibers. These results were further pronounced in multilayer 0° aligned fiber scaffold/cell assembly. The specific components like collagen concentration and cell infiltration however may remain neutral with respect to scaffold fiber alignment. Collagen deposition of MSCs although unenhanced with scaffold design but had followed the fiber alignment (Fisher et al. 2015). Cross-linking of hydrogel scaffolds like hyaluronic acid and tyramine (HA-Ty) conjugate tends to have a considerable impact on MSCs localization, condensation, matrix biosynthesis, and on their fate (Vickers et al. 2010; Toh et al. 2012). Cross-linking of cartilage specific scaffolds (collagen type II and glycosaminoglycan) that had allowed cell mediated contraction had promoted goat BM-MSCs chondrogenic expression. Further increase in such cross-linking had resulted in decreased cell mediated contraction and thereby inhibited their chondrogenic expression (Vickers et al. 2010).

5.3.1.3 Various Combined Factors and MSCs Chondrogenesis

Mechanical factor like hydraulic pressure may not have any observable effect on cattle MSCs (cMSCs) differentiation (Zeiter et al. 2009). Contrarily hydrodynamic pressure had differentiated them towards musculoskeletal lineage (chondrogenic and/or osteogenic lineages) (Goldman and Barabino 2016). Even the adverse effects of inflammatory mediator (interleukin 1β) on cMSCs chondrogenesis had been prevented by combined action of electromagnetism and transforming growth factor $\beta 3$ (TGF- $\beta 3$) (Ongaro et al. 2012). The shear stress may prone MSCs to undergo hypertrophic pathway under insufficient TGF- $\beta 3$ concentration (Goldman and Barabino 2016). Mechanical compression on hydrogel embedded goat BM-MSCs carrying TGF- $\beta 1$ had upregulated their chondrogenic expression. However, in the absence of TGF- $\beta 1$ mechanical compression had inhibited such a differentiation. It may be inferred that growth factor induced MSCs chondrogenesis may be hastened in the presence of mechanical compression (Terraciano et al. 2007). Chondrogenic differentiation of cattle bone marrow MSCs (cBM-MSCs) through mechanical loading may occur by rapid chromatin condensation that remains coincident with the fibro-chondrogenic marker upregulation. This has been observed with the blockade of histone methyltransferase EZH2, a promoter of chromatin condensation and gene silencing (Heo et al. 2015).

5.3.1.4 Chondrocytes and MSCs Chondrogenesis

Resident chondrocytes too favor MSCs chondrogenic differentiation. Fresh chondrocytes undergo superior chondrogenesis than cultured MSCs. There expression of chondrogenic matrices like aggrecan and collagen II remains high. Chondrocyte secretion of aggrecan and collagen II had been two and 30 fold higher as compared to the alginate loaded MSCs in the presence of BMP-2 (Park et al. 2005). Even agarose gel (4% w/v) laden cattle BM-MSCs (cBM-MSCs) had secreted mechanically inferior cartilage matrix as compared to that of chondrocytes. cBM-MSCs laden hydrogel constructs had secreted matrices like glycosaminoglycan (GAG) and its equilibrium modulus had attained plateau with the passage of time, inferring

that delayed differentiation may not be the reason behind their diminished chondrogenic capacity (Mauck et al. 2006). Despite these properties of chondrocytes, their culture expansion deleteriously affects their chondrogenic potential. Culture expanded chondrocytes had secreted mechanically inferior (more Col I and less Col II) matrices that lacked integration with native tissue. Contrarily, cBM-MSCs had secreted cartilage specific matrices of superior mechanical properties (Rackwitz et al. 2014). MSCs chondrogenic potential may be further promoted in the presence of chondrocytes. Co-culture of cBM-MSCs and fibrochondrocytes had favored latter's chondrogenic expression. The cBM-MSCs:fibrochondrocytes ratio in co-culture, however, may affect MSCs differentiation. In monoculture a significant GAG content was produced in a cell ratio of 100:0, while highest secretory activity had been demonstrated at equal cellular concentrations (50:50). Such a combined co-culture chondrogenesis may also prevent hypertrophic chondrocyte formation (type X collagen) (McCorry et al. 2016). Similarly, infrapatellar fat pad derived gMSCs in the presence of chondrocytes had shown improved secretion of cartilage specific matrices (Arora et al. 2017). In vitro mono and co-cultures too had supported these results (McCorry and Bonassar 2017). It may be inferred that MSCs chondrogenesis improves in the presence of chondrocytes and as such results may be more encouraging in less damaged cartilage than extensively damaging one. To enhance MSCs in vitro chondrogenic matrices synthesis, co-culture may be an effective option (McCorry and Bonassar 2017).

5.3.1.5 Hypoxia and MSCs Chondrogenesis

Sustained hypoxia tends to enhance MSCs chondrogenesis (Zscharnack et al. 2009; Bornes et al. 2015). cBM-MSCs laden in collagen/hyaluronic acid scaffold incubated under hypoxic conditions had expressed significantly superior chondrogenic matrices in comparison to those cultured under normoxia. The enhanced expression of collagen X in the presence of hypoxia however needs to be checked (Bornes et al. 2015). Hypoxia may enhance sheep BM-MSCs population doubling (Bornes et al. 2015) but might induce sheep MSCs apoptosis (Zhao et al. 2014). Such an adverse effect may be reduced by culture incorporation of proto-oncogenic serine/threonine kinase, Pim-1 (Zhao et al. 2014). Acid ceramidase incorporation to the culture medium had led to the two fold increase in equine and feline MSCs growth. On addition to the chondrogenic media, the chondrogenic potential of these cells had increased and was synergistic with TGF- β 1 (Simonaro et al. 2013).

5.3.1.6 Miscellaneous Factors Affecting MSCs Chondrogenesis

Growth factor- and scaffold-induced MSCs chondrogenesis may also be affected by the donor age and source location. cBM-MSCs harvested from different age donors had variable chondrogenic potential. MSCs from immature donors had greater chondrogenic expression in the presence of TGF- β 3 and hyaluronic acid hydrogels as compared to the mature MSCs (Erickson et al. 2011). MSCs from the same tissue source but at different locations of an individual may or may not have comparable in vitro chondrogenic potential. Intra-articular fat pad derived MSCs had been demonstrated to exhibit superior chondrogenic expression potential in comparison to the

non-articular AD-MSCs (Stewart 2011). Synovial fluid and synovial membrane MSCs of osteoarthritic and osteochondritis dissecans horses and healthy tissues tend to show comparable phenotypic and multipotency potentials. However, the chondrogenic differentiation potential of MSCs from healthy joints had been better (Fulber et al. 2016). Breed wise variation also ensues as chondrogenic differentiation potential of BM-MSCs from Labrador retriever and Hovawart dog breed had been superior to that from Border collie and German shepherd breeds (Bertolo et al. 2015). Thus, allogeneic sources may show variable results.

In general, MSCs chondrogenesis may be promoted through Sox9 expression. Pyruvate dehydrogenase kinase isoform 2 (PDK2) is possibly upregulating Sox9 gene from upregulated Sox6 (Wang et al. 2017). Enhanced Sox9 expression tends to repress chondrocyte hypertrophy possibly through inhibition of Runx2 (Zhou et al. 2006), Wnt (Topol et al. 2009), Col10a1, and VEGFA (Hattori et al. 2010; Leung et al. 2011) pathways.

5.3.2 Osteogenesis of MSCs

MSCs osteogenic lineage differentiation may occur in the presence of relevant cues eliciting from available environment. In vitro MSCs osteogenic studies have utilized commercially available medium (containing growth factors). However, various other factors, either alone or in combination, including matrices or growth factors or cells, in addition to mechanical forces also direct such differentiation. It may be emphasized that β -glycerolphosphate appears to be unsuitable phosphate ion source for sheep adipose tissue MSCs (sAD-MSCs) and sBM-MSCs. These cells had failed to show alkaline phosphatase activity (Kalaszczynska et al. 2013). Human MSCs although do show such activity and thus, MSCs mineralization status may sometimes be misleading.

5.3.2.1 Growth Factors and MSCs Osteogenesis

Growth factors like dexamethasone, ascorbic-acid-2-phosphate, monosodium phosphate, β -glycerolphosphate, 1,25-dihydroxyvitamin-D3 (1,25(OH)₂), basic fibroblast growth factor (bFGF), BMP-2, BMP-7 and osteogenic growth peptide (OGP) favor MSCs osteogenesis (Knippenberg et al. 2006; Jäger et al. 2006; Zhang and Jiang 2006; Tjabringa et al. 2008; Zhu et al. 2008; Huang et al. 2013; Kalaszczynska et al. 2013; Randau et al. 2013; Bottagisio et al. 2015; Hu et al. 2015b; Elkhenany et al. 2016). As mentioned above the growth factors like BMP-2 and BMP-7 may have variable effects on MSCs differentiation especially in relation to source. Goat BM-MSCs (gBM-MSCs) osteogenesis may potentially be mediated through p38 mitogen-activated protein kinase (MAPK) pathway and in the goat AD-MSCs p44/42 MAPK pathway might be involved (Elkhenany et al. 2016). OGP may favor cBM-MSCs osteogenic differentiation through endogenous nitric oxide synthases (eNOS) (Zhu et al. 2008). gAD-MSCs osteogenesis under the influence of 1,25-dihydroxyvitamin-D3 may be mediated through polyamine metabolism (Tjabringa et al. 2008). Thus, specific growth factors may promote MSCs osteogenesis and the involved mechanism may vary.

5.3.2.2 Scaffold and MSCs Osteogenesis

Osteogenic differentiation potential of goat, sheep and dog BM-MSCs and horse AD-MSCs had been demonstrated to enhance in the presence of various scaffolds like chitosan, chitosan and poly ϵ -caprolactone, demineralized bone matrix, hydroxyapatite and hyaluronic acid, methacrylate-endcapped caprolactone, nano-bioactive glass ceramic coated PLLA, oxidized graphene and porous tantalum, silica-coated bioactive ceramic, starch and poly-caprolactone, zinc silicate mineral (Fischer et al. 2004; Nair et al. 2009a, b; Ivirico et al. 2009; García Cruz et al. 2010; Hwang et al. 2011; Pereira-Junior et al. 2013; Rodrigues et al. 2014; Elkhenany et al. 2015; Lee et al. 2015a, b; Fang et al. 2017; Bwalya et al. 2017; Das et al. 2017; Jo et al. 2017; Mahdavia et al. 2017; Trindade et al. 2017; Bageshlooyafshar et al. 2019; Wei et al. 2019).

sBM-MSCs had differentiated into osteoblasts and chondrocytes when placed on porous calcium polyphosphate along with tri-iodothyronine (T3). The assembly had led to the formation of cartilaginous structure at the top and osteogenic tissue at bottom near osteo-conductive calcium polyphosphate (Lee et al. 2015a, b). The cells may remain aligned or scattered depending upon the scaffold type. gMSCs cultured on tricalcium phosphate and hydroxyapatite had undergone osteogenic differentiation; however, the cellular interconnection and alignment were maintained in former scaffold and scattered in the latter (Prins et al. 2016). Scaffold or implant biodegradability needs special emphasis as degraded products tend to alter local pH and thereby MSCs viability (Liu 2011; Johnson et al. 2012). Completely demineralized bone matrix may act as more favorable osteogenic agent for MSCs as compared to partial or intact bone matrix (Jo et al. 2017). Comparing hydroxyapatite and silica-coated bioactive ceramic scaffolds gBM-MSCs osteogenic potential was more enhanced under the latter scaffold (Nair et al. 2009a, b). Scaffold matrices like bovine teeth hydroxyapatite had been demonstrated to be biocompatible to cBM-MSCs with the ability to improve MSCs osteogenesis. The scaffold concentration, however, had affected the cell viability being better at 10% than at 50% and 100% (Setiawatie et al. 2017). Even cross-linked natural biological scaffolds like small intestine submucosa and goat acellular lung scaffold had favored gBM-MSCs osteogenesis (Li et al. 2006; Gupta et al. 2017).

Scaffold synthesis technique and composition have direct effect on scaffold design that in turn affects MSCs growth and differentiation. Freeze gelation and particle leaching out prepared chitosan had supported gBM-MSCs osteogenic differentiation (García Cruz et al. 2010). Scaffold hydrophilicity too affects MSCs differentiation. Higher hydration content (50%) had decreased osteogenic activity of gBM-MSCs as depicted by their compromised alkaline phosphatase activity (Ivirico et al. 2009). Dog BM-MSCs (dBM-MSCs) on nanomaterial based thin film scaffolds of carbon nanotubes (CNT), chitosan, and poly ϵ -caprolactone had shown slow proliferation but without decrease in overall number. Although initially cells had shown slow proliferation on CNT but that had improved as the time progressed. This depicted adaptation of dBM-MSCs (Das et al. 2017).

5.3.2.3 Miscellaneous Factors and MSCs Osteogenesis

Mechanical factors may have direct effect upon MSCs differentiation (Versari et al. 2007). Pulsatile fluid flow and extracorporeal shock wave therapy had favored gMSCs osteogenic differentiation (Knippenberg et al. 2005; Tan et al. 2017). This might probably have been through adenosine release regulation and A2B receptor activation (Tan et al. 2017). Thus, the cells may show better clinical response in the case of a specific mechanical force.

Some of the ailments and aging may also affect MSCs osteogenic potential. BM-MSCs in an osteoporotic goat had shown restricted osteogenesis and had been compensated with the addition of beta tricalcium phosphate (β -TCP) (Cao et al. 2012). Platelet plasma although promotes gBM-MSCs proliferation but may inhibit their osteogenesis (Cheng et al. 2007). An age related reduction in the proliferation (40% reduction) and osteogenic potential of dBM-MSCs had been demonstrated (Volk et al. 2012).

5.3.3 Adipogenesis of MSCs

Adipogenesis is achieved with commercial adipogenic differentiation media preparations. The culture ingredients that favor MSCs adipogenesis include indomethacin, insulin, 3-isobutyl-1-methylxanthine, dexamethasone (Jäger et al. 2006; Martinello et al. 2011; Oh et al. 2011; Kalaszczynska et al. 2013; Bottagisio et al. 2015; Hu et al. 2015a, b). One of the studies had been unsuccessful to obtain optimal cBM-MSCs adipogenesis but could be achieved with the addition of insulin (Lee et al. 2015a, b). These differentiated cells express peroxisome proliferator-activated receptor gamma (PPAR- γ , preadipocytic commitment) and lipoprotein lipase (LPL) genes in the early adipogenic differentiation stage (Jiang et al. 2002; Martinello et al. 2011; Oh et al. 2011).

Various conventional chemicals/drugs have been demonstrated to affect MSCs adipogenesis. Among notable ones are telmisartan (antihypertensive drug), capsaicin, and epigallocatechin gallate. The former drug had promoted cBM-MSCs (Ramirez-Espinosa et al. 2015), while latter two components had inhibited such a differentiation (Jeong et al. 2014; Jeong et al. 2015). The decreased adipogenic expression may occur by decreased expression of PPAR- γ , cytosine-cytosine-adenosine-adenosine thymidine/enhancer binding protein alpha, fatty acid binding protein-4, and stearyl CoA desaturase (Jeong et al. 2014; Jeong et al. 2015). In clinical endometritis adipogenesis of cMSCs may also reduce (Lara et al. 2017).

5.4 Extended Differentiation Potential of MSCs

MSCs in addition to tri-lineage differentiation show extended differentiation potential as depicted by their ability to express pluripotency markers.

5.4.1 Neural Cell like Differentiation

MSCs neural cell like trans-differentiation has been achieved by various factors, although the actual differentiation is questioned. Cattle umbilical cord MSCs (cUC-MSCs) had differentiated into the neural like cells under low glucose Dulbecco's modified Eagle's medium (L-DMEM), fetal bovine serum (FBS), and basal eagle medium (BME) (Xiong et al. 2014). Neural induction medium for dog AD-MSCs included FBS, dimethyl sulfoxide, butylated hydroxyanisole, hydrocortisone, insulin, 3-isobutyl-1-methylxanthine, and adenosine 3'5'-cyclic monophosphate sodium salt monohydrate (Oh et al. 2011). Likewise, other specific medium had promoted neurogenesis in cattle Wharton's jelly (cWJ-MSCs). Neural differentiation was characterized by their ability to express neuronal/glial markers like neurofilament 200 (N200), neuronal microtubule associated protein 2 (MAP2), neurotrophin 3 (NT3), Tau, glial fibrillary acidic protein (GFAP) (Cardoso et al. 2012). Neural like cell trans-differentiation of gAD-MSCs had also been achieved with BIX-01294, a specific inhibitor of methyltransferase G9a. gAD-MSCs trans-differentiation achieved with methyltransferase inhibitor relies on Nanog regulatory network (Wang et al. 2018). The neural cell like differentiation of bufAm-MSCs had been promoted by glycogen synthase kinase 3 inhibitor (kenpaulone) (Deng et al. 2018). The neurogenic markers are variably expressed at different time intervals. Nestin and Map-2 expression mostly occurs at 24 hr. culture period while tropomyosin receptor kinase A (TRKA) and PRion Protein Cellular (PrP^c) expression is seen at 144 hrs (Dueñas et al. 2014). Therefore, at different time intervals MSCs neural expression profile is variable making it important to study their differentiation as per the developmental phase of an individual.

5.4.2 Differentiation into Islet Like Cells

Bovine umbilical cord MSCs (cUC-MSCs) differentiate into the islet like cells upon supplementation with media like H-DMEM, HGF, mercaptoethanol, and niacinamide. The trans-differentiation had been demonstrated through pancreatic and duodenal homeobox 1 (PDX-1) and insulin secretion (Xiong et al. 2014). Similarly, a culture medium containing nicotinamide, extendin-4, glucose, and poly-D-lysine had promoted cattle placental MSCs islet like cell differentiation. The differentiated cells had exhibited special characteristics including zinc ion staining, secretion of insulin, glucagon, paired box protein (Pax-4), Pax-6, homeobox protein Nkx6.1 and Forkhead box protein (Fox) (Peng et al. 2017). During MSCs islet-like cell differentiation, glucose promotes growth of pancreatic β -cells through phosphatidylinositol 3-kinase pathway (Wu et al. 1997). During islet like cells trans-differentiation of MSCs poly-D-lysine acts an important ingredient. It provides an intercellular connection and support, water retention, compression resistance, cell migration, cell physiology, and finally cell differentiation (Peng et al. 2017). These in vitro studies need to be translated under in vivo applications to evaluate actual feasibility and utility.

5.4.3 Differentiation into Hepatocyte like Cells

cUC-MSCs tend to differentiate into the hepatocyte like cells upon culturing in L-DMEM, FBS, hepatocyte growth factor (HGF), fibroblast growth factor 4 (FGF-4), dexamethasone, and ITS (Xiong et al. 2014). FGF-4 acts as an important growth and development factor for hepatocytes, while HGF increases their mitosis and resists their apoptosis (Jiang et al. 2002). cBM-MSCs upon culture supplementation with α -fetoprotein, albumin, α 1-antitrypsin, connexin 32, tyrosine aminotransferase, and cytochrome P450 had differentiated to hepatogenic lineage. MSCs hepatogenesis was characterized on the basis of their significant increase in the expression of genes like cytochrome P34A4 (CYP3A4), ALB, α 1-AT, CNX32, and TAT. CYP3A4 mRNA expression was higher as early as day 7 up to day 28 (Scott and Halpert 2005; Dueñas et al. 2014). α 1AT and TAT, late markers of the hepatocyte lineage, play the role of tyrosine degradation and inhibit proteases (Rehman et al. 2004; Jedicke et al. 2014). CNX32 accounts for majority of liver connexin proteins (90%) (Nakashima et al. 2004) whose expression occurs at the later stage of differentiation. It points out that MSCs must be exposed to hepatogenic factors for longer time to trigger them towards the hepatic line.

5.4.4 Differentiation into Myocyte like Cells

Myogenic differentiation of bovine fetal BM-MSCs has been achieved with various protocols like incorporation of 5-Aza-2'-deoxycytidine (5-Aza), myoblast-secreted factor Galectin-1 (Gal-1), and/or myoblast culture medium SkGM-2 BulletKit (Oh et al. 2011; Okamura et al. 2017). Variable concentration of 5-Aza has bearing on cell viability. Higher concentration (100 μ M) may increase cellular differentiation but may be more toxic to cells. It had been concluded that cBM-MSCs were myogenically differentiated to some extent (Okamura et al. 2017). Similarly, bezafibrate had promoted myogenesis of cBM-MSCs (Ramirez-Espinosa et al. 2015). Muscle derived cells (MDCs) had differentiated gBM-MSCs through their fusion producing myotubes (Kulesza et al. 2016). Sheep fetal derived BM-MSCs supplemented with reversine and 5-azacytidine had differentiated into cardiomyocyte like cells (Soltani et al. 2016). Arterial matrix supplementation to culture had directed sheep AD-MSCs differentiation towards endothelial like cells (Zhang et al. 2016).

5.4.5 Differentiation into Germ Cell like Cells

In vitro cultured sheep MSCs and bovine MSCs under the influence of TGF- β 1, BMP-4, BMP8b, and retinoic acid transdifferentiate into the germ cells (Ghasemzadeh-Hasankolaei et al. 2014; Ghasemzadeh-Hasankolaei et al. 2015). Dog adipose tissue and ovarian derived MSCs were transdifferentiated into primordial germ cell like cells and male germ cell like cells under the influence of insulin, epidermal growth factor (EGF), glial derived nerve factor (GDNF), basic fibroblast growth factor (bFGF), and BMP-4 (Wei et al. 2016; Trindade et al. 2017).

TGF- β 1 supplementation had differentiated sheep BM-MSCs to spermatogonia stem cell like cells and spermatogonia-like cells. These cells were differentiated to primordial germ cell like cells through BMP-4 and BMP-8 supplementation (Ghasemzadeh-Hasankolaei et al. 2014). Zinc supplementation had changed gene expression of sheep BM-MSCs approximating germinal cell genes but had failed to promote germinal cell like trans-differentiation (Ghasemzadeh-Hasankolaei et al. 2012). Under the influence of above mentioned factors cBM-MSCs had expressed higher levels of pluripotency markers and deleted in azoospermia-like (*Dazl*) but failed to transcribe mRNA of germinal cell genes like *Vasa*, *Fragilis*, *Stella* male germinal cell genes (*Stra8* and *Piwil2*). TGF- β 1 and BMP-4 had transiently increased expression of *Nanog* and *Dazl*, while retinoic acid had effectively promoted *Dazl* expression. Such expression pattern had signified early germ cell like differentiation of cBM-MSCs (Cortez et al. 2018). gBM-MSCs had been trans-differentiated into the germ cell-like cells exhibited by expression of both early as well as late stage germ cell markers. Even limited number of differentiated cells had a potential to enter meiosis (Zhang et al. 2019). *Dazl* gene ectopic expression in gBM-MSCs had improved their ability to transdifferentiate to the putative germ cells as compared to the effect of retinoic acid which in turn had better effect than BMP-4 (Yan et al. 2015).

Apart from ectopic expression of germ cell like cells, follicular fluid may also favor MSCs germinal cell trans-differentiation. Higher concentration (20%) of follicular fluid had been able to promote gUC-MSCs differentiation towards oocyte like cells (Qiu et al. 2012). However, these transdifferentiated cells actual identity and utility can be evaluated under in vivo studies.

Goat mammary fat pad adipose stem cells had been transdifferentiated towards epithelial lineage with an initial cocktail of insulin, hydrocortisone, and epidermal growth factor and subsequently with keratinocyte growth factor (Reza et al. 2014). All these studies are promising but in infancy and need extensive studies before any conclusion can be drawn.

5.5 Conclusion(s)

MSCs characteristic features are controlled by the available microenvironment/ niche. An extensive work on MSCs differentiation has been undertaken. These cells may undergo differentiation towards particular lineage based on the available cues in the form of available matrix, cells, humoral/growth factors, and mechanical factors. Most of the studies in available literature have mainly focused on individual cues, while in vivo cues are diverse and can act simultaneously leading to diverse results. Variable mechanisms have been demonstrated in differentiation of MSCs and have varied even on the basis of cell source. It is important to understand MSCs differentiation with respect to the developmental stages. Commonly used antihypertensive drugs may have effect on adipogenicity of MSCs. Effect of these drugs on the possible obesity in such individuals may be evaluated. A little evidence on hepatocyte, islet like cell, neural and germinal differentiation could be seen though preliminary studies support such cell type

differentiations of MSCs. Thus, further studies are desired to be conducted in order to understand the particular lineage differentiation. Besides, multiple factorial studies may be conducted to understand combined effect of these factors on MSCs expression.

References

- Arora A, Sriram M, Kothari A, Katti DS (2017) Co-culture of infrapatellar fat pad-derived mesenchymal stromal cells and articular chondrocytes in plasma clot for cartilage tissue engineering. *Cytotherapy* 19(7):881–894
- Atala A, Lanza RP (2001) Preface. In: Atala A, Lanza RP (eds) *Methods of tissue engineering*. Academic Press, San Diego
- Bageshlooyafshar B, Isangcheshmeh AM, Vakilian S et al (2019) Zinc silicate mineral-coated scaffold improved in vitro osteogenic differentiation of equine adipose-derived mesenchymal stem cells. *Res Vet Sci* 124:444–451
- Bertolo A, Steffen F, Malonzo-Marty C, Stoyanov J (2015) Canine mesenchymal stem cell potential and the importance of dog breed: implication for cell-based therapies. *Cell Transplant* 24(10):1969–1980
- Bornes TD, Jomha NM, Mulet-Sierra A, Adesida AB (2015) Hypoxic culture of bone marrow-derived mesenchymal stromal stem cells differentially enhances in vitro chondrogenesis within cell-seeded collagen and hyaluronic acid porous scaffolds. *Stem Cell Res Ther* 6:84
- Bornes TD, Jomha NM, Mulet-Sierra A, Adesida AB (2016) Optimal seeding densities for *In Vitro* Chondrogenesis of two- and three-dimensional-isolated and -expanded bone marrow-derived Mesenchymal stromal stem cells within a porous collagen scaffold. *Tissue Eng Part C Methods* 22(3):208–220
- Bosnakovski D, Mizuno M, Kim G, Ishiguro T, Okumura M, Iwanaga T, Kadosawa T, Fujinaga T (2004) (2004) Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells in pellet cultural system. *Exp Hematol* 32(5):502–509
- Bosnakovski D, Mizuno M, Kim G, Takagi S, Okumura M, Fujinaga T (2005) Isolation and multilineage differentiation of bovine bone marrow mesenchymal stem cells. *Cell Tissue Research* 319(2):243–253
- Bosnakovski D, Mizuno M, Kim G, Takagi S, Okumura M, Fujinaga T (2006) Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells (MSCs) in different hydrogels: influence of collagen type II extracellular matrix on MSC chondrogenesis. *Biotechnol Bioeng* 93(6):1152–1163
- Bottagisio M, Lovati AB, Lopa S, Moretti M (2015) Osteogenic differentiation of human and ovine bone marrow stromal cells in response to b-Glycerophosphate and monosodium phosphate. *Cell Reprogram* 17(4):235–242
- Bwalya EC, Kim S, Fang J, Wijekoon HMS, Hosoya K, Okumura M (2017) Effects of pentosan polysulfate and polysulfated glycosaminoglycan on chondrogenesis of canine bone marrow-derived mesenchymal stem cells in alginate and micromass culture. *J Vet Med Sci* 79(7):1182–1190
- Cao L, Liu G, Gan Y, Fan Q, Yang F, Zhang X, Tang T, Dai K (2012) The use of autologous enriched bone marrow MSCs to enhance osteoporotic bone defect repair in long-term estrogen deficient goats. *Biomaterials* 33(20):5076–5084
- Cardoso TC, Novais JB, Antello TF, Silva-Frade C, Ferrarezi MC, Ferrari HF, Gameiro R, Flores EF (2012) Susceptibility of neuron-like cells derived from bovine Wharton's jelly to bovine herpesvirus type 5 infections. *BMC Veterinary Research* 10(8):242
- Cheng W, Jin D, Zhao Y (2007) Effect of platelet-rich plasma on proliferation and osteogenic differentiation of bone marrow stem cells in China goats. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi* 21(4):386–389

- Colosimo A, Russo V, Mauro A, Curini V, Marchisio M, Bernabò N, Alfonsi M, Mattioli M, Barboni B (2013) Prolonged in vitro expansion partially affects phenotypic features and osteogenic potential of ovine amniotic fluid-derived mesenchymal stromal cells. *Cytotherapy* 15:930–950
- Cortez J, Bahamonde J, De los Reyes M, Palomino J, Torres CG, Peralta OA (2018) In vitro differentiation of bovine bone marrow-derived mesenchymal stem cells into male germ cells by exposure to exogenous bioactive factors. *Reprod Domest Anim* 53(3):700–709
- Das K, Mili B, Madhusoodan AP, Saxena AC, Kumar A, Singh P, Vermae MR, Sarkara M, Bag S (2017) Proliferation of canine bone marrow derived mesenchymal stem cells on different nanomaterial based thin film scaffolds. *Tissue Cell* 49(2):270–274
- Deng Y, Huang G, Zou L, Nong T, Yang X, Cui J, Wei Y, Yang S, Shi D (2018) Isolation and characterization of buffalo (*bubalus bubalis*) amniotic mesenchymal stem cells derived from amnion from the first trimester pregnancy. *J Vet Med Sci* 80(4):710–719
- Desancé M, Contentin R, Bertoni L, Gomez-Leduc T, Branly T, Jacquet S, Betsch J-M, Batho A, Legendre F, Audigé F, Galéra P, Demoor M (2018) Chondrogenic differentiation of defined equine Mesenchymal stem cells derived from umbilical cord blood for use in cartilage repair therapy. *Int J Mol Sci* 19:537
- Dueñas F, Becerra V, Cortes Y, Vidal S, Sáenz L, Palomino J, De Los Reyes M, Peralta OA (2014) Hepatogenic and neurogenic differentiation of bone marrow mesenchymal stem cells from abattoir-derived bovine fetuses. *BMC Vet Res* 10:154
- Elkhenany H, Amelse L, Lafont A, Bourdo S, Caldwell M, Neilsen N, Dervishi E, Derek O, Biris AS, Anderson D, Dhar M (2015) Graphene supports in vitro proliferation and osteogenic differentiation of goat adult mesenchymal stem cells: potential for bone tissue engineering. *J Appl Toxicol* 35(4):367–374
- Elkhenany H, Amelse L, Caldwell M, Abdelwahed R, Dhar M (2016) Impact of the source and serial passaging of goat mesenchymal stem cells on osteogenic differentiation potential: implications for bone tissue engineering. *J Anim Sci Biotechnol* 7:16
- Erickson IE, van Veen SC, Sengupta S, Kestle SR, Mauck RL (2011) Cartilage matrix formation by bovine Mesenchymal stem cells in three-dimensional culture is age-dependent. *Clin Orthop Relat Res* 469:2744–2753
- Fang J, Wei Y, Teng X, Zhao S, Hua J (2017) Immortalization of canine adipose-derived mesenchymal stem cells and their seminiferous tubule transplantation. *J Cell Biochem* 119(4):3663–3670
- Fischer EM, Layrolle P, Van Blitterswijk CA, De Bruijn JD (2004) Bone formation by Mesenchymal progenitor cells cultured on dense and microporous hydroxyapatite particles. *Tissue Eng* 9(6):1179–1188
- Fisher MB, Henning EA, Sögaard N, Bostrom M, Esterhai JL, Mauck RL (2015) Engineering meniscus structure and function via multi-layered Mesenchymal stem cell-seeded Nanofibrous scaffolds. *J Biomech* 48(8):1412–1419
- Fulber J, Maria DA, Silva LCLCD, Massoco CO, Agreste F, Baccarin RYA (2016) Comparative study of equine mesenchymal stem cells from healthy and injured synovial tissues: an in vitro assessment. *Stem Cell Res Ther* 7(1):35
- García Cruz DM, Gomes M, Reis RL, Moratal D, Salmerón-Sánchez M, Gómez Ribelles JL, Mano JF (2010) Differentiation of mesenchymal stem cells in chitosan scaffolds with double micro and macroporosity. *J Biomed Mat Res Part A*. 95A:1182–1193
- Ghasemzadeh-Hasankolaei M, Sedighi-Gilani MA, Eslaminejad MB (2014) Induction of ram bone marrow Mesenchymal stem cells into germ cell lineage using transforming growth factor- β superfamily growth factors. *Reprod Dom Anim* 49:588–598
- Ghasemzadeh-Hasankolaei M, Eslaminejad BM, Sedighi-Gilani M (2015) Derivation of male germ cells from ram bone marrow mesenchymal stem cells by three different methods and evaluation of their fate after transplantation into the testis. *In Vitro Cell Dev Biol Anim* 52(1):49–61
- Ghasemzadeh-Hasankolaei M, Batavani R, Eslaminejad BM, Sedighi-Gilani M (2012) Effect of zinc ions on differentiation of bone marrow-derived Mesenchymal stem cells to male germ cells and some germ cell-specific gene expression in rams. *Biol Trace Elem Res* 150:137–146

- Goldman SM, Barabino GA (2016) Hydrodynamic loading in concomitance with exogenous cytokine stimulation modulates differentiation of bovine mesenchymal stem cells towards osteochondral lineages. *BMC Biotechnol* 16:10
- Gregor A, Filová E, Novák M et al (2017) Designing of PLA scaffolds for bone tissue replacement fabricated by ordinary commercial 3D printer. *J Biol Eng* 11(1):31
- Gugjoo MB, Amarpal (2018) Mesenchymal stem cell research in sheep: current status and future prospects. *Small Rum Res* 169:46–56
- Gugjoo MB, Amarpal, Sharma GT, Kinjavdekar P, Aithal HP, Pawde AM (2016) Cartilage tissue engineering: role of mesenchymal stem cells along with growth factors and scaffolds. *Indian J Med Res* 144(3):339–347
- Gugjoo MB, Amarpal, Abdelbaset-Ismail A, Aithal HP, Kinjavdekar P, Pawde AM, SaiKumar G, Sharma GT (2017) Mesenchymal stem cells with IGF-1 and TGF- β 1 in laminin gel for osteochondral defects in rabbits. *Biomed Pharmacother* 93:1165–1174
- Gugjoo MB, Amarpal, Chandra V, Wani MY, Dhama K, Sharma GT (2018) Mesenchymal stem cell research in veterinary medicine. *Curr Stem Cell Res Ther* 13(8):645–657
- Gugjoo MB, Amarpal, Makhdoomi DM, Sharma GT (2019a) Equine mesenchymal stem cells: properties, sources, characterization and potential therapeutic applications. *J Equine Vet Sci* 72:16–27
- Gugjoo MB, Amarpal, Fazili MR, Shah RA, Sharma GT (2019b) Mesenchymal stem cell: basic research and potential applications in cattle and buffalo. *J Cell Physiol* 234(6):8618–8635
- Gugjoo MB, Amarpal, Sharma GT (2019c) Mesenchymal stem cell basic research and applications in dog medicine. *J Cell Physiol* 234(10):16779–16811
- Gugjoo MB, Amarpal, Fazili MR, Gayas MA, Ahmad RA, Dhama K (2019d) Animal mesenchymal stem cell research in cartilage regenerative medicine – a review. *Vet Q* 39(1):95–120
- Gugjoo MB, Amarpal AAI, Aithal HP, Kinjavdekar P, Saikumar GS, Sharma GT (2020) Allogeneic Mesenchymal stem cells and growth factors in gel scaffold repair Osteochondral defect in rabbit. *Regen Med* 14. <https://doi.org/10.2217/rme-2018-0138>
- Gupta SK, Kumar R, Mishra NC (2017) Influence of quercetin and nanohydroxyapatite modifications of decellularized goat-lung scaffold for bone regeneration. *Mat Sci Eng C* 71:919–928
- Hattori T, Muller C, Gebhard S, Bauer E, Pausch F, Schlund B, Bosl MR, Hess A, Surmann-Schmitt C, von der Mark H et al (2010) SOX9 is a major negative regulator of cartilage vascularization, bone marrow formation and endochondral ossification. *Development* 137(6):901–911
- Hegewald AA, Ringe J, Bartel J (2004) Krüger I, Notter M, Barnewitz D, Kaps C, Sittlinger M. hyaluronic acid and autologous synovial fluid induce chondrogenic differentiation of equine mesenchymal stem cells: a preliminary study. *Tissue Cell* 36:431–438
- Heo S-J, Thorpe SD, Driscoll TP, Duncan RL, Lee DA, Mauck RL (2015) Biophysical regulation of chromatin architecture instills a mechanical memory in Mesenchymal stem cells. *Sci Rep* 5:16895
- Hu P, Pu Y, Li X, Zhu Z, Zhao Y, Guan W, Ma Y (2015a) Isolation, *in vitro* culture and identification of a new type of mesenchymal stem cell derived from fetal bovine lung tissues. *Mol Med Rep* 12:3331–3338
- Hu Y, Zhang Q, Zhang L, Tang X-X, He H-Y (2015b) Basic fibroblast growth factor lentiviral vector-transfected sheep bone marrow mesenchymal stem cells and non-specific osteogenic gene expression. *Mol Med Rep* 12:267–272
- Huang Y-Z, Cai J-Q, Lv F-J, Xie H-L, Yang Z-M, Huang Y-C, Deng L (2013) Species variation in the spontaneous calcification of bone marrow-derived mesenchymal stem cells. *Cytherapy* 15:323e329
- Hwang NS, Varghese S, Li H, Elisseeff J (2011) Regulation of osteogenic and chondrogenic differentiation of mesenchymal stem cells in PEG-ECM hydrogels. *Cell Tissue Res* 344:499–509
- Ivirico JL, Salmerón-Sánchez M, Ribelles JL, Pradas MM, Soria JM, Gomes ME, Reis RL, Mano JF (2009) Proliferation and differentiation of goat bone marrow stromal cells in 3D scaffolds with tunable hydrophilicity. *J Biomed Mat Res B Appl Biomater* 91(1):277–286
- Jäger M, Bachmann R, Scharfstädt A, Krauspe R (2006) Ovine cord blood accommodates multipotent Mesenchymal progenitor cells. *In Vivo* 20:205–214

- Jedicke N, Struever N, Aggrawal N, Welte T, Manns MP, Malek NP, Zender L, Janciauskiene S, Wuestefeld T (2014) Alpha-1-antitrypsin inhibits acute liver failure in mice. *Hepatology* 59:2299–2308
- Jeong JY, Suresh S, Park MN, Jang M, Park S, Gobianand K, You S, Yeon S-H, Lee H-J (2014) Effects of capsaicin on adipogenic differentiation of bovine bone marrow Mesenchymal stem cell. *Asian Australas J Anim Sci* 27(12):1783–1793
- Jeong JY, Suresh S, Jang M, Park MN, Gobianand K, Seungkwon Y, Yeon S-H, Lee H-J (2015) Epigallocatechin-3-gallate suppresses the lipid deposition through the apoptosis during differentiation in bovine bone marrow mesenchymal stem cells. *Cell Biol Int* 39:52–64
- Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, OrtizGonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 418:41–49
- Jo K, Kim Y, Lee SH, Yoon YS, Kim WH, Kweon O-K (2017) Effect of canine cortical bone demineralization on osteogenic differentiation of adipose- derived mesenchymal stromal cells. *Heliyon* 3(8):e00383
- Johnson I, Perchy D, Liu H (2012) In vitro evaluation of the surface effects on magnesium-yttrium alloy degradation and mesenchymal stem cell adhesion. *J Biomed Mat Res Part A* 100A:477–485
- Kalaszczynska I, Ruminski S, Platek AE, Bissenik I, Zakrzewski P, Noszczyk M, Lewandowska-Szumiel M (2013) Substantial differences between human and ovine mesenchymal stem cells in response to osteogenic media: how to explain and how to manage? *Biores Open Access* 2(5):356–363
- Katja N, Michaela E, Jochen R, Bernd F, Rudi M, Thomas H et al (2007) BMP7 promotes adipogenic but not osteo-/chondrogenic differentiation of adult human bone marrow-derived stem cells in high-density micro-mass culture. *J Cell Biochem* 102:3,626–3,637
- Knippenberg M, Helder M, Doulabi BZ, Semeins CM, Wuisman PIJM, Klein-Nulend J (2005) Adipose tissue-derived Mesenchymal stem cells acquire bone cell-like responsiveness to fluid shear stress on Osteogenic stimulation. *Tissue Eng* 11(11/12):1780–1787
- Knippenberg M, Helder M, Doulabi BZ, Semeins CM, Wuisman PIJM, Klein-Nulend J (2006) Osteogenesis versus chondrogenesis by BMP-2 and BMP-7 in adipose stem cells. *Biochem Biophys Res Commun* 342:902–908
- Kol A, Walker NJ, Galuppo LD, Clark KC, Buerchler S, Bernanke A, Borjesson DL (2012) Autologous point-of-care cellular therapies variably induce equine mesenchymal stem cell migration, proliferation and cytokine expression. *Equine Vet J* 45(2):193–198
- Kulesza A, Burdzinska A, Szczepanska I, Zarychta-Wisniewska W, Pajak B, Bojarczuk K, Dybowski B, Paczek L (2016) The mutual interactions between Mesenchymal stem cells and myoblasts in an autologous co-culture model. *PLoS One* 11(8):e0161693
- Lanza R, Langer R, Vacanti JP (2011) Principles of tissue engineering. Academic Press, Cambridge
- Lara E, Velásquez A, Cabezas J, Rivera N, Pacha P, Rodríguez-Alvarez L, Saravia F, Castro FO (2017) Endometritis and in vitro PGE2 challenge modify properties of cattle endometrial Mesenchymal stem cells and their Transcriptomic profile. *Stem Cells Int* 2017:4297639
- Lee SH, Cha S-H, Kim C-L, Lillehoj HS, Song J-Y, Lee K-W (2015a) Enhanced adipogenic differentiation of bovine bone marrow derived mesenchymal stem cells. *J Appl Anim Res* 43(1):15–21
- Lee WD, Hurtig MB, Pilliar RM, Stanford WL, Kandel RA (2015b) Engineering of hyaline cartilage with a calcified zone using bone marrow stromal cells. *Osteoarthritis Cartil* 23(8):1307–1315
- Leung VYL, Gao B, Leung KKH, Melhado IG, Wynn SL, Au TYK, Dung NWF, Lau JYB, Mak ACY, Chan D et al (2011) SOX9 governs differentiation stage-specific gene expression in growth plate chondrocytes via direct concomitant transactivation and repression. *PLoS Genet* 7(11):e1002356
- Li H, Ji T, Xu LQ, Hu YJ, Li SY, Zhang CP (2006) Study on the osteogenesis ability of co-culturing bone marrow stromal cells (BMSCs) and small intestinal submucosa. *Shanghai Kou Qiang Yi Xue* 15(2):167–171

- Liu H (2011) The effects of surface and biomolecules on magnesium degradation and mesenchymal stem cell adhesion. *J Biomed Mat Res Part A* 99A:249–260
- Mahdavia FS, Salehia A, Seyedjafarib E, Mohammadi-Sangcheshmeha A, Ardeshtyrlajimi A (2017) Bioactive glass ceramic nanoparticles-coated poly(L-lactic acid) scaffold improved osteogenic differentiation of adipose stem cells in equine. *Tissue Cell* 49(5):565–572
- Martinello T, Bronzini I, Maccatrozzo L, Mollo A, Sampaolesi M, Mascarello F, Decaminada M, Patruno M (2011) Canine adipose-derived-mesenchymal stem cells do not lose stem features after a long-term cryopreservation. *Res Vet Sci* 91(1):18–24
- Mauck RL, Yuan X, Tuan RS (2006) Chondrogenic differentiation and functional maturation of bovine mesenchymal stem cells in long-term agarose culture. *Osteoarthritis Cartil* 14(2):179–189
- McCorry MC, Bonassar LJ (2017) Fiber development and matrix production in tissue engineered menisci using bovine Mesenchymal stem cells and Fibrochondrocytes. *Connect Tissue Res* 58(3–4):329–341
- McCorry MC, Puetzer JL, Bonassar LJ (2016) Characterization of mesenchymal stem cells and fibrochondrocytes in three-dimensional co-culture: analysis of cell shape, matrix production, and mechanical performance. *Stem Cell Res Ther* 12(7):39
- Meyer U, Meyer T, Handschel J, Wiesmann HP (2009) Fundamentals of tissue engineering and regenerative medicine. Springer, Berlin
- Nair MB, Varma HK, John A (2009a) Triphasic ceramic coated hydroxyapatite as a niche for goat stem cell-derived osteoblasts for bone regeneration and repair. *J Mat Sci Mat Med* 20:S251–S258
- Nair MB, Varma HK, Menon KV, Shenoy SJ, John A (2009b) Tissue regeneration and repair of goat segmental femur defect with bioactive triphasic ceramic-coated hydroxyapatite scaffold. *Biomed Mat Res* 91A:855–865
- Nakashima Y, Ono T, Yamanoi A, El-Assal ON, Kohno H, Nagasue N (2004) Expression of gap junction protein connexin32 in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. *J Gastroenterol* 39:763–768
- Oh HJ, Park JE, Kim MJ, Hong SG, Ra JC, Jo JY et al (2011) Recloned dogs derived from adipose stem cells of a transgenic cloned beagle. *Theriogenology* 75(7):1221–1231
- Okamura LH, Cordero P, Palomino J, Parraguez VH, Torres CG, Peralta OA (2017) Myogenic differentiation potential of Mesenchymal stem cells derived from fetal bovine bone marrow. *Anim Biotechnol* 29(1):1–11
- Ongaro A, Pellati A, Setti S, Masieri FF, Aquila G, Fini M, Caruso A, De Mattei M (2012) Electromagnetic fields counteract IL-1b activity during chondrogenesis of bovine mesenchymal stem cells. *J Tissue Eng Regen Med* 9(12):E229–E238
- Park Y, Sugimoto M, Watrin A, Chiquet M, Hunziker EB (2005) BMP-2 induces the expression of chondrocyte-specific genes bovine synovium-derived progenitor cells cultured in three-dimensional alginate hydrogel. *Osteoarthritis Cartil* 13:527e536
- Peng S-Y, Chou C-W, Kuo Y-H, Shen P-C, Steven Shaw S-W (2017) Potential differentiation of islet like cells from pregnant cow derived placental stem cells. *Taiwan J Obstet Gynaecol* 56:306–311
- Pereira-Junior OCM, Rahal SCJ, Lima-Neto F, da Cruz Landim-Alvarenga F, Monteiro FOB (2013) In vitro evaluation of three different biomaterials as scaffolds for canine mesenchymal stem cells. *Acta Cir Bras* 28(5):353–360
- Place ES, Evans ND, Stevens MM (2009) Complexity in biomaterials for tissue engineering. *Nat Mater* 8:457–470
- Prins H-J, Fernandes H, Rozemuller H, van Blitterswijk C, de Boer J, Martens ACM (2016) Spatial distribution and survival of human and goat mesenchymal stromal cells on hydroxyapatite and β -tricalcium phosphate. *J Tissue Eng Reg Med* 10:233–244
- Qiu P, Bai Y, Liu C, He X, Cao H, Li M, Zhu H, Hua J (2012) A dose-dependent function of follicular fluid on the proliferation and differentiation of umbilical cord mesenchymal stem cells (MSCs) of goat. *Histochem Cell Biol* 138:593–603

- Rackwitz L, Djouad F, Janjanin S, Nöth U, Tuan RS (2014) Functional cartilage repair capacity of de-differentiated, chondrocyte- and mesenchymal stem cell-laden hydrogels in vitro. *Osteoarthritis Cartil* 22(8):1148–1157
- Ramirez-Espinosa JJ, Gonzalez-Davalos L, Shimada A, Pina E, Varela-Echavarria A, Mora O (2015) Bovine (*Bos taurus*) bone marrow mesenchymal cell differentiation to Adipogenic and myogenic lineages. *Cells Tissues Organs* 201:51–64
- Randau TM, Schildberg FA, Alini M, Wimmer MD, Haddouti E-M, Gravius S, Ito KJ, Stoddart M (2013) The effect of dexamethasone and Triiodothyronine on terminal differentiation of primary bovine chondrocytes and Chondrogenically differentiated Mesenchymal stem cells. *PLoS One* 8(8):e72973
- Rehman KK, Ayesha Q, Khan AA, Ahmed N, Habibullah CM (2004) Tyrosine aminotransferase and gamma-glutamyl transferase activity in human fetal hepatocyte primary cultures under proliferative conditions. *Cell Biochem Funct* 22:89–96
- Reza AMMT, Shiwani S, Singh NK, Lohakare JD, Lee SJ, Jeong DK, Han JY, Rengaraj D, Lee BW (2014) Keratinocyte growth factor and thiazolidinediones and linolenic acid differentiate characterized mammary fat pad adipose stem cells isolated from prepubertal Korean black goat to epithelial and adipogenic lineage. *In Vitro Cell Dev Biol-Anim* 50:194–206
- Rodrigues MT, Leonor IB, Gröen N, Viegas CA, Dias IR, Caridade SG, Mano JF, Gomes ME, Reis RL (2014) Bone marrow stromal cells on a 3D bioactive fiber mesh undergo osteogenic differentiation in the absence of osteogenic media supplements: the effect of silanol groups. *Acta Biomater* 10(10):4175–4185
- Santos VH, Pfeifer JPH, de Souza JB, Milani BHG, de Oliveira RA, Assis MG, Deffune E, Moroz A, Garcia Alves AL (2018) Culture of mesenchymal stem cells derived from equine synovial membrane in alginate hydrogel microcapsules. *BMC Vet Res* 14:114
- Schop D, Janssen FW, Borgart E, de Bruijn JD, van Dijkhuizen-Radersma R (2008) Expansion of mesenchymal stem cells using a microcarrier-based cultivation system: growth and metabolism. *J Tissue Eng Reg Med* 2:126–135
- Scott EE, Halpert JR (2005) Structure of cytochrome P450 3A4. *Trends Biochem Sci* 30:5–7
- Setiawatie EM, Ulfah N, Wahjuningrum DA, Sari DS, Rubianto M (2017) Viability bovine tooth Hydroxyapatite on bone marrow Mesenchymal stem cells. In: International medical device and technology conference. Universiti Teknologi Malaysia, Johor Bahru, pp 78–81
- Simonaro CM, Sachot S, Ge Y, He X, DeAngelis VA, Eliyahu E, Leong D, Sun HB, Mason JB, Haskins ME, Richardson DW, Schuchman EH (2013) Acid Ceramidase maintains the Chondrogenic phenotype of expanded primary chondrocytes and improves the Chondrogenic differentiation of bone marrow-derived Mesenchymal stem cells. *PLoS One* 8(4):e62715
- Soltani L, Rahmani HR, Joupri MD, Ghaneialvar H, Mahdavi AH, Shamsara M (2016) Ovine fetal mesenchymal stem cell differentiation to cardiomyocytes, effects of co-culture, role of small molecules; reversine and 5-azacytidine. *Cell Biochem Funct* 34:250–261
- Stewart MC (2011) Cell-based therapies: current issues and future directions. *Vet Clin North Am Equine Pract* 27(2):393–399
- Tan L, Zhao B, Ge FT, Sun DH, Yu T (2017) Shockwaves inhibit chondrogenic differentiation of human mesenchymal stem cells in association with adenosine and A2B receptors. *Sci Rep* 7:1437
- Terraciano V, Hwang N, Moroni L, Park HB, Zhang Z, Mizrahi J, Seliktart D, Elisseeff J (2007) Differential response of adult and embryonic Mesenchymal progenitor cells to mechanical compression in hydrogels. *Stem Cells* 25:2730–2738
- Tjabringa GS, Zandieh-Doulabi B, Helder MN, Knippenberg M, Wuisman PIJM, Klein-Nulend J (2008) The polyamine spermine regulates osteogenic differentiation in adipose stem cells. *J Cell Mol Med* 12(5A):1710–1717
- Toh WS, Lim TC, Kurisawa M, Spector M (2012) Modulation of mesenchymal stem cell chondrogenesis in a tunable hyaluronic acid hydrogel microenvironment. *Biomaterials* 33:3835e3845
- Toh WS, Foldager CB, Olsen BR, Spector M (2013) Basement membrane molecule expression attendant to Chondrogenesis by nucleus Pulposus cells and Mesenchymal stem cells. *J Orthop Res* 31:1136–1143

- Topol L, Chen W, Song H, Day TF, Yang Y (2009) Sox9 inhibits Wnt signaling by promoting beta-catenin phosphorylation in the nucleus. *J Biol Chem* 284(5):3323–3333
- Trindade AB, Therrien J, Garcia JM, Smith LC (2017) Mesenchymal-like stem cells in canine ovary show high differentiation potential. *Cell Prolif* 50(6):e12391
- Varghese S, Hwang NS, Canver AC, Theprungsrikul P, Lin DW, Elisseeff J (2008) Chondroitin sulphate based niches for chondrogenic differentiation of mesenchymal stem cells. *Matrix Biol* 27:12–21
- Versari S, Villa A, Helder MN, Doulabi BZ, van Loon J, Bradamante S (2007) Effects of gravity on proliferation and differentiation of adipose tissue-derived stem cells. *J Gravit Physiol* 14(1):P127–P128
- Vickers SM, Gotterbarm T, Spector M (2010) Cross-linking affects cellular condensation and chondrogenesis in type II collagen-GAG scaffolds seeded with bone marrow-derived mesenchymal stem cells. *J Orthop Res* 28(9):1184–1192
- Volk SW, Wang Y, Hankenson KD (2012) Effects of donor characteristics and ex vivo expansion on canine mesenchymal stem cell properties: implications for MSC-based therapies. *Cell Transplant* 21(10):2189–2200
- Wang L, Gordon RA, Huynh L, Su X, Parkmin KY, Han J, Arthur JS, Kalliolias GD (2010) Ivashkiv L.B. indirect inhibition of toll-like receptor and type I interferon responses by ITAM-coupled receptors and integrins. *Immunity* 32:518–530
- Wang M, Yuan Z, Ma N, Hao C, Guo W, Zou G, Zhang Y, Chen M, Gao S, Peng J et al (2017) Advances and prospects in stem cells for cartilage regeneration. *Stem Cells Int* 2017:4130607
- Wang Q, Wang X, Lai D, Deng J, Hou Z, Liang H, Liu D (2018) BIX-01294 promotes the differentiation of adipose mesenchymal stem cells into adipocytes and neural cells in Arbas cashmere goats. *Res Vet Sci* 119:9–18
- Wei Y, Fang J, Cai S, Lv C, Zhang S, Hua J (2016) Primordial germ cell-like cells derived from canine adipose mesenchymal stem cells. *Cell Prolif* 49(4):503–511
- Wei X, Liu B, Liu G, Yang F, Cao F, Dou X (2019) Mesenchymal stem cell-loaded porous tantalum integrated with biomimetic 3D collagen-based scaffold to repair large osteochondral defects in goats. *Stem Cell Res Ther* 10:72
- Wu KL, Gannon M, Peshavaria M, Offield MF, Henderson E, Ray M et al (1997) Hepatocyte nuclear factor 3beta is involved in pancreatic beta-cell-specific transcription of the pdx-1 gene. *Mol Cell Biol* 17(10):6002e13
- Xiong H, Bai C, Wu S, Gao Y, Lu T, Hu Q, Guan W, Ma Y (2014) Biological characterization of Mesenchymal stem cells from bovine umbilical cord. *Anim Cells Syst* 1(1):59–67
- Yan G, Fan Y, Li P, Zhang Y, Wang F (2015) Ectopic expression of DAZL gene in goat bone marrow-derived mesenchymal stem cells enhances the trans-differentiation to putative germ cells compared to the exogenous treatment of retinoic acid or bone morphogenetic protein 4 signalling molecules. *Cell Biol Int* 39(1):74–83
- Yin H, Wang Y, Sun Z, Sun X, Xu Y, Li P, Meng H, Yu X, Xiao B, Fan T, Wang Y, Xu W, Wang A, Guo Q, Peng J, Lu S (2016) Induction of mesenchymal stem cell chondrogenic differentiation and functional cartilage microtissue formation for in vivo cartilage regeneration by cartilage extracellular matrix-derived particles. *Acta Biomater* 33:96–109
- Zeiter S, Lezuo P, Ito K (2009) Effect of TGF- β 1, BMP-2 and hydraulic pressure on chondrogenic differentiation of bovine bone marrow mesenchymal stromal cells. *Biorheology* 46:45–55
- Zhang XL, Jiang XQ (2006) Osteoblastic differentiation of goat bone marrow stromal cells after AdBMP-2 mediated transduction in vitro. *Shanghai Kou Qiang Yi Xue* 15(6):610–613
- Zhang W, Huo Y, Wang X, Jia Y, Su L, Wang C, Li Y, Yang Y, Liu Y (2016) Decellularized ovine arteries as biomatrix scaffold support endothelial of mesenchymal stem cells. *Heart Vessel* 31(11):1874–1881
- Zhang YL, Li PZ, Pang J, Wan YJ, Zhang GM, Fan YX, Wang ZY, Tao NH, Wang F (2019) Induction of goat bone marrow mesenchymal stem cells into putative male germ cells using mRNA for STRA8, BOULE and DAZL. *Cytotechnology* 71(2):563–572

- Zhao Y, Hu J, Buckingham B, Pittenger MF, Wu ZJ, Griffith BP (2014) Pim-1 kinase cooperates with serum signals supporting Mesenchymal stem cell propagation. *Cells Tissues Organs* 199:140–149
- Zhou G, Zheng Q, Engin F, Munivez E, Chen Y, Sebald E, Krakow D, Lee B (2006) Dominance of SOX9 function over RUNX2 during skeletogenesis. *Proc Natl Acad Sci U S A* 103(50):19004–19009
- Zhu HB, Guo DZ, Yang SJ, Zhang YH, Wang H, Guo HT, Zhang Y, Cheng DC (2008) Osteogenic actions of the osteogenic growth peptide on bovine marrow mesenchymal stromal cells in culture. *Vet Med* 53(9):501–509
- Zscharnack M, Poesel C, Galle J, Bader A (2009) Low oxygen expansion improves subsequent Chondrogenesis of ovine bone-marrow-derived Mesenchymal stem cells in collagen type I hydrogel. *Cells Tissues Organs* 190:81–93



Mesenchymal Stem Cell Genetic Engineering and Regenerative Medicine

6

M. B. Gugjoo, E. Rasool, and Amar Pal

Abstract

Characteristic properties of mesenchymal stem cells (MSCs) make them a suitable candidate in regenerative medicine. There are various associated limitations in MSCs in vivo applications like their short survival and limited expression of particular genes. Genetic engineering of MSCs may be employed to address such issues. Various genetic engineering methods have been incorporated although without any established standard technique. Genetically engineered MSCs are being studied in various kinds of ailments of different tissues with variably promising results. The current chapter focusses on the MSC genetic engineering in relation to the regenerative medicine.

6.1 Introduction

Regenerative medicine aims to etch away the concerns related to diseased/non-functional and/or mal-functional tissues/organs. Mesenchymal stem cells (MSCs) are frequently studied for their therapeutic applications attributed to their characteristic properties and lack of any ethical and teratogenic concerns (Gugjoo et al.

M. B. Gugjoo (✉)

Division of Veterinary Clinical Complex, FVSc & AH, SKUAST-Kashmir,
Jammu and Kashmir, India

E. Rasool

Division of Surgery & Radiology, FVSc & AH, SKUAST-K,
Srinagar, Jammu and Kashmir, India

A. Pal

Division of Surgery, Indian Veterinary Research Institute, Izatnagar, UP, India

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2020

M. B. Gugjoo, A. Pal (eds.), *Mesenchymal Stem Cell in Veterinary Sciences*,
https://doi.org/10.1007/978-981-15-6037-8_6

2018). MSCs supposedly work via trophic effects through plethora of compounds, immune-modulatory/anti-inflammatory and/or by cell replacement activities. Their trophic action and differentiation is, however, determined by the available microenvironment upon which a limited control can be exerted (Gugjoo et al. 2019, 2020). MSCs show poor in vivo survival and weak host tissue engraftment especially after local implantation (Wagner and Ho 2007). To realize MSCs full therapeutic potential and tailor them to the specific diseases, the cells are being genetically engineered (Bersenev and Levine 2012, Gage 2012; Roís et al. 2012; Lu et al. 2013; Yan et al. 2015). This avenue has generated much interest among researchers, and the initial reports demonstrated are also promising (Kode et al. 2009; Griffin et al. 2010; Hang et al. 2012).

Genetic engineering/genetic modification/genetic manipulation involves a set of technologies to alter genetic makeup of cells. It is aimed to deliver proteins to neighbouring cells, kill cancer cells, reduce immune rejection, secrete regulatory and pro-healing factors and increase survival of engrafted cells. In genetic engineering, a specific gene cassette is formed to be loaded into a carrier/vehicle (vector). Vectors are the carriers to deliver gene cassette (containing transgene) to the cells (Phillips and Tang 2008; Li et al. 2017; Zhang et al. 2019). These include viruses like retroviruses (Boura et al. 2014), DNA plasmids (Marquez-Curtis et al. 2013; Watkins et al. 2014), mRNA (Rejman et al. 2010), miRNA (Meng et al. 2015) and siRNA (Roís et al. 2012), constructs of minimalistic, immunologically defined gene expression (MIDGE) (Mok et al. 2012; Mählmann et al. 2015) and transposons construct like the Sleeping Beauty System (Martin et al. 2014). Viral vector transduction directly transduces cells while non-viral transfection requires specific methods like lipofection or electroporation to cross the cell membrane (Papanikolaou et al. 2011).

Viral vectors may be genome integrating like lentivirus and adeno-associated virus or non-integrating like adeno and Sendai virus vectors. The integrating viral vectors tend to yield transgene for longer period, while non-integrating vectors express the gene of interest transiently. The genome-integrating viral vectors though may have long-lasting effects but tend to compromise the safety concerns, especially in relation to the insertional mutagenesis and associated potential malignancy (Hacein-Bey-Abina et al. 2003a, b). Inside the cells, the transgene may overexpress unphysiologically (constitutive synthesis) or can express within the physiological limits (controlled by a gene switch). The constitutive transgene expression leads to constant unphysiological oversynthesis of specific proteins. This down-regulates receptors and renders gene expression ineffective. Gene switch transgene expression can be well controlled. The cell responds to the physiological stimulus like oxygen level, concentration of hormones, drugs or chemical agents (Phillips and Tang 2008; Nowakowski et al. 2013). These techniques have their pros and cons and need to be fully weighed before any in vivo application.

MSCs are also being virally transduced but due to the above-mentioned concerns, a safer approach is desired. MSCs characteristic properties including the one of homing make them a suitable non-viral vector candidate. Add-on genetic engineering if well controlled and understood can make MSCs application a game changer in regenerative medicine. The genetically engineered MSCs currently

remain to be approved for clinical trials (Nowakowski et al. 2015). The main aim currently remains to develop genetic engineering technique that does not change basic stem cell properties, induce cytotoxicity and should be rescuable after in vivo transplantation (Papanikolaou et al. 2011). The current chapter focuses on the genetically engineered MSCs of veterinary species and their utility in regenerative medicine.

6.2 Genetic Engineering of MSCs (GE-MSCs)

Non-viral transfected MSCs in comparison to the viral transduced ones may offer a plausibly safe option in regenerative medicine. The current gene transfection efficiency of MSCs is not at par to that of the MSCs viral vector transduction. One of the reasons that might affect their efficiency is transfection technique. Transfection of cattle bone marrow MSCs (cBM-MSCs) by the electroporation had yielded better (58%) gene transfer efficiency (Colleoni et al. 2005) in comparison to the lipofection (15%) (Díaz et al. 2015) demanding further incites.

GE-MSCs should be ensured to maintain their basic properties (Pinel and Pluhar 2012). MSCs in comparison to other stem cell types have limited culture lifespan. The cell proliferation may cause telomere shortening, and as such the extended culture passaging might lead to genetic changes. In order to improve MSCs survival, genetic engineering has been tried. Sheep bone marrow MSCs (sBM-MSCs) transfected with telomerase reverse transcriptase (TERT) gene had been cultured up to 40 passages. These cells had normal karyotype rate of 88.24% with unaffected differentiation properties (Zhu et al. 2017). Thus, further MSC studies may be conducted to determine the reasons behind their limited self-renewal properties and finally the possible addressal.

Furthermore, it is imperative to confirm MSCs properties remain unaffected with genetic engineering. In a mongrel dog study, VEGF165-transfected BM-MSCs have been demonstrated to express the surface markers as per International Society of Cellular Therapy (ISCT) (Hang et al. 2012). A comparison had been made through the characterization of dog AD-MSCs (dAD-MSCs) and SV40-T retrovirus-transfected dAD-MSCs (tdAD-MSCs). Both these cell lines had characteristic properties as laid down by ISCT. tdAD-MSCs though had higher colony forming potential; pluripotency markers (Sox2 and Nanog) were expressed in both cell lines with stable karyotype. In conclusion, tdAD-MSCs and dAD-MSCs had comparable characteristics (Ayala-Cuellar et al. 2019).

6.3 GE-MSCs as Therapeutics

In veterinary science, the genetically engineered MSC studies are very limited. However, the available literature favours their utilization under in vivo conditions. Below are described some of the in vivo GE-MSCs studies conducted in animals.

6.3.1 GE-MSCs in Germ Cell Differentiation

There is a growing need to produce elite germplasm in productive animals. Sheep and cattle MSCs have been demonstrated to transdifferentiate into germ cell-like cells (Ghasemzadeh-Hasankolaie et al. 2012, 2014, 2015; Cortez et al. 2018). To boost that potential, genetically engineered MSCs may prove useful. In two goat studies, only a small fraction of Stra8, Boule- and Dazl-transfected BM-MSCs had transdifferentiated to putative germ cells. These differentiated cells could express primordial germ cell-specific genes and premeiotic genes. The cells that co-overexpressed all the three genes (Stra8, Boule and Dazl) were more close to germ cell-like cells (Li et al. 2017; Zhang et al. 2019). This demands further in vitro studies followed by in vivo experimental studies to determine their clinical utility.

6.3.2 GE-MSCs and Mastitis Control

Stem cell therapy has a significant potential to regenerate the udder tissues (Sharma et al. 2017; Gugjoo et al. 2019). Mammary stem cells are currently difficult to harvest and as replacement MSCs can be utilized. One of the studies had created bovine lactoferricin (LFcinB) transgene to confirm feasibility of creating genetically engineered cattle or buffalo MSCs. These modified cells had variable expression of the LFcinB, an antibacterial peptide (Sharma et al. 2017). Such LFcinB-cloned MSCs may be injected into the mammary gland for their higher antibacterial properties especially against *Staphylococcus aureus* and *Escherichia coli* (Sharma et al. 2017).

6.3.3 GE-MSCs and Bone Healing

Genetically engineered MSCs may be employed to enhance the bone healing. Among various factors, MSCs transfection with BMP-2 has been comparably common. Human BMP-2-transfected goat BM-MSCs had been demonstrated to express and secrete higher concentration of rhBMP-2 as compared to simple BM-MSCs. Goat tibial bone defects treated with calcined bone and hBMP-2-transduced BM-MSCs had complete healing in 5 out of 8 animals. Contrarily, tibial defects treated with scaffold and simple BM-MSCs had slight to almost nil healing. The callus formed in engineered cell-implanted tibial bones had significantly better bio-mechanical strength as compared to the simple cell-treated bone defects (Dai et al. 2005). In goat femoral head osteonecrosis model, the BMP-2- or β -gal-modified BM-MSCs along with β -tri-calcium phosphate (β -TCP) have been evaluated. Comparing these two cell engineering options, femoral bone density and surface was much improved with BMP-2-transfected cells at 3-month period. The regenerated bone mechanical testing had demonstrated significantly higher compression strength and Young's modulus in the BMP-2 group approaching to that of normal bone (Tang et al. 2007). In dog left radial oligotrophic non-union fracture, BMP-7-transfected dog AD-MSC sheets had led to the early bone healing (9 weeks) (Song

et al. 2017). Thus, the combined use of genetic and tissue engineering may provide an innovative way to treat bone defects/fractures. Although these studies have demonstrated improved bone healing results, however, the dose titration of these factors is required. An excess bone formation leading to tumour formation may arise in case higher dose of BMPs is dissipated by these transfected cells (Pinel and Pluhar 2012). Similarly, transplantation of VEGF-transduced autologous BM-MSCs in experimentally induced dog osteonecrotic femoral head had led to better healing in comparison to the simple BM-MSCs (Hang et al. 2012).

6.3.4 GM-MSCs in Cartilage and Tendon Repair

Transforming growth factor-beta (TGF- β) plays a crucial role in MSCs proliferation and differentiation, while Nell-1 specifically target cells that are committed to osteochondral lineage. TGF- β up-regulates chondrogenic gene expression through enhanced expression of the Sox9. Under in vitro study, TGF- β 1-transduced human synovial-derived MSCs (hSD-MSCs) had undergone effective chondrogenesis as revealed by increased chondrogenic marker gene expression and glycosaminoglycan secretion (Kim et al. 2014). In vitro studies have demonstrated that TGF- β 1 transduction of MSCs together with scaffolds like calcium alginate gel creates the chondrogenic micro-environment. This had been confirmed in an in vitro study wherein BM-MSCs had enhanced type II collagen synthesis ability (Zhu et al. 2013). Nell-1-transfected BM-MSCs in the presence of poly-lactic-polyglycolic acid (PLGA) scaffold too had hastened repair of goat mandibular condyle defect. At sixth week fibrocartilage formation while at 24th week complete repair of articular cartilage along with the subchondral bone had been demonstrated. In contrast, simple BM-MSCs/PLGA implants had repaired defects with fibrocartilage at 24 weeks. Overall both the cell lines had improved bone healing as compared to PLGA or empty defects (Zhu et al. 2011).

Insulin-like growth factor 1 (IGF-1) is considered to have stimulating effect on collagen synthesis (Hansen et al. 2013). As such, IGF-1-transfected MSCs may prove useful. In an equine study, IGF-1-transfected BM-MSCs had shown to have significantly higher IGF-1 secretion in comparison to the normal BM-MSCs. Implantation of BM-MSCs and IGF-1-induced BM-MSCs in an equine superficial flexor tendinitis had improved healing of the tendon at 8 weeks. The differences in healing however were statistically non-significant between the cell-treated animals (Schnabel et al. 2009).

6.3.5 GM-MSCs in Neural Differentiation and Repair

Brain-derived neurotrophic factor (BDNF) serves as a neurotransmitter modulator and has a crucial role in survival and growth of neurons. It also takes part in neuronal plasticity essentially required in learning and memory (Bathina and Das 2015). HO-1-MSCs reduce inflammation, which favours BDNF-induced neuro-regeneration

(Araujo et al. 2012). In peripheral nerve regeneration experimental study, the BDNF-transfected AD-MSCs were engaged in nerve regeneration. The cells had differentiated into various cell types including Schwann cells and had encouraged functional recovery of the rat sciatic nerve (Tseng and Hsu 2014). Adipose-derived mesenchymal stem cells (AD-MSCs) transfected with BDNF and heme oxygenase-1 (HO-1) were utilized had led to a significant improvement in hind limb functions of spinal cord-injured dogs. Co-transfection of AD-MSCs had been more effective. An increase in clinical evaluation scores and a robust increase in neuronal regeneration were demonstrated. Thus, MSCs transfection with multiple factors may be more effective in promoting the healing of spinal cord injury (SCI) (Khan et al. 2018).

6.3.6 GM-MSCs in Cardiac Ailments

Stem cell therapy in myocardial infarction offers an exciting research area. The current issues like stem cell retention and their efficacy in the heart remains to be solved. MSCs genetic modification has been achieved with various factors like glucagon-like peptide-1 (GLP-1), and VEGF and hypoxia-inducible factor 1- α (HIF1- α) are considered to favour better cardiac repair and function. Glucagon-like peptide-1 (GLP-1) is an incretin hormone with cardio-protective properties (Wright et al. 2012), VEGF improves angiogenesis (Locatelli et al. 2015) and hypoxia-inducible factor 1- α is considered to mediate overexpression of erythropoietin, vascular endothelial growth factor, angiopoietin-1 and inducible nitrous oxide synthase (Hnatiuk et al. 2016).

Transfection of human BM-MSC with GLP-1 has been demonstrated as beneficial adjunct in pig myocardial infarction model. GLP-1-transfected human MSCs encapsulated in alginate (bead-GLP-1 MSC) after coronary artery injection had led to the better-left ventricular function and a decrease in infarct area as compared to control. The GLP-1-transfected cell-treated infarcts had reduced inflammation and apoptotic cells together with an increased collagen deposition and fewer myofibroblasts (Wright et al. 2012). In sheep acute myocardial infarction (AMI) model, vascular endothelial growth factor (VEGF)-transfected MSCs had been demonstrated as useful. An increased angiogenesis together with decreased infarct size and improved left ventricular (LV) function was demonstrated with intracardiac implantation of these cells (Locatelli et al. 2015). Similarly, HIF1- α -transfected allogeneic BM-MSCs as compared to the simple BM-MSCs had led to significant reduction of infarct size in a sheep acute myocardial infarction (AMI) model. BM-MSCs too had decreased infarct size although lower than transfected cells but better than the control (Hnatiuk et al. 2016).

6.3.7 GE-MSCs in Skin Wounds

In skin wounds the main concern remains to improve angiogenesis along with the collagen deposition. Although skin healing is good, but in some cases it takes longer

time especially in diabetes or at the joint areas. VEGF-modified human umbilical cord-derived MSCs (VEGF-UC-MSCs) along with acellular dermal matrix scaffolds evaluated in pig skin wounds had effectively promoted vascularization of the graft and led to an improved quality of wound healing (Han et al. 2014).

Apart from these studies, numerous other animal studies like corneal regeneration, specific diseases among others may be conducted to evaluate beneficial effects of engineered cells. The preliminary studies involving angiopoietin-1 (Piao et al. 2010) and ACE2 (He et al. 2014) among others also have been conducted in human. These studies are preliminary, case uncontrolled and without any uniformity to be able to draw any conclusion.

6.4 Conclusion

MSCs are being either transduced virally or transfected by non-viral methods. Either of the methods have their prospects and constraints. Currently, the genetic engineering of MSCs is under experimental phase, and the standardization of these techniques needs further understanding. The genetically engineered MSCs have been studied under in vitro conditions with reasonably better results. Their in vivo applications also have appear promising although variably. Further studies that are aimed at their safe and effective genetic engineering need to be conducted. The engineered cells should then be evaluated under in vivo conditions for their actual safety, utility and efficacy.

References

- Araujo JA, Zhang M, Yin F (2012) Heme oxygenase-1, oxidation, inflammation, and atherosclerosis. *Front Pharmacol* 3:119
- Ayala-Cuellar AP, Kim CW, Hwang KA, Kang JH, Lee G, Cho J, Choi KC (2019) Characterization of canine adipose tissue-derived mesenchymal stem cells immortalized by SV40-T retrovirus for therapeutic use. *J Cell Physiol* 234(9):16630–16642
- Bathina S, Das UN (2015) Brain-derived neurotrophic factor and its clinical implications. *Arch Med Sci* 11(6):1164–1178
- Bersenev A, Levine BL (2012) Convergence of gene and cell therapy. *Regen Med* 7:50–56
- Boura JS, Vance M, Yin W, Madeira C, Lobato da Silva C, Porada CD, Almeida Porada G (2014) Evaluation of gene delivery strategies to efficiently overexpress functional HLA-G on human bone marrow stromal cells. *Mol Ther Methods Clin Dev* 2014(1):14041
- Colleoni S, Donofrio G, Lagutina I, Duchi R, Galli C, Lazzari G (2005) Establishment, differentiation, electroporation, viral transduction, and nuclear transfer of bovine and porcine mesenchymal stem cells. *Cloning Stem Cells* 7(3):154–166. <https://doi.org/10.1089/clo.2005.7.154>
- Cortez J, Bahamonde J, De los Reyes M, Palomino J, Torres CG, Peralta OA (2018) In vitro differentiation of bovine bone marrow-derived mesenchymal stem cells into male germ cells by exposure to exogenous bioactive factors. *Reprod Domest Anim* 53(3):700–709
- Dai KR, Xu XL, Tang TT, Zhu ZA, Yu CF, Lou JR, Zhang XL (2005) Repairing of goat Tibial bone defects with BMP-2 gene-modified tissue-engineered bone. *Calcif Tissue Int* 77:55–61
- Díaz P, Cuevas F, Peralta OA (2015) GFP labelling and epigenetic enzyme expression of bone marrow-derived mesenchymal stem cells from bovine foetuses. *Res Vet Sci* 99:120–128

- Gage FH (2012) Transplantation in the future. *Prog Brain Res* 200:7–13
- Ghasemzadeh-Hasankolaei M, Sedighi-Gilani MA, Eslaminejad MB (2014) Induction of ram bone marrow mesenchymal stem cells into germ cell lineage using transforming growth factor- β superfamily growth factors. *Reprod Dom Anim* 49:588–598
- Ghasemzadeh-Hasankolaei M, Eslaminejad BM, Sedighi-Gilani M (2015) Derivation of male germ cells from ram bone marrow mesenchymal stem cells by three different methods and evaluation of their fate after transplantation into the testis. *In Vitro Cell Dev Biol Anim* 52(1):49–61
- Ghasemzadeh-Hasankolaei M, Batavani R, Eslaminejad BM, Sedighi-Gilani M (2012) Effect of zinc ions on differentiation of bone marrow-derived mesenchymal stem cells to male germ cells and some germ cell-specific gene expression in rams. *Biol Trace Elem Res* 150:137–146
- Griffin M, Greiser U, Barry F, O'Brien T, Ritter T (2010) Genetically modified mesenchymal stem cells and their clinical potential in acute cardiovascular disease. *Discov Med* 9:219–223
- Gugjoo MB, Amarpal, Chandra V, Wani MY, Dhama K, Sharma GT (2018) Mesenchymal stem cell research in veterinary medicine. *Current Stem Cell Research and Therapy* 13(8):645–657
- Gugjoo MB, Amarpal, Fazili MR, Shah RA, Sharma GT (2019) Mesenchymal stem cell: basic research and potential applications in cattle and buffalo. *J Cell Physiol* 234(6):8618–8635
- Gugjoo MB, Amarpal, Fazili MR, Shah RA, Mir MS, Sharma GT (2020) Goat mesenchymal stem cell basic research and potential applications. *Small Rum Res* 183:106045
- Hacein-Bey-Abina S, von Kalle C, Schmidt M, Le Deist F, Wulffraat N, McIntyre E (2003a) A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* 348:255–256
- Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P (2003b) LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 302:415–419
- Han Y, Tao R, Han Y, Sun T, Chai J, Xu G, Liu J (2014) Microencapsulated *VEGF* gene-modified umbilical cord mesenchymal stromal cells promote the vascularization of tissue-engineered dermis: an experimental study. *Cytherapy* 16(2):160–169
- Hang D, Wang Q, Guo C, Chen Z, Yan Z (2012) Treatment of osteonecrosis of the femoral head with VEGF165 transgenic bone marrow mesenchymal stem cells in mongrel dogs. *Cells Tissues Organs* 195:495–506
- Hansen M, Boesen A, Holm L, Flyvbjerg A, Langberg H, Kjaer M (2013) Local administration of insulin-like growth factor-I (IGF-I) stimulates tendon collagen synthesis in humans. *Scand J Med Sci Sports* 23(5):614–619
- He HL, Liu L, Chen QH, Cai SX, Han JB, Hu SL, Chun P, Yang Y, Guo FM, Huang YZ, Qiu HB (2014) MSCs modified with ACE2 restore endothelial function following LPS challenge by inhibiting the activation of RAS. *J Cell Physiol* 230(3):691–701
- Hnatiuk AP, Ong S-G, Olea FD, Locatelli P, Riegler J, Lee WH, Jen CH, De Lorenzi A, Gimenez CS, Laguens R, Wu JC, Crottogini A (2016) Allogeneic Mesenchymal stromal cells overexpressing mutant human hypoxia-inducible factor 1- α (HIF1- α) in a sheep model of acute myocardial infarction. *J Am Heart Assoc* 5:e003714
- Khan IU, Yoon Y, Kim A, Jo KR, Choi KU, Jung T, Kim N, Son Y, Kim WH, Kweon O (2018) Improved healing after the co-transplantation of HO-1 and BDNF overexpressed mesenchymal stem cells in the subacute spinal cord injury of dogs. *Cell Transplant* 27:1140–1153
- Kim YI, Ryu JS, Yeo JE, Choi YJ, Kim YS, Ko K, Koh YG (2014) Overexpression of TGF- β 1 enhances chondrogenic differentiation and proliferation of human synovium-derived stem cells. *Biochem Biophys Res Commun* 450(4):1593–1599
- Kode JA, Mukherjee S, Joglekar MV, Hardikar AA (2009) Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration. *Cytherapy* 11:377–391
- Li PZ, Yan GY, Han L, Pang J, Zhong BS, Zhang GM, Wang F, Zhang YL (2017) Overexpression of STRA8, BOULE, and DAZL genes promotes goat bone marrow-derived mesenchymal stem cells in vitro trans-differentiation toward putative male germ cells. *Reprod Sci* 24(2):300–312
- Locatelli P, Olea FD, Hnatiuk A, De Lorenzi A, Cerdá M, Giménez CS, Sepúlveda D, Laguens R, Crottogini A (2015) Mesenchymal stromal cells overexpressing vascular endothelial growth factor in sheep myocardial infarction. *Gene Ther* 22(6):449–457

- Lu W, Yaoming N, Boli R, Jun C, Changhai Z, Yang Z, Zhiyuan S (2013) mHCN4 genetically modified canine mesenchymal stem cells provide biological pacemaking function in complete dogs with atrioventricular block. *Pacing Clin Electrophysiol* 36(9):1138–1149
- Mählmann K, Feige K, Juhls C, Endmann A, Schuberth H-J, Oswald D, Hellige M, Doherr M, Cavalleri J-MV (2015) Local and systemic effect of transfection-reagent formulated DNA vectors on equine melanoma. *BMC Vet Res* 11:107
- Marquez-Curtis LA, Gul-Uludag H, Xu P, Chen J, Janowska-Wieczorek A (2013) CXCR4 transfection of cord blood mesenchymal stromal cells with the use of cationic liposome enhances their migration toward stromal cell-derived factor-1. *Cytotherapy* 15(7):840–849
- Martin PK, Stilhano RS, Samoto VY, Takiya CM, Peres GB, da Silva Michelacci YM, da Silva FH, Pereira VG, D'Almeida V, Marques FL, Otake AH, Chammas R, Han SW (2014) Mesenchymal stem cells do not prevent antibody responses against human α -L-iduronidase when used to treat mucopolysaccharidosis type I. *PLoS One* 9(3):e92420
- Meng YB, Li X, Li ZY, Zhao J, Yuan XB, Ren Y, Cui ZD, Liu YD, Yang XJ (2015) microRNA-21 promotes osteogenic differentiation of mesenchymal stem cells by the PI3K/ β catenin pathway. *J Orthop Res* 33(7):957–964
- Mok PL, Cheong SK, Leong CF, Chua KH, Ainoon O (2012) Extended and stable gene expression via nucleofection of MIDGE construct into adult human marrow mesenchymal stromal cells. *Cytotechnology* 64(2):203–216
- Nowakowski A, Andrzejewska A, Janowski M, Walczak P, Lukomska B (2013) Genetic engineering of stem cells for enhanced therapy. *Acta Neurobiol Exp* 73:1–18
- Nowakowski A, Walczak P, Janowski M, Lukomska B (2015) Genetic engineering of mesenchymal stem cells for regenerative medicine. *Stem Cells Dev* 24(19):2219–2242
- Papanikolaou E, Pappa KI, Anagnou NP (2011) Genetic manipulation of stem cells. *Gynecol Obstetric S6*:001
- Phillips MI, Tang YL (2008) Genetic modification of stem cells for transplantation. *Adv Drug Deliv Rev* 60(2):160–172
- Piao W, Wang H, Inoue M, Hasegawa M, Hamada H, Huang J (2010) Transplantation of Sendai viral angiopoietin-1-modified mesenchymal stem cells for ischemic limb disease. *Angiogenesis* 13(3):203–210
- Pinel CB, Pluhar GE (2012) Clinical application of recombinant human bone morphogenetic protein in cats and dogs: a review of 13 cases. *Can Vet J* 53:767–774
- Rejman J, Tavernier G, Bavarsad N, Demeester J, de Smedt SC (2010) mRNA transfection of cervical carcinoma and mesenchymal stem cells mediated by cationic carriers. *J Control Release* 147(3):385–391
- Rois CN, Skoracki RJ, Mathur AB (2012) GNAS1 and PHD2 short-interfering RNA support bone regeneration in vitro and in an in vivo sheep model. *Clin Orthop Relat Res* 470(9):2541–2553
- Schnabel L, Lynch M, Van der Meulen M, Yeager A, Kornatowski M, Nixon A (2009) Mesenchymal stem cells and insulin-like growth factor-I gene-enhanced mesenchymal stem cells improve structural aspects of healing in equine flexor digitorum superficialis tendons. *J Orthop Res* 27:1392–1398
- Sharma N, SWK DLH, Ghosh M, Sodhi SS, Singh AK, Kim NE et al (2017) A PiggyBac mediated approach for lactoferricin gene transfer in bovine mammary epithelial stem cells for management of bovine mastitis. *Oncotarget* 8(61):104272
- Song J, Kim Y, Kweon O-K, Kang B-J (2017) Use of stem-cell sheets expressing bone morphogenetic protein-7 in the management of a nonunion radial fracture in a toy poodle. *J Vet Sci* 18(4):555–558
- Tang TT, Lu B, Yue B, Xie XH, Xie YZ, Dai KR, Lu JX, Lou JR (2007) Treatment of osteonecrosis of the femoral head with hBMP-2-gene-modified tissue-engineered bone in goats. *J Bone Joint Surg Br* 89(1):127–129
- Tseng TC, Hsu SH (2014) Substrate-mediated nanoparticle/gene delivery to MSC spheroids and their applications in peripheral nerve regeneration. *Biomaterials* 35(9):2630–2641
- Wagner W, Ho AD (2007) Mesenchymal stem cell preparations—comparing apples and oranges. *Stem Cell Rev* 3:239–248

- Watkins DJ, Zhou Y, Matthews MA, Chen L, Besner GE (2014) HB-EGF augments the ability of mesenchymal stem cells to attenuate intestinal injury. *J Pediatr Surg* 49(6):938–944
- Wright EJ, Farrell KA, Malik N, Kassem M, Lewis AL, Wallrapp C, Holt CM (2012) Encapsulated glucagon-like peptide-1-producing mesenchymal stem cells have a beneficial effect on failing pig hearts. *Stem Cells Transl Med* 1(10):759–769
- Yan G, Fan Y, Li P, Zhang Y, Wang F (2015) Ectopic expression of DAZL gene in goat bone marrow-derived mesenchymal stem cells enhances the trans-differentiation to putative germ cells compared to the exogenous treatment of retinoic acid or bone morphogenetic protein 4 signalling molecules. *Cell Biol Int* 39(1):74–83
- Zhang YL, Li PZ, Pang J, Wan YJ, Zhang GM, Fan YX, Wang ZY, Tao NH (2019) Induction of goat bone marrow mesenchymal stem cells into putative male germ cells using mRNA for STRA8, BOULE and DAZL. *Cytotechnol* 71(2):563–572
- Zhu S, Zhang B, Man C, Ma Y, Hu J (2011) NELL like molecule-1 modified bone marrow mesenchymal stem cells/poly-lactic-co-glycolic acid composite improves repair of large osteochondral defects in mandibular condyle. *Osteoarthritis Cartilage* 19:743–750
- Zhu S, Zhang T, Sun C, Yu A, Qi B, Cheng H (2013) Bone marrow mesenchymal stem cells combined with calcium alginate gel modified by hTGF- β 1 for the construction of tissue-engineered cartilage in three-dimensional conditions. *Exp Ther Med* 5(1):95–101
- Zhu X, Liu Z, Deng W, Zhang Z, Liu Y, Wei L, Zhang Y, Zhou L, Wang Y (2017) Derivation and characterization of sheep bone marrow-derived mesenchymal stem cells induced with telomerase reverse transcriptase. *Saudi J Biol Sci* 24:519–525



Sheep Mesenchymal Stem Cell Basic Research and Potential Applications

7

M. B. Gugjoo and Amar Pal

Abstract

Mesenchymal stem cells (MSCs) act as reservoir of repair system in an adult tissue. These cells are available in almost all the tissues. The cells have been harvested and isolated from numerous tissue sources. Due to their miniscule number in tissues culture expansion followed by characterization becomes imperative. Sheep MSCs (sMSCs) too have been obtained from numerous sources including foetal membranes. These cells have been evaluated in various human translational models and have variably proven to be useful. Such studies additionally form basis of the MSCs therapeutic applications in veterinary practice. The present chapter throws light on various aspects of sheep mesenchymal stem cells including their potential therapeutic applications.

7.1 Introduction

Veterinary and human medicine is intricately dependant and linked to each other, forming important constituents of “One Health” initiative (Gugjoo et al. 2018). Various ailments among the animals and human have similar pathophysiology and established therapeutics in one species can form basis of its applications in other (Gugjoo et al. 2018). Sheep acts as a suitable model animal for various ailments of epithelium, cardiovascular, skeletal and neurological systems for human (DiVincenti

M. B. Gugjoo

Division of Veterinary Clinical Complex, FVSc & AH, SKUAST-Kashmir,
Jammu and Kashmir, India

A. Pal (✉)

Division of Surgery, Indian Veterinary Research Institute, Izatnagar, UP, India

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2020

M. B. Gugjoo, A. Pal (eds.), *Mesenchymal Stem Cell in Veterinary Sciences*,
https://doi.org/10.1007/978-981-15-6037-8_7

et al. 2014; Pelagalli et al. 2018). Sheep being neurologically less developed than carnivores and equines has an edge in the particular area. Sheep body features like long bone size and its macrostructure structure are approximate to that of human (Anderson et al. 1999; Van Der Donk et al. 2001). Due to their comparable bone biomechanics, biochemistry and histology sheep bone fracture healing turn over markers may be adopted for human (Sousa et al. 2015). Sheep have sufficient superficial space on their backs that may be utilized to study MSCs regeneration potential in experimental lesions (Chevrier et al. 2009; Music et al. 2018; Yr et al. 2018). The current chapter details sheep mesenchymal stem cell (sMSCs) characteristics and their potential therapeutic applications.

7.2 MSCs Characterization

sMSCs are characterized as per the recommendations of International Society for Cellular Therapy (ISCT) (Dominici et al. 2006). Plastic adherent sMSCs show surface marker(s) expression, although variably and differentiate into adipogenic, chondrogenic and osteogenic lineages (Table 7.1) (Colosimo et al. 2013; Liu et al. 2014 Tian et al. 2016).

Sheep bone marrow derived mesenchymal stem cells (sBM-MSCs) grow as morphologically large adherent cells and exhibit a fibroblastic morphology. sBM-MSCs are bigger (diameter: $18.1 \pm 3.3 \mu\text{m}$ volume: $3.4 \pm 1.7\text{pl}$) than the mononuclear cells (diameter: $8.6 \pm 1.0 \mu\text{m}$ in volume: $0.34 \pm 0.12\text{pl}$) (Caminal et al. 2017). Cell morphology under transmission electron microscopy (TEM) had appeared as cuboidal electron-lucent and spindle shaped electron-dense cells similar to human MSCs. The surface marker expression especially CD90 was comparable in these cell types. The characterization of glycoprotein secretory system in these cells demonstrates their potential secretory properties (Desantis et al. 2015).

7.3 sMSCs Number and Proliferation

Different tissue sources have variable sMSCs population and proliferation potential. These cells become senescent after certain passages. Among various sources, sheep peripheral blood harboured lowest number of MSCs (sPB-MSCs) and had shown lower ability to proliferate (Lyahyai et al. 2012). MSCs from various sources may have different proliferation properties. Comparative studies have demonstrated that sBM-MSCs had higher proliferation potential as compared to cotyledon derived MSCs (Cot-MSCs) (Ribitsch et al. 2017) and sheep adipose tissue MSCs (sAD-MSCs) (Heidari et al. 2013). Apart from source based differences, culture expansion may also affect their proliferation. In initial passages, sheep dermis derived MSCs (sD-MSCs) in comparison to sBM-MSCs may show lower proliferation potential but that becomes higher with extended passaging (Jahroomishirazi et al. 2014). Likewise sheep liver-MSCs had shorter population doubling time as compared to sAD-MSCs (Heidari et al. 2013). Further incites in this regard are required to confirm the reason behind such variabilities.

Table 7.1 Sheep mesenchymal stem cell (MSC) sources and their characteristics (Gugjoo and Amarpal 2018)

Positive markers	Negative markers	Differentiation characteristics	References
<i>Adipose tissue MSCs (AD-MSCs)</i>			
CD29, CD44	–	–	Grzesiak et al. (2011)
CD90, CD105, CD73, CD29	HLA-DR, CD45, CD34, STRO-1	Adipogenic, chondrogenic, osteogenic	Zorzi et al. (2015)
CD44, CD90	CD31, CD45		Vahedi et al. (2016)
CD44	CD31, CD45	Adipogenic, chondrogenic, osteogenic	Feng et al. (2018)
<i>Amniotic fluid MSCs (am-MSCs)</i>			
CD13, CD29, CD44, CD90, CD106, OCT4	CD45	Adipogenic, chondrogenic, osteogenic	Tian et al. (2016)
CD44, CD58, CD166	CD14, CD31, CD45	–	Shaw et al. (2011)
<i>Amnion MSCs (A-MSCs)</i>			
CD29, CD44, CD105, CD90	CD31	Adipogenic, osteogenic	Klein et al. (2011)
CD166 antigen at low levels and CD29 and CD58 intermediate levels	CD14, CD31, CD45, CD49f	–	Colosimo et al. (2013)
<i>Bone marrow MSCs (BM-MSCs)</i>			
CD44, CD105, Vimentin	CD45, CD34	Adipogenic, chondrogenic, osteogenic	Mrugala et al. (2008)
CD29, CD44, CD166	CD14, CD31, CD45	Adipogenic, chondrogenic, osteogenic	Mccarty et al. (2009)
CD9, CD44, CD54, CD73, CD90, CD105, CD166	CD45	–	Rentsch et al. (2010)
CD44	CD34	Adipogenic, chondrogenic, osteogenic	Czernik et al. (2013)
CD44, CD90 (low expression)	CD45, HLA-DR	Adipogenic, chondrogenic, osteogenic	Caminal et al. (2014)
CD44, CD58, HLA-I, CD90	CD31, CD34, CD45, HLA-DR	–	Desantis et al. (2015)
CD29, CD36, CD73, CD90, CD166, CD29	CD45 and CD34 (variable expression)	Adipogenic, chondrogenic, osteogenic, neuronal like cells (downregulation)	Mediano et al. (2015a, b)
CD44, CD90, CD140a, CD105 and CD166	CD45, CD73	Adipogenic, chondrogenic, osteogenic	Caminal et al. (2017)
CD146	CD34, CD45	Adipogenic, chondrogenic, osteogenic	Hindle et al. (2016)

(continued)

Table 7.1 (continued)

Positive markers	Negative markers	Differentiation characteristics	References
CD73 (96.9 ± 5.9), CD90 (99.6% ± 0.3), CD105 (99.16 ± 1.5), CD271 (97.762.0) and MHCII (94.0% 67.2)	Negative (CD117 (0.1%60.1) or low (MHCII (10.5% ± 16.0); CD31 (14.6% ± 4.2) and CD45 (39.4% ± 31.8)	Adipogenic, chondrogenic, osteogenic	Khan et al. (2016)
CD90	–	Adipogenic, chondrogenic, osteogenic	Landa-Solis et al. (2016)
CD44, CD90, CD140a, CD105 and CD166	–	Adipogenic, chondrogenic, osteogenic	Vivas et al. (2018)
<i>Cotyledonary MSCs</i>			
CD29, CD33 and CD166	CD31, CD45	Adipogenic (weak), chondrogenic, osteogenic	Ribitsch et al. (2017)
<i>Dermal MSCs (D-MSCs)</i>			
β-integrin, CD71, CD44 and CD73	–	Adipogenic, chondrogenic, osteogenic, neurogenic	Cui et al. (2014)
CD271	CD45	Adipogenic, chondrogenic, osteogenic	Jahroomishirazi et al. (2014)
<i>Endometrium MSCs (end-MSCs)</i>			
CD271	CD49f	Adipogenic, chondrogenic, osteogenic, smooth muscle	Letouzey et al. (2015)
<i>Kidney MSCs (K-MSCs)</i>			
Oct-4, VIM, CD44, FN1, CD73, CD44 and PAX2	CD34	Adipogenic, chondrogenic, hepatocellular	Ji et al. (2016)
<i>Lung MSCs (L-MSCs)</i>			
Oct4, Nanog, CD29, CD44, CD71, CD73 and CD90	CD34, CD45	Adipogenic, chondrogenic, osteogenic, hepatocyte	Ma et al. (2017)
<i>Periodontal ligament MSCs (PL-MSCs)</i>			
CD106, CD44, CD166, CBFA-1, collagen-I, bone sialoprotein	–	–	Gronthos et al. (2006)
<i>Peripheral blood MSCs (PB-MSCs)</i>			
CD29, CD73, CD90 and CD105 (low expression)	CD45, and CD34 (variable expression)	Adipogenic, chondrogenic, osteogenic, neurogenic	Lyahyai et al. (2012)
CD29, CD36, CD73, CD90, CD166, CD105	CD45 and CD34 (variable expression)	Adipogenic, chondrogenic, osteogenic, neuronal like cells (downregulation)	Mediano et al. (2015a, b)
Stro-1, CD90, CD106, CD105, CD146, CD166, CD44	CD34, CD45	Adipogenic, Chondrogenic, Osteogenic,	Hopper et al. (2015)

(continued)

Table 7.1 (continued)

Positive markers	Negative markers	Differentiation characteristics		References
Synovial MSCs (Sy-MSCs)				
CD73, CD90, CD105	–	Adipogenic, chondrogenic, osteogenic		Burk et al. (2017)
Umbilical cord MSCs (UC-MSCs)				
CD44	CD38, CD45, CD41/61.	–		Fadel et al. (2011)
CD29, CD44, CD73, CD90	CD34	Adipogenic, chondrogenic, osteogenic		Chen et al. (2018)
Placental MSCs (Pl-MSCs)				
CD29, CD44, CD73, CD90, CD105, Sox2	CD31, CD45	Early stage gestation	Adipogenic, chondrogenic, osteogenic	Brown et al. (2016)
CD29, CD44, CD73, CD90, CD105	CD31, CD45, Sox2	Late stage gestation		

AD-MSCs adipose tissue MSCs, *Am-MSCs* amniotic fluid MSCs, *A-MSCs* amnion MSCs, *BM-MSCs* bone marrow MSCs, *D-MSCs* dermal MSCs, *End-MSCs* endometrium MSCs, *K-MSCs* Kidney MSCs, *L-MSCs* lung MSCs, *Pl-MSCs* periodontal ligament MSCs, *PB-MSCs* peripheral blood MSCs, *Sy-MSCs* synovial MSCs, *UC-MSCs* umbilical cord MSCs, *Pl-MSCs* placental MSCs

sMSCs proliferation potential decreases with extended passaging (Colosimo et al. 2013; Ji et al. 2016; Tian et al. 2016). sAD-MSCs and umbilical cord derived MSCs (sUC-MSCs) had normally proliferated up to passage 6 (Vahedi et al. 2016) and passage 10 (Chen et al. 2018), respectively, and became senescent afterwards. This depicts variability in proliferation abilities of the sMSCs at different passages, though various other factors like culture conditions and donor tissue type may also affect these properties.

sMSCs karyotypic features may remain unaffected in >90% of the cells up to as high as 20 passages (Ji et al. 2016; Tian et al. 2016). The cells (sD-MSCs) harvested from young ones (1 month old) may show even more extended passaging (48 passages) with normal karyotype (Cui et al. 2014). Among various reasons of limited self-renewal property, telomere length shortening with cell proliferation appears to be important. This has been confirmed in telomerase reverse transcriptase (TERT) transfected sBM-MSCs. These transfected cells could be cultured up to 40 passages with normal karyotype rate of 88.24% (Zhu et al. 2017).

7.4 Tissue Source and Marker Expression

sMSCs express various surface markers and lack haematopoietic markers. Variability in surface markers of the sMSCs with respect to the tissue source has been demonstrated (Table 7.1). The variability in marker expression is attributed to the lack of species specific antibodies, besides differences in the type of tissue sources and harvesting methods (Gugjoo et al. 2020a, b). Additionally, sMSCs have also been demonstrated to express various pluripotency markers like Oct4, Nanog and Stro-1 (Hopper et al. 2015; Brown et al. 2016; Ji et al. 2016; Ma et al. 2017).

7.5 Source and Differentiation Potential

sMSCs exhibit variability in their differentiation potential on the basis of their tissue source. The musculoskeletal differentiation potential of sCot-MSCs and sBM-MSCs had been akin but sBM-MSCs adipogenic potential was more pronounced (Ribitsch et al. 2017). Foetal MSCs usually show low PPAR γ pathway and as such may be less demanding for adipogenic activity (Ragni et al. 2013). Among various musculoskeletal tissues, sheep synovial fluid derived MSCs (sSy-MSCs) had shown higher chondrogenic expression in comparison to sBM-MSCs (Burk et al. 2017). However, MSCs from adipose tissue, bone marrow and liver-MSCs had comparable differentiation activity (Heidari et al. 2013). Such variabilities in differentiation potential of MSCs with respect to source need further investigation especially on molecular basis. This may help to better utilize MSCs for therapeutic applications.

7.6 Miscellaneous Factors Affecting MSCs Characteristics

Scrapie infected sheep carry MSCs with normal tri-lineage differentiation ability. However, their proliferation and neurocyte like cell differentiation may be reduced (Mediano et al. 2015a, b) and as such may offer limited therapeutic benefits in neural ailments. The cellular characteristics of MSCs may also be influenced by the factors like feeding standard, age of the animal and disease status of the animal. Low feeding status of ewe may affect their foetal MSCs characteristics (Pillai et al. 2016). Ageing may or may not decrease MSCs proliferation and clonogenic potential (Rhodes et al. 2004; Han et al. 2010). In sheep unlike dog, breed variation had non-significant effect on BM-MSCs proliferation (Rhodes et al. 2004).

7.7 Potential Therapeutic Applications

MSCs in sheep have been implanted through various routes including parenteral considering their homing property (Gugjoo and Amarpal 2019). MSCs secretory properties and differentiation in particular tissue is being determined by the available local milieu. The cells (including of xenogenic and/or allogeneic sources) have been well tolerated under *in vivo* conditions. In a pre-immune sheep foetus, xenogenic MSCs (human source) had differentiated into hepatic cells upon intrahepatic/intraperitoneal implantation (Chamberlain et al. 2007) or secreted insulin upon pancreatic transplantation (Ersek et al. 2011). Xenogenic MSCs too had been demonstrated to have tissue specific trans-differentiation even in immuno-potent sheep (Liechty et al. 2000; Mackenzie and Flake 2001; Shaw et al. 2011). Likewise, allogeneic applications of these sMSCs had been demonstrated to be safe (Berner et al. 2013; Dooley et al. 2015; McDonald et al. 2015; Zorzi et al. 2015). Apart from being immuno-compromised, the cells have been demonstrated to have immune-modulatory properties (Dooley et al. 2015). Considering such immuno-compromised and immuno-modulatory properties, allogeneic MSCs are increasingly being studied to make ready-to-use therapy possible.

sMSCs have been mainly studied in experimental human translational models without any direct sheep clinical trial (Gugjoo and Amarpal 2018). This poses an impediment in their definitive therapeutic applications in veterinary practice, in general and in sheep clinical practice, in particular. For *in vivo* identification, sMSCs may be labelled with superparamagnetic iron oxide (SIPO), though its concentration must be standardized (Scharf et al. 2015).

7.7.1 Wound Healing

In foetal tissues wound healing occurs through regeneration and in adults by repair (Klein et al. 2011). Sheep has been chosen as wound model as it has sufficient space on back and limited neurological development in comparison to the canine and equine (Martinello et al. 2018). In utero implanted xenogeneic or autologous MSCs have been demonstrated to home to the site of foetal wounds. The implanted cells have been able to expedite wound healing by increasing the secretion of extracellular matrix (Table 7.2) (Mackenzie and Flake 2001; Klein et al. 2011). Apart from foetal wounds, adult sheep wounds too tend to heal faster with allogeneic PB-MSCs. The wound had closed within 15 days and might have been due to the cell induced inflammation inhibition and enhanced re-epithelialization, neo-vascularization and contraction. These treated wounds had significantly higher expression of hair-keratine (hKER) and collagen type I (Col1 α 1) being observed from day 15 up to day 42 (Martinello et al. 2018). It may be concluded that MSCs application are quite promising for wound management. Further studies are desired to confirm the posology of the MSCs applications.

7.7.2 Osteochondral Defects

An extensive literature on MSCs osteochondral studies exist. The results have been mostly favourable and without any serious adverse effect as detailed in Table 7.3. A single study however had failed to demonstrate any obvious improvement with MSCs as compared to the control (Delling et al. 2015a).

MSCs intra-articular implantation remains safe (Caminal et al. 2014; Abdalmula et al. 2017). Intra-articularly implanted cells remain viable and attached to the joint structures (Delling et al. 2015b; Feng et al. 2018; Markides et al. 2019). The available inflammatory environment however, may reduce their chondrogenic potential, although without affecting their phenotype (Ando et al. 2012). MSCs possible pathway of neo-cartilage formation may be ensued via endochondral ossification process (Lydon et al. 2019). MSCs tend to undergo hypertrophic chondrocyte formation and may lead to osteogenesis (Gugjoo et al. 2018).

Intravenous implantation of BM-MSCs had been able to reduce lameness, joint pain and swelling in a sheep mono-arthritis model. The experimental joints had less cartilage erosions, limited inflammatory cells and were supplemented with activated stromal cells and angiogenesis (Abdalmula et al. 2017). In chronic induced osteoarthritis models, improved parameters (histological and/micro-CT scores) of healed

Table 7.2 In vivo preclinical experimental mesenchymal stem cell studies on cutaneous wounds

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
In utero tracking of cells to wounds or developing organs	29 animals	13 months	Xenogeneic (human) MSCs	5–20 × 10 ⁶ early gestation (65 days gestation); 1 × 10 ⁸ cells/kg (mid-gestation, 85 days gestation)	Polymerase chain reaction (PCR), immuno-histochemistry and in situ hybridization	PCR confirmed cellular engraftment to one or more tissues. Relatively large engraftment was seen in wounded areas. Cells persisted in foetal tissue up to a year	Mackenzie and Flake (2001)
Full thickness square wound (4 × 4 cm, six wounds)	Six wounds (three wounds treated with conventional topical cream or gel, four with cold ionized plasma, fifth with PBS and sixth with MSCs)	15 and 42 days	Allogeneic PB-MSCs, hyaluronic acid	1 × 10 ⁶ (injected in wound margins), 1 × 10 ⁶ (loaded with hyaluronic acid and implanted at wound centre)	Clinical, histopathological, and molecular analysis	At 15 days post-lesion, the wounds treated with MSCs showed a higher degree of wound closure, a higher percentage of re-epithelialization, proliferation, neo-vascularization and increased contraction in comparison to a control group. Later, the wounds treated with MSCs had more mature and denser cutaneous adhexa as compared to the control group with absence of inflammation and expression of CD3+ and CD20+. Moreover, the mRNA expression of hair-keratine (hKER) and collagen I was observed in the MSCs treated group 15 days	Martinello et al. (2018)

Table 7.3 In vivo chondrogenic preclinical experimental mesenchymal stem cell studies in sheep

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Medial femoral condyle defect	28 ($n = 16$, cell + β -TCP; $n = 8$ in β -TCP only and $n = 4$ in control)	8 mm (diameter) and 4 mm (depth)/24 weeks	Autologous BM-MSCs + beta-tricalcium phosphate (β -TCP)	3×10^7	Macroscopic observation, histological, immunohistochemical, biochemical analysis	Experimental animal defects were resurfaced with hyaline-like tissue. An ideal interface formed between the engineered cartilage, adjacent normal cartilage and the underlying bone	Guo et al. (2004)
Osteonecrosis of femoral head	8 animals ($n = 4$ in control group; $n = 2$ each in sheep MSC group and human MSC group treated after 8 weeks of induced necrosis)	10 ml of absolute ethanol induced	Sheep BM-MSCs (transfected) and human dental stem cells	1×10^6 (each cell type)	Light microscopy	Better bone regeneration in cell treated group animals	Feitosa et al. (2010)
Chronic model of medial femoral condyles osteochondral lesions	10 animals ($n = 40$ defects; group I: chondrogenically differentiated MSC/hydrogel constructs; group II: Undifferentiated ovine MSC/hydrogel constructs; group III: Cell-free hydrogel; group IV: control)	7 mm/6 months	Autologous BM-MSCs/collagen I hydrogel constructs	4×10^5 MSCs mixed with collagen I	Histopathology	Group I had significantly better histologic scores with morphologic characteristics of hyaline cartilage such as columnarization and presence of collagen type II as compared to others. However, each group showed variability in results	Zscharnack et al. (2010)

(continued)

Table 7.3 (continued)

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Chronic model of anterior cruciate ligament excision	16 animals ($n = 6$ animals in treatment group I; pre-differentiated MSCs and group II: undifferentiated MSCs) and $n = 4$ (control group)	6 weeks	Chondrogenically differentiated MSCs or undifferentiated MSCs	10×10^6 per joint	Gross, histological and clinical observation	Retardation of osteoarthritis in cell treated groups. Non-significant difference in group I and II except for macroscopic observations of meniscus repair. Severe osteoarthritis in control	Al Faqeh et al. (2012)
Chronic model full thickness medial femoro-tibial condyles and meniscal tear	10 ($n = 20$ defects; 10 studied at 6 months period while other 10 at 12 months period) one of the limbs remained control	60 mm defect size/6 month or 12 months	BM-MSCs	1.1×10^7 (6 month period animals) or 1.2×10^7 (12 month period animals)	Radiography, MRI, ultrasound, macroscopic and histological analyses	Regeneration of articular cartilage and meniscus was case-dependent but statistically significant improvement was found in specific macroscopic and histological parameters	Caminal et al. (2014)

Medial femoro-tibial condyle defect	9 (<i>n</i> = 18 defects)	7 mm defect size/4 months and 12 months	BM-MSCs alone or seeded on co-polymeric poly-lactide:Polglycolic acid scaffolds either	$3.3 \times 10^6 \pm 0.4 \times 10^6$ cells	Biomechanical testing, macroscopic and histological analyses	Better macroscopic scores at 4 months in cell treated as compared to 12 months evaluation period. Non-significant histopathological scores at 12 months between cell treated and cell-free groups	Caminal et al. (2014a)
Chronic anterior cruciate ligament transection and medial meniscectomy	18 (<i>n</i> = 6 animals in each group) group I: BM-MSCs; group II: Bone marrow mononuclear cells; group III: Control	8 weeks	Autologous BM-MSCs	10 x 10 ⁶ after 12 weeks of model creation	Macroscopically and histologically, and glycosaminoglycan (GAG) contents, gene expression levels (collagen II, Aggrecan and matrix metalloproteinase-13), tumour necrosis factor- α (TNF- α) and transforming growth factor beta	Significantly higher cartilage regeneration and lower proteoglycan loss in group I than group II. Comparable inhibition of PGE ₂ , TNF- α and TGF- β levels in synovial fluid and promotion of higher levels of aggrecan and col II in two cell treated groups. Downregulation of MMP-13 also comparable. Both the cell treated groups had significantly better cartilage than control	Song et al. (2014)
(continued)							

Table 7.3 (continued)

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Full thickness lateral femoral condyle defect	12 animals ($n = 3$ in each group); group I (amniotic membrane); group II (cryopreserved amniotic membrane previously cultivated with BM-MSCs); Group III (cryopreserved amniotic membrane alone); group IV (control)	7×5 mm/8 weeks	BM-MSCs and amniotic membrane	2×10^6 cells and amniotic membrane	Gross and histopathology	Significant difference in healing between treatment and control groups. Non-significant differences between treatment groups. The samples of groups II, III and IV reported a ICRS grade II (similar to healthy cartilage). The mean value of O'Driscoll scale in the control group (3.3) was significantly lower as compared to the treatment groups (group II: 10.7; G III: 8; G IV: 11.3). Non-significant differences were found between the experimental groups	Garcia et al. (2015)
Partial thickness medial femoral condyle defect	15 animals ($n = 30$ knees (group I: Scaffold plus cell; group II; scaffold only; group III control)	10 mm/6 months	Xenogenic AD-MSCs and collagen/chitosan scaffold	1×10^6 along with scaffold	Microscopic and macroscopic analysis	Significantly higher histological scores in cell treated group as compared to others	Zorzi et al. (2015)

Unilateral medial meniscectomy	20 (group I: $n = 6$ animals, BM-MSCs + scaffold; group II: $n = 6$ animals BM concentrate + scaffold; group III: $n = 4$ animals scaffold treated group IV: $n = 4$ animals, control)	12 weeks	BM-MSCs + scaffold (Hyaff [®] -11) and BM concentrate + scaffold (Hyaff [®] -11)	6×10^6 seeded on scaffold	Macroscopy, histology, immuno-histochemistry and micro-computed tomography	BM concentrate better inhibited inflammation in cartilage, meniscus and synovium. It also improved cartilage healing. Subchondral bone thickness decreased in both the cell treated groups	Desando et al. (2016)
Meniscal cartilage tear model	30 animals (three groups with $n = 10$ animals in each group): Group I: Scaffold laden MSCs; group II: Scaffold only and group III: Suturing only	5 mm $\times$ 3 mm/13 weeks and 6 months	BM-MSCs collagen I scaffold	$1 \times 10^6/\text{cm}^2$	Macroscopy and histopathology	Statistically significant improvement in cell treated as compared to control at 13 weeks. But no difference at 6 months period	Whitehouse et al. (2017)
Anterior cruciate ligament resection and medial meniscectomy	24 ($n = 6$ in each group) group I: Control; group II: HA; group III: Low dose AD-MSCs + HA; group IV: High dose MSCs + hyaluronic acid	14 weeks after treatment	Allogeneic AD-MSCs and hyaluronic acid	5×10^7 (high dose) at 3 weeks and $(1 \times 10^7$ cells (low dose) at 6 weeks	Magnetic resonance imaging (MRI), macroscopy, micro-computed tomography and cartilage-specific staining	AD-MSCs + hyaluronic acid could efficiently block osteoarthritis progression and promoted cartilage regeneration	Feng et al. (2018)

tissue had been observed with the implantation of BM-MSCs (Caminal et al. 2014) and AD-MSCs (Feng et al. 2018). The variable concentrations of AD-MSCs implanted along with hyaluronic acid at 3 and 6 weeks post injury were viable for more than 2 months and had restricted osteoarthritis progression (Feng et al. 2018). Even xenogenic AD-MSCs (human) along with scaffold (chitosan/collagen) had significantly improved ICRS-I score in a femoral condyle partial thickness defect as compared to the scaffold treated and control (Zorzi et al. 2015).

The hyaline tissue formation has not been reported in all the studies and individual cases with some demonstrating chondroid metaplasia. The integration of healed tissue to native cartilage too had been the problem (Caminal et al. 2014). It may thus, be concluded that MSCs may repair the osteochondral injuries but does not guarantee the same (Gugjoo et al. 2020a, b).

One of the sheep osteoarthritis model studies had failed to yield improved results at 12 weeks post autologous MSCs implantation (Delling et al. 2015a). This may have been due to the weak joint injury failing to induce obvious osteoarthritic changes or else due to the less concentration of implanted cells (Feng et al. 2018).

7.7.2.1 Comparative osteochondral Studies

The *ex vivo* studies have demonstrated higher chondrogenic gene expression of BM-MSCs cultured under hypoxia (chondrogenically primed) as compared to the normoxia cultured BM-MSCs (Bornes et al. 2017). These chondrogenically primed MSCs however, have failed to yield any significant improvement in tissue healing as compared to non-primed cells (Al Fagheh et al. 2012; Bornes et al. 2017). The cryopreservation does not seem to have any adverse effect on MSCs. Amniotic-MSCs as fresh and cryopreserved cells had shown comparable healing in a sheep full thickness lateral femoral condyle defect model (Garcia et al. 2015).

Cultured BM-MSCs and AD-MSCs tend to have an edge over bone marrow mononuclear cells (MNCs) or stromal vascular fraction (SVF), respectively. Implantation of both the former cultured cell types had shown improved cartilage regeneration together with little loss of proteoglycan in a sheep osteoarthritis model (Song et al. 2014; Lv et al. 2018) in comparison to the non-cultured cell mix. The mononuclear cells though still had some positive impact on healing as compared to control. The inflammatory mediators were inhibited in synovial fluid (Song et al. 2014). Since MNCs and SVF contain little concentration of MSCs, comparably little beneficial effects with these cell types may be expected. Contrarily, bone marrow concentrate (BMC) along with hyaluronan had shown greater repair ability in comparison to the BM-MSCs/hyaluronan. However, both cells types had reduced inflammation and thus, may be effective to prevent fibrosis and hypertrophy (Desando et al. 2016). Scaffolds as such may play an important role in stem cell effectiveness.

There is need to conduct studies that incorporate similar source, passage cells at uniform concentrations. The studies have to be case controlled with an extended study period. Furthermore, role of incorporated scaffolds need to be considered.

Comparative studies against the established surgical techniques like microfracture must also be made to evaluate feasibility and efficacy of MSC therapy.

7.7.3 Bone Defects

Bone tissue engineering is increasing by each passing day to ensure early and better healing (Chandran et al. 2017). MSCs due to their characteristic properties are being made subject. Most of the sheep preclinical experimental bone model studies have shown better and early bone healing upon MSCs implantation as enlisted in table (Table 7.4). One of the sheep study had shown early osteogenic synthesis with scaffold laden sMSCs, implanted subcutaneously (Boos et al. 2014). Apart from culture expanded MSCs, bone marrow mononuclear cells (contains MSCs) along with sheep bone mineral too have favoured improved sinus augmentation at 8 and 16 weeks in comparison to the autogenous graft (Gutwald et al. 2010).

MSCs bone healing potential has been evaluated in different sheep bone models. Among various bones studied are metatarsal-, tibial- mandible- and cranial-critical bone defects. Mostly autologous or allogeneic BM-MSCs have been evaluated in these studies. BM-MSCs along with coral granules (Viateau et al. 2007; Manassero et al. 2013), cortical allograft (Fernandes et al. 2014), polycaprolactone and hydroxyapatite (Berner et al. 2015), serum scaffold (Gallego et al. 2015) and calcium alginate (Shang et al. 2001) have been implanted to evaluate healing potential in critical size bone defects. The implantation of scaffold laden cells had led to an improved early bone healing with better structural and morphological features (Fernandes et al. 2014; Gallego et al. 2015). The healed tissue had better quality and approximated to normal bone tissue (Gallego et al. 2015). The healed tissue strength could commensurate with clinical standards (Berner et al. 2015). In all these cell and scaffold treated defects healing was much improved as compared to those of scaffold treated. Incorporation of growth factor like rhBMP-2 to BM-MSCs and/ autologous serum loaded on to the scaffold and implanted subcutaneously had led to subcutaneous ossification (Boos et al. 2014).

Cell immunogenicity may or may not affect healing potential of MSCs. Autologous and allogeneic MSCs had shown comparable tibial bone healing (Berner et al. 2013) while xenogeneic BM-MSCs had failed to improve sheep tibial bone healing (Niemeyer et al. 2010). Mononuclear cells (MNCs) may show improved healing over raw bone marrow concentrate (Ardjomandi et al. 2015). MSCs may also be effective to repair osteoporotic bone (Chandran et al. 2017). MSCs along with the tricalcium phosphate scaffold or graft may also be used to promote interbody spinal fusion and spinal arthrodesis (Table 7.4).

Combination of MSCs and other implants/scaffolds like TiAl6V4 constructs with a calcium-phosphate coating (Garcia-Gareta et al. 2015) and polyetherketoneketone (Adamzyk et al. 2016) had failed to enhance healing in a sheep trabecular bone and calvarial defects, respectively. The scaffolds although had favoured *in vitro* cell adhesion, growth and osteogenic differentiation of MSCs (Adamzyk et al.

Table 7.4 In vivo preclinical experimental osteogenic mesenchymal stem cell studies in sheep

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose/assembly	Evaluation criteria	Overall result	References
Tibial defects	4 animals (cell-loaded implants, $n = 2$; cell-free HAC cylinders, $n = 2$)	2-month	Autologous BM-MSCs, hydroxyapatite ceramic (HAC)	$0.5\text{--}1.0 \times 10^6$ in fibrinogen	Indentation assay	In cell-loaded implants, bone formation occurred both within the internal macropore space and around the HAC cylinder while in control cell-free implants, bone formation was limited mostly to the outer surface and was not observed in most of the inner pores. As tested in an indentation assay, the stiffness of the complex HAC-bone material was found to be higher in cell-loaded implants as compared to controls	Kon et al. (2000)
Cranial bone defect (parietal bone, 20 mm in diameter, bilateral)	8 animals with 16 defects (MSCs and calcium alginate, $n = 8$; calcium alginate, $n = 4$; control unrepaired, $n = 4$)	6 weeks and 18 weeks post repair	Autologous BM-MSCs and calcium alginate	–	Gross, histological and computerized tomography (CT) scan	New bone tissues were observed with MSCs/scaffold as early as 6 weeks post-repairing but not in control groups. The engineered bone tissue became more mature at 18 weeks post-repairing. CT scan revealed an almost complete repair of the defect of MSCs/scaffold at 18 weeks	Shang et al. (2001)

Left metatarsal bone defect (25 mm) stabilized with plate and PMMA space being replaced after 6 weeks	21 animals (group I: Empty, $n = 5$; group II: Coral scaffold, $n = 4$; group III: Coral scaffold/MSCs, $n = 6$; autogenous cortico-cancellous graft, $n = 6$)	6 months	Autologous BM-MSCs	138 ± 22 (coral pieces), $8.28 \pm 1.32 \times 10^6$ (BM-MSCs)	Radiology, histology and CT	Non-significant differences in newly formed bone in defects were seen in coral/MSCs and autograft groups. Significant differences in radiological scores in MSCs/coral and autograft groups were seen (21% and 100%, respectively)	Viateau et al. (2007)
Postero-lateral lumbar fusion	24 (autograft (AG), $n = 6$; cell enriched tricalcium phosphate (TCP), $n = 6$; TCP alone, $n = 6$; TCP/10 ml bone marrow, $n = 6$)	6 months	Bone marrow (selective cell retention technology), tricalcium phosphate	–	Micro-CT, manual palpation and histology	33% and 25% of the MSCs and AG groups had fused vertebrae while bone marrow/TCP had 8% and TCP alone had 0% fusion	Gupta et al. (2007)
Critical sized tibial defect (stabilized by internal locking compression plate and external skeletal fixator)	45 animals ($n = 15$ in each group) autologous bone chips of iliac crest graft; ceramic scaffold only; ceramic scaffold + cells	20–24 weeks	Autologous BM-MSCs, silicon stabilized tricalcium phosphate	$70\text{--}100 \times 10^6$	Micro-radiography, histology and vascular density	Radiography revealed best healing in cell/scaffold treated group. Histology showed better vascular density in cell/scaffold group. Partial bone deposition occurred at periphery of bone stumps (scaffold group)	Giannoni et al. (2008)

(continued)

Table 7.4 (continued)

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Anterior cervical discectomy (C3-C4 and C4-C5)	18 animals (gelfoam sponge, $n = 6$; MSCs + gelfoam, $n = 6$; gelfoam sponge + MSCs + pentosan polysulphate, $n = 6$)	3 months	Allogeneic MSCs, Gelfoam sponge, pentosan polysulphate (PPS)	1×10^6	Radiology and histology	New bone formation in gelfoam sponge only and MSCs/gelfoam group was observed in 9/12, 11/12 animals, respectively. In group additionally implanted with PPS had significantly higher cartilaginous tissue within the interbody cages as compared to other groups. Only 1/12 in the PPS group had new bone formation	Goldschlager et al. (2010)
Critical sized tibial bone defect (3 cm, stabilized by plate)	21 animals (treated with mineralized collagen, $n = 7$; human MSCs, $n = 7$ and ovine MSCs, $n = 7$)	3 months ($n = 2$ each group) and 6 months ($n = 5$)	Human BM-MSCs and oBM-MSCs and mineralized collagen scaffold (matrices)	2×10^7 (oBM-MSCs or human BM-MSCs)	Radiology and histology	Radiology and histology revealed significantly better bone formation upon oBM-MSCs seeded on matrices implantation compared to matrices and human BM-MSCs seeded matrices implantation. However, human BM-MSCs did not evoke any local or systemic reaction	Niemeyer et al. (2010)

Critical size tibial segmental defect (3cm length and 8 mm diameter) (stabilized by interlocking nail)	24 animals (control scaffold only, $n = 12$; cell + scaffold)	9 months	BM-MSCs	225×10^6	Radiology, CT, biomechanical, histology	The cell treated group had significantly higher callus formation and rate of bone apposition in the defect	Field et al. (2011)
Cervical interbody fusion (C3-C4)	30 animals (autograft (AG) implantation, $n = 6$; hydroxyapatite/tricalcium phosphate (HA/TCP), $n = 6$; HA/TCP + 5million cells, $n = 6$; HA/TCP + ten million cells, $n = 6$; control unoperated sham, $n = 6$)	3 months	Allogeneic BM-MSCs + hydroxyapatite/tricalcium phosphate (HA/TCP)	5×10^6 and 10×10^6	CT, functional radiography, histomorphological, biomechanical analysis	9/12 of the cell/scaffold treated animals had continuous bony bridging while AG had 1/6 and HA/TCP had 2/6. New bone density was 121% higher in cell groups compared to HA/TCP and 128% as compared to AG groups. Functional radiography revealed significantly reduced macromotion between C3/C4	Goldschlager et al. (2011)
Critical sized tibial segmental bone defect	24 animals (6 in allogeneic MSCs treated + scaffold; 6 in autologous cell treated + scaffold; 6 scaffold only; 6 autologous graft)	12 weeks	BM-MSCs + scaffold (polycaprolactone + β -tricalcium phosphate)	35×10^6	Radiology, micro-computed tomography (micro-CT), biomechanical testing, histology	No significant differences in bone formation in allogeneic and autologous groups but better bone formation than scaffold treated. However, biomechanical testing failed to show any differences in bone formation in cell loaded and scaffold loaded	Berner et al. (2013)

(continued)

Table 7.4 (continued)

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose/assembly	Evaluation criteria	Overall result	References
Metatarsal bone defect (25 mm) supported by 3.5 mm dynamic compression plate	10 animals (scaffold, $n = 4$; MSCs on scaffold, $n = 5$)	6 months	BM-MSCs + acropora coral scaffold	$7.5 \pm 1.2 \times 10^6$	Micro-CT and histology	Two-fold increase in bone formation with cell + scaffold transplantation as compared to scaffold only at 6 months	Manassero et al. (2013)
Radial and tibial nerve section-resection (1 cm)	7 animals ($n = 2$ tibial nerve and $n = 2$ radial nerves: Treated with scaffold + PRP; $n = 4$ tibial nerve and $n = 2$ radial nerves; control $n = 1$ tibial nerve and $n = 3$ radial nerves: Scaffold + pooled human serum)	3 and 6 months	BM-MSCs, PRP and scaffold (neurolac ^{ex} biodegradable scaffold)	$30\text{--}50 \times 10^6$ (repair of nerve after 3 weeks)	Clinical evaluation, neuro-physiological study, morphology study	At 6 months significant myelinated nerve fibres and increased conduction in MSCs treated with or without PRP	Casanas et al. (2014)
Postero-lateral lumbar fusion (L4–L5)	34 animals (lumbar fusions: Treated with autograft $n = 17$ on right half and allograft on left half $n = 17$; hydroxyapatite $n = 17$ on left half; hydroxyapatite + cells (hybrid scaffold) on right half $n = 17$)	6 months	Osteo-induced BM-MSCs, scaffolds, mineral scaffold and hybrid scaffold	50×10^6	Clinical observation, radiography, CT, histology, histomorphometry	Lumbar fusion rates were higher for auto and allograft (70%) than mineral scaffold (22%) and hybrid scaffold (35%) based on histology and CT. Quantity of bone formation is also higher in autograft and allograft. Hybrid scaffold had higher but statistically insignificant differences from hydroxyapatite scaffold based on histology	Cuenca-Lopez et al. (2014)

Midshaft critical sized tibial bone defect	8 animals ($n = 4$ control; $n = 4$ treatment group)	18 weeks	Autologous BM-MSCs on allogeneic graft	6×10^7	Radiography, CT and histology	More conspicuous healing and union between host bone and allograft in cell treated group. Complete remodelling at 18 weeks in cell treated groups. Histologically marrow cavity re-established, intertrabecular spaces filled with adipose marrow	Fernandes et al. (2014)
Femoral defect (10 mm diameter and 3 cm deep)	12 animals (right femur treated with scaffold plus cells; left femur with scaffold)	8 and 16 week	BM-MSCs derived osteoblasts + β -TCP	2×10^6 loaded on β -TCP	Radiography, histology	Bone defect was successfully repaired at 16 week. Sufficient volume of bone tissue volume formed in cell groups compared to scaffold only	Li et al. (2014)
Postero-lateral spinal fusion (three-level bilateral postero-lateral inter-transverse fusion, L1–L6)	6 animals (group I: Cell-seeded hydroxyapatite; at L2–L3; group II: Cell-seeded HA/tricalcium phosphate at L2–L3; autograft at L5–L6)	12 weeks	BM-MSCs, hydroxyapatite (HA), tricalcium phosphate (TCP)	$5-6 \times 10^7$	Radiography, manual palpation, histology, scanning electron microscopy	TCP/HA achieved superior lumbar inter-transverse fusion compared to HA but autograft had best fusion	Shamsul et al. (2014)

(continued)

Table 7.4 (continued)

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Sinus augmentation	16 animals (ficol density isolated MSCs, $n = 8$; bone marrow aspirate, $n = 8$)	8 and 16 weeks	BM-MSCs, bone marrow aspirate, bovine bone mineral	–	Histological and histomorphometrical, CT	The approach of using BBM and MSCs in combination with fibrin adhesive was sufficient for new bone formation as the FICOLL. However, due to significantly lower cell numbers isolated using the BMAC in sheep, less new bone was formed in the test arm	Ardjomandi et al. (2015)
Critical sized tibial defect (3 cm, plate stabilized)	24 animals (polycaprolactone-hydroxyapatite scaffold (PCL/HA), $n = 8$; PCL/HA + 100x10 ⁶ , $n = 8$; autologous bone defect, $n = 6$)	12 months	Allogeneic BM-MSCs, PCL/HA (4 weeks after defect surgery)	100 × 10 ⁶ cells	Biomechanical testing and micro-CT; histology and immune-histochemistry, scanning electron microscopy	Improved bone regeneration in cell/scaffold group comparable to autograft group	Berner et al. (2015)
Segmental mandibular bone defect	15 animals ($n = 5$ control, serum scaffold; $n = 10$ treatment: cell + scaffold)	12 and 32 weeks	Autologous BM-MSCs and autologous serum scaffold	25–30 × 10 ⁶	Computed and micro-CT	Complete bone healing in treatment group was observed at 12 and 32 weeks but only at 32 week in control	Gallego et al. (2015)

Lumbar interbody spine fusion	36 animals [$n = 8$ cortico-cancellous autograft; $n = 8$ in lower concentration (2.5×10^6) cell group; $n = 8$ in medium cell concentration (6.25×10^6) group; $n = 8$ in higher cell concentration (12.5×10^6) group]	16 weeks	Allogeneic BM-MSCs, scaffold (hydroxyapatite, tricalcium phosphate and type I collagen)	2.5×10^6 ; 6.25×10^6 ; 12.5×10^6	Plain radiography, CT, histopathology, histomorphometric and biomechanical competency	No adverse reaction to cell implantation. The cell/scaffold implantation achieved similar or better fusion as that of autograft after 16 weeks	Wheeler et al. (2016)
Tibial (2 holes each in right and left) and femoral defects (2 holes each in right and left) (8 mm diameter and 4 mm deep)	10 ($n = 8$ defects per animals, group I: Scaffold without cells; group II: Scaffold seeded with cells in static conditions; group III: Scaffold seeded with cells in dynamic conditions; group IV: Control empty defects)	2 and 4 months	Autologous osteodifferentiated BM-MSCs + hydroxyapatite	2×10^6 seeded on scaffold	Macroscopy, radiography, micro-CT and histology	Tibial bone is characterized by limited repair capability as compared to femur in which cell implantation is not crucial. Dynamic cell-loaded implants performed better compared to groups I and II	Lovati et al. (2016)

(continued)

Table 7.4 (continued)

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Osteoporotic bone model (ovariectomy induced, 12 mm × 4 mm defect size)	8 animals	10 months	AD-MSCs, strontium hydroxyapatite (SrHA)	–	Histological, histomorphometry, micro-CT and elemental analysis	cSrHA along with MSCs enhanced osteogenic ability with mature lamellar bone formation as compared to bare HA, SrHA and tissue engineered HA implanted groups. Histomorphometry data substantiated improved osteogenesis on par with material resorption, as cSrHA implanted group exhibited highest regeneration ratio. Density histograms from micro-CT further signified the enhanced osteointegrative ability of cSrHA implants. The therapeutic potential of cSrHA in osteoporotic bone healing and proposes the use of allogeneic AD-MSCs for fabricating “off the shelf tissue engineered products”	Chandran et al. (2017)

2016). Thus, *ex vivo* cell compatible tissue engineered constructs may not necessarily prove useful under *in vivo* environment.

7.7.4 Tendon Defects

The tendons and ligaments have limited ability to self-heal due to low cell concentration and associated low matrix turnover. For proper functioning, the healed tissue should be free of adhesions (Gugjoo et al. 2018a). Healing potential of stem cells has been studied in various types of sheep tendon models. An improved healing but variably has been demonstrated (Table 7.5).

MSCs from bone marrow (Crovace et al. 2008; Lacitignola et al. 2014), amniotic fluid (Colosimo et al. 2013), and peripheral blood (Martinello et al. 2013) has been demonstrated to improve tendon healing in sheep collagenase-induced Achilles and digital flexor tendinitis, respectively. Tissue remodelling and improved structural organization was observed in healed tendons (Martinello et al. 2013). Even mononuclear cells (MNCs) too had improved tendon healing (Crovace et al. 2008). BM-MSCs implanted locally were demonstrated to survive up to 6 weeks and engraft efficiently (Colosimo et al. 2013; Lacitignola et al. 2014). MSCs and MNCs had shown comparable fibre and extracellular matrix restoration in treated tendons (Table 7.5). However, the CD34 positive cells were highly appreciable in the BM-MNCs treated cases (Crovace et al. 2008). Similarly, PB-MSCs implantation in sheep digital flexor tendon (DFT) model had improved the healing of tendon.

MSCs from amnion (Am-MSCs) together with scaffold had improved diaphragmatic tendon repair. The repaired tendon tissues had better functional and mechanical parameters in comparison to the tendons treated with acellular grafts (Fuchs et al. 2004; Turner et al. 2011). Implantation of MSCs along with demineralised bone matrix in a sheep tendon-bone defect model also had promoted healing. Tissue repaired with MSCs laden on allogeneic graft had better mechanical and histological properties as compared to that of the xenogeneic graft assembly (Thangarajah et al. 2016).

7.7.5 Cardiac Ailments

Sheep outperform other species as cardiovascular model animal (DiVincenti et al. 2014). Among various implantation routes, intracoronary implantation of allogeneic BM-MSCs had been demonstrated to be safe with marked efficacy in sheep model of acute myocardial infarction (AMI) (Table 7.6) (Zhao et al. 2012; Houtgraaf et al. 2013). 3-D echo guided intramyocardially delivered BM-MSCs also offer safety with better results (Cheng et al. 2013). Intra-myocardial BM-MSCs delivery in MI however, may attain therapeutic threshold. The higher cell concentration may not always be useful rather might further damage the area (Dixon et al. 2009; Hamamoto et al. 2009).

Table 7.5 In vivo preclinical experimental tenogenic mesenchymal stem cell studies in sheep

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Tendinitis of the Achilles tendon (collagenase-induced)	30 (<i>n</i> = 6 each group: BM-MSCs; BM-MNCs; fibrin; saline; sham control)	8 weeks after treatment	BM-MSCs, BM-MNCs and fibrin glue after 2 weeks of induced tendinitis	$1.1 \times 10^6 \pm 1.4 \times 10^5$ (MSCs); $101 \times 10^6 \pm 26 \times 10^6$ (MNCs)	Histomorphology, immuno-histochemistry of collagen type I, III, cartilage Oligomeric matrix protein (COMP) and CD34 positive cells expression	BM-MSCs and BM-MNCs showed similar restoration of the architecture of fibres and extra cellular matrix (ECM), with a high expression of collagen type I and COMP and a very low expression of collagen type III in treated tendons. The presence of CD34 positive cells was appreciable in the BMNC group while few cBMSC showed this cluster of differentiation, not expressed in tendons treated with fibrin or saline	Crovace et al. (2008)

Deep digital flexor tendons lesions (bilateral)	18 animals (groups 1 and 4 received injections of 1 ml of PRP into the tendon; groups 2 and 5 1 ml of PRP + autologous MSCs; groups 3 and 6 received autologous PB-MSCs in 1 ml of hyaluronic acid)	30 and 120 days	Autologous PB-MSCs, PRP, hyaluronic acid (only left limb lesions treated while contralateral limb served as control)	PRP (882 ± 199 × 10 ³ /ml); MSCs (10 × 10 ⁶)	Clinical, ultrasonographic, histology, immuno-histochemical expression	Significant differences were found between treated groups and their corresponding controls (placebo) regarding tendon morphology and extracellular matrix (ECM) composition. However, our results indicate that the combined use of PRP and MSCs did not produce an additive or synergistic regenerative response and highlighted the predominant effect of MSCs on tendon healing, enhanced tissue remodelling and improved structural organization	Martinello et al. (2013)
---	---	-----------------	--	---	--	--	--------------------------

(continued)

Table 7.5 (continued)

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Tendinitis of the Achilles tendon (collagenase-induced, bilateral)	9 animals (BM-MSCs/fibrin glue, $n = 9$; fibrin glue, $n = 9$)	3, 4 and 6 weeks	Allogeneic BM-MSC (transfected with red fluorescent protein), fibrin glue	6×10^7 after 2 weeks	Evaluation for morphology, collagen I deposition and presence of CD34 + cells	BM-MSCs into tendon lesions had positive effects on the injured tendons. The BM-MSCs survived at three, four and 6 weeks after treatment. These cells enhanced quality healing of tendons as compared to the controls, where no labelled cells were detected. Interestingly, we demonstrated high expression of CD34+ cells in tendons that had been treated with BM-MSCs	Lacitignola et al. (2014)

Tendon defect model of retraction	10 animals (cells + Xenogenic demineralised bone matrix, <i>n</i> = 5; cells + allogeneic demineralised bone matrix, <i>n</i> = 5)	6, 9 and 12 weeks	BM-MNCs	–	Force plate analysis, CT, histology	Allograft was associated with significantly higher weight bearing as compared to xenograft. Greater remodelling of demineralised bone matrix into tendon like tissue at defect area and more direct type of enthesis characterized by significantly more fibrocartilage. No failure of tendon-bone healing was noted in either group	Thangarajah et al. (2016)
-----------------------------------	--	-------------------	---------	---	-------------------------------------	--	---------------------------

(continued)

Table 7.5 (continued)

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Lesion of lateral branch of the ovine deep digital flexor tendon (lateral border)	50 (group 1: Sacrificed at 1 day ($n = 2$), treated with MION-labelled MSCs. Group 2: Sacrificed 1–2 weeks ($n = 4$), three sheep treated with MION-labelled MSCs and one sheep with PBS. Group 3: 4 weeks ($n = 12$), six sheep treated with non-labelled MSCs and six sheep with PBS. Group 4: 12 weeks ($n = 16$), eight sheep treated with non-labelled MSCs and eight sheep with PBS. Group 5: 24 weeks ($n = 16$), eight sheep treated with non-labelled MSCs and eight sheep with PBS)	1 day, and 1–2, 4 12 and 24 weeks	Magnetic iron-oxide nanoparticles labelled autologous BM-MSCs (implanted 2 weeks after lesion)	5×10^6	MRI, histology and flow cytometry	Cell treated limbs indicated cellular distribution throughout the tendon synovial sheath but restricted to the synovial tissues, with no MSCs detected in the tendon or the surgical lesion. The lesion was associated with negligible morbidity with minimal inflammation post-surgery. Evaluation of both cell treated and control lesions showed no evidence of healing of the lesion at 4, 12 and 24 weeks on gross and histological examination	Khan et al. (2018)

Table 7.6 In vivo preclinical mesenchymal stem cell studies on cardiac/arterial models in sheep

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose/assembly	Evaluation criteria	Overall result	References
Foetal cardiac development (intraparitoneal implantation of MSCs into sheep foetus at 55–62 days of gestation)	21 animals	Analysis done at 115–131 days of gestation (approx 50–55 days later)	GFP transduced MSCs from human BM, foetal brain and liver	–	Immunohistochemistry	Cells were detected in 20/21 foetal hearts. At least 10 areas of cell engraftment were reported. Human areas represented 43.2% areas of the total Purkinje fibre areas. No difference in engraftment could be observed based on cell type. ~0.01% cardiomyocytes were from human origin in sheep foetal heart. In conclusion, stem cells take part in development of heart	Airey et al. (2004)
Myocardial infarction (coronary ligation)	47 animals (group I: 25 million cells, n = 7; group II: 75 Million cells, n = 7; group III: 225 Million cells, n = 10; group IV: 450 Million cells, n = 8; group V: Free media group)	8 weeks	Stro-3 positive cells derived from BM	25 × 10 ⁶ ; 75 × 10 ⁶ ; 225 × 10 ⁶ ; 450 × 10 ⁶ (injected at border zone after 1 h)	Echocardiography, histology, collagen content and MMPs level of myocardium	End diastolic volume increased in all groups but in group I and II, it was attenuated. ICTP, an index of collagen degradation was more in group I. Matrix metalloproteinases (MMPs) were higher in group I as compared to other groups including group V	Dixon et al. (2009)
Transmural myocardial infarction (coronary ligation)	46 animals (group I: 25 million cells, n = 7; group II: 75 Million cells, n = 7; group III: 225 million cells, n = 10; group IV: 450 Million cells, n = 8; group V: Free media group)	4 and 8 weeks	Stro-3 positive cells derived from BM	25 × 10 ⁶ ; 75 × 10 ⁶ ; 225 × 10 ⁶ ; 450 × 10 ⁶ (injected at border zone after 1 h)	Echocardiography, CD31 and α smooth muscle actin immunohistochemistry	No evidence of myocardial regeneration. Low dose cell groups had better ejection fraction and CD31 and α smooth muscle actin immunohistochemistry showed increased vascular density at border zone	Hamamoto et al. (2009)

(continued)

Table 7.6 (continued)

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose/assembly	Evaluation criteria	Overall result	References
Myocardial infarction (MI)	10 animals (MSCs treated, $n = 5$; MI only, $n = 5$)	12 weeks	Allogeneic BM-MSCs	2×10^6 (six injections each containing 3.33×10^7)	Histology, determination of apoptosis, Western blot, determination of SERCA2a activity and calcium uptake	Remodelling and contractile strain alteration was reduced in cell treated cases. Cardiomyocyte hypertrophy was significantly attenuated with normalization of hypertrophy of related signalling proteins like phosphatidylinositol 3-kinase- α (PI3K- α), PI3K- γ , extracellular signal-related kinase (ERK), phosphorylated ERK as compared to control. Imbalance of calcium handling proteins was prevented by MSCs. Upregulation of pro-apoptotic protein BCL-2 was normalized, apoptosis of cardiomyocyte was reduced and there was less fibrosis	Zhao et al. (2012)
Chronic myocardial injury (anterior wall myocardial infarction)	39 animals ($n = 10$: 25×10^6 ; $n = 9$: 75×10^6 ; $n = 10$: 225×10^6 ; $n = 10$ non-conditioned control media)	8 weeks after study design	Allogeneic MSCs	25×10^6 ; 75×10^6 ; 225×10^6	Histological, echocardiography	Highest cell dose lead to incremental increase in ejection fraction and wall thickening in infarct and border zones as compared to control. Highest density of arteriole density in infarct and border zone in highest cell treated as compared to control	Cheng et al. (2013)

Acute myocardial infarction	68 [34 died and 4 placebo (10 animals); 12.5 (7 animals); 25 (7 animals); 37.5 (6 animals) million cells]	8 weeks	Mesenchymal precursor cells	12.5 × 10 ⁶ ; 25 × 10 ⁶ ; 37.5 × 10 ⁶	Invasive haemodynamics, echocardiography	Global left ventricular ejection fraction as measured by pressure-volume loop analysis deteriorated in controls to 40.7 ± 2.6% after 8 weeks. In contrast, MPC treatment improved cardiac function to 52.8 ± 0.7%. Echocardiography revealed significant improvement of both global and regional cardiac functions. Infarct size decreased by 40% in treated sheep, whereas infarct and border zone thickness were enhanced. Left ventricular adverse remodelling was abrogated by MPC therapy, resulting in a marked reduction of left ventricular volumes. Blood vessel density increased by >50% in the infarct and border areas. Compensatory cardiomyocyte hypertrophy was reduced in border and remote segments, accompanied by reduced collagen deposition and apoptosis. No microinfarctions in remote myocardial segments or histological abnormalities in unrelated organs were found	Houtgraaf et al. (2013)
-----------------------------	---	---------	-----------------------------	--	--	---	-------------------------

(continued)

Table 7.6 (continued)

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose/assembly	Evaluation criteria	Overall result	References
Acute myocardial infarction (anteroapical)	32 animals ($n = 8$ each in transfected MSCs treated; MSCs treated and PBS injected; VEGF treated groups)	1 month	VEGF transfected BM-MSCs (1 week after defect)	2×10^7	Magnetic resonance, echocardiography	31% decrease in infarct size post treatment. Myocardial salvage occurred mostly at subendocardium level. Ejection fraction recovered to baseline value. All these effects were predominantly observed in transfected MSCs treated animals	Locatelli et al. (2015)
Acute myocardial infarction (diagonal branch)	10 animals ($n = 5$: MSCs; $n = 5$: PBS control)	1 month	Human MSCs	1×10^6 cells per kg body weight	Echocardiography and perfusion scintigraphy	Cell treated animals had significant improvement in myocardial perfusion compared with baseline measurements. No change in ventricular dimensions in either group	Dayan et al. (2016)
Acute myocardial infarction (anterior apical infarct)	18 animals (control, PBS $n = 6$; transfected BM-MSCs, $n = 6$; BM-MSCs, $n = 6$)	2 months	Allogeneic human hypoxia-inducible factor 1- α transfected BM-MSCs, simple BM-MSCs	2×10^7 (BM-MSCs); 2×10^7 (transfected BM-MSCs)	Cardiac magnetic resonance, echocardiography, histology	Infarct volume decreased and increased ejection fraction with transfected BM-MSCs compared to BM-MSCs was seen on cardiac magnetic resonance and echocardiography. Histology showed improved angio/arteriogenesis in transfected cell group. In control neither parameter improved	Hnatiuk et al. (2016)
Acute myocardial infarction	18 animals (MSCs treated group, $n = 6$; endothelial cell (ECs) treated, $n = 6$; control, $n = 6$)	2 months	BM-MSCs and endothelial cells	27×10^6 (MSCs); 27×10^6 (ECs)	Echocardiography, electron microscopy and immune-histochemistry	Cell treated groups showed significant improvement in ejection fraction as compared to control. Vascular density also improved with cardiomyocytes in variable stages of development	Rabbani et al. (2017)

Pulmonary artery patching	7 animals (1–2 weeks, $n = 4$; 4–6 weeks, $n = 3$)	2 and 6 weeks	Autologous MSCs and endothelial progenitor cells (EPCs) seeded onto poly-4-hydroxybutyrate (P4HB)-coated polyglycolic acid (PGA) nonwoven biodegradable mesh scaffolds (10×20 mm)	2×10^5 MSCs and 2×10^6 EPCs per square centimetre.	Histology and immunohistochemistry	One week after implantation, seeded patches had surface thrombus formation and macrophage infiltration. Seeded patches implanted for 2 weeks showed granulation tissue, early pannus formation, macrophages, foreign body giant cells around disintegrating polymer and early angiogenesis (microvessel formation). After 4 weeks in vivo, seeded patches contained glycosaminoglycans, collagen and coverage of the luminal surface by host artery-derived pannus containing α -SMA-positive cells and laminated elastin; polymer scaffold degradation was almost complete with replacement by fibrous tissue containing viable cells	Mendelson et al. (2007)
Carotid artery resection (40 mm)	7 animals ($n = 4$, treated with cells/scaffold and each 2 animals evaluated at 2 month and 5 month; control, $n = 3$)	2 and 5 months	BM-MSCs differentiated smooth muscle like cells (SMCs) and endothelial cells (ECs)	SMCs (2×10^5) in collagen gel and ECs (1×10^5) implanted on decellularized scaffold	Histology; immunohistochemistry, electron microscopy	Tissue engineered blood vessels (TEBVs) were patent, anti-thrombotic and stable up to 5 months. TEBVs had smooth muscle, endothelium and collagen and elastin at both the time points (2 and 5 months). Non-seeded grafts occluded within 2 weeks	Zhao et al. (2010)

(continued)

Table 7.6 (continued)

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose/assembly	Evaluation criteria	Overall result	References
Surgical resection of carotid artery (5 cm)	24 animals (n=6 animals, groups implanted either with PVA/dx along with MSCs or with cells and anticoagulant or PTFE grafts ⁽⁸³⁾)	24 weeks (each animal sacrificed at 4, 8, 12, 16, 20 and 24 weeks)	Human WJ-MSCs along with polyvinyl alcohol and dextran (PVA/dx; 90/10 v/v)	10 ⁴	Graft explanation, Doppler studies, histopathology, immune-histochemistry, scanning electron microscopy, digital image analysis	PVA/dx grafts could maintain similar or higher patency rate, systolic/diastolic laminar blood flow velocities comparable to PTFE grafts. CD12 and α -actin staining presented similar results in PVA/dx/MSCs and PTFE groups. Fibrosis layer was lower and endothelial cells were only detected at graft artery transition sites near the MSCs implantation sites	Alexandre et al. (2014)

The infusion rate may also affect the results. In normal heart, 1.25 million cells per minute had appeared safer as compared to the 2.5 million cells that had led to the myocardial necrosis. In an infarcted myocardium, MSCs at the rate of 01 million cells/min had led to sluggish coronary flow after 25 million cell implantation while the cell implantation at 0.5 million cells had led to sluggish flow after 40 million dose (Houtgraaf et al. 2013).

Intraperitoneal implantation of xenogeneic MSCs from BM, foetal liver at 55–62 days of gestation had engrafted into heart and exhibited properties of various cardiac cells (Airey et al. 2004). These xenogenic MSCs too had enhanced myocardial perfusion although without any significant effect on ejection fraction (Dayan et al. 2016). To evaluate long term effects of MSCs extended studies are desired. Bone marrow implantation may not be feasible option to treat myocardial infarction (Bel et al. 2003).

Even transfection of MSCs with some specific factors may improve their healing potential. Among notable options are hypoxia-inducible factor 1- α (HIF1- α) and vascular endothelial growth factor (VEGF). BMSCs transfected with these factors had significantly reduced infarct size in a sheep acute myocardial infarction (AMI) model. Improved results might have been due to enhanced angio-/arteriogenesis through cell paracrine secretion (Locatelli et al. 2015; Hnatiuk et al. 2016).

Apart from myocardial repair, MSCs have been evaluated in skeletal and blood vessel repair. MSCs had significantly improved rotator muscle cuff repair irrespective of their source. Experimental muscles had increased contraction force, vascularity, and increased myocyte concentration over adipocytes (Coleman et al. 2011). For blood vessel repair, MSCs have been employed along with tissue engineered scaffold patches (Mendelson et al. 2007; Zhao et al. 2010). The cell-seeded scaffold patches implanted in sheep pulmonary artery had remodelled to a layered and viable tissue. The tissue had been well integrated into the native arterial wall. The key remodelling processes included are intimal overgrowth at the luminal surface (pannus formation; neointima) and granulation tissue formation, and fibrosis with foreign body reaction (Mendelson et al. 2007). MSCs laden tissue engineered blood vessels (TEBVs) had also been implanted in case of carotid artery resection model. The constructs were patent, anti-thrombotic and stable up to 5 months. TEBVs had smooth muscle, endothelium and collagen and elastin up to 5 months. Non-seeded grafts contrarily were occluded within 2 weeks (Zhao et al. 2010).

7.7.6 Nervous System

MSCs may trans-differentiate into neuron-like and glial-like cells, in addition to PAX3 expressing progenitor cells (Chen et al. 2001; Ceccarelli et al. 2015). The differentiated cell functionality remains controversial (Przyborski et al. 2008). Due to these properties MSCs have been evaluated in various nerve ailments as detailed in Table 7.7. Additionally MSCs carry potential to be evaluated for scrapie and post hypoxia sequelae of brain (Mediano et al. 2015a, b; Ophelders et al. 2016).

Table 7.7 In vivo preclinical experimental studies of mesenchymal stem cell studies on neurological ailments in sheep

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Radial and tibial nerve resection (1 cm)	7 animals (MSCs + scaffolds)	3 and 6 months	BM-MSCs, repapmen with Neurolac™ biocamera scaffold	$30-50 \times 10^6$	Histological, immuno-histochemistry and Morphometry	At 3 months period results were not good. At 6 months results showed significantly myelinated nerve fibres with increased conduction as compared to control in both radial and tibial nerves	Casañas et al. (2014)
In utero repair of Myelomeningocele (at 75 days of pregnancy)	4 animals (1 case treated with term derived placental MSCs + foetal membrane (FM) patch; other with early gestation placental MSCs + FM patch; 1 case with FM only; 1 case with skin closure only)	145 days of pregnancy at delivery time	Placenta derived MSCs in collagen gel	5×10^5 (early gestation MSCs); 1×10^6 (term gestation MSCs)	Histology and motor assessment	Increased preservation of spinal cord architecture and large neurons in early gestation MSCs treated compared to others. The animal treated with early gestation MSCs was capable of normal ambulation. Limited distal motor function in others	Brown et al. (2016)
Myelomeningocele (created at 75 day of pregnancy)	12 animals (cell treated, $n = 6$; control, $n = 6$)	At term (145 days)	Human placental MSCs	5×10^5	Motor functional, histological and immuno-histochemical analysis	Significant and clinically relevant improvement of motor function with increased preservation of large neurons with spinal cord	Wang et al. (2015)

Foetal Myelomeningocele (created midgestational - 76-81 days)	21 animals	25 days	Placental MSCs + porcine small intestinal derived extracellular matrix		Sheep Locomotor rating (SLR) scale and histology of spinal cord	Foetal viability of 71% (5/8) in group treated with cells while only 17% (1/6) in matrix treated group. SLR and large neuron density was higher in cell treated animals. The cross sectional areas of spinal cord and grey matter were equally preserved. Cell treated animals improve hind limb motor function in lambs	Kabagambe et al. (2017)
Global hypoxia- ischemic injury in preterm foetus (umbilical cord occlusion, UCO)	24 animals (sham UCO, $n = 6$; sham UCO and cell derived vesicle treatment, $n = 6$; UCO treated with saline, $n = 6$; UCO with cell derived vesicle treatment, $n = 6$)	7 days	Human BM-MSCs derived extracellular vesicles	4×10^7 (2×10^7 intrauterine injection twice; one 1 h after UCO and other 4 days later)	Electrophysiological parameters and histology of brain	Improved brain function by reducing number and duration of seizures and by preserving baroreceptor reflex sensitivity. Cerebral inflammation remained unaffected	Ophelders et al. (2016)

(continued)

Table 7.7 (continued)

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Intervertebral disc degeneration (IVD) (Postero-lateral annulotomy at 3 levels: L3/L4, L4/L5, L5/L6)	8 animals (MSCs into nucleosus pulposus; MSCs into annulus fibrosus and phosphate saline control)	6, 9 and 12 months	Allogeneic BM-MSCs 6 months after defect creation	1×10^6	Radiography, MRI, biochemical and histology	Significant improvement in disc height index and disc height in both cell treatment groups at 6 months. Mean Pfirrmann and histopathological grade improved in cell treated animals. Spontaneous repair of antero-lateral annular lesion in cell treated groups	Freeman et al. (2016)
Intervertebral disc degeneration (antero-lateral annulus fibrosus lesion; 20 mm wide 6 mm deep: Lesion at L1–L2, L3–L4 and L5–L6)	45 animals (control $n = 10$; early treatment group at 4 weeks after lesion creation, $n = 24$; late treatment group at 12 weeks, $n = 12$)	3 and 6 months	Allogeneic BM-MSCs	1×10^7	Histology and gene expression	MSC treatment prevented increased adipose and connective tissue formation, normally forms after damage. MSCs treatment did not prevent slow-to-fast muscle fibre formation. Alteration in gene expression of pro-inflammatory cytokines within the muscle. Increased interleukin-1 expression was reduced by early MSCs treatment. TNF and TGF- β 1 expression was increased at 6 months	Dowdell et al. (2017)

Lumbar disc herniation (repair by microdiscectomy)	18 animals (pMSCs, $n = 6$; MSCs, $n = 6$; control, $n = 6$)	6 months	Pentosan polysulphate-primed mesenchymal progenitor cells (pMSCs) and MSCs	5×10^5	3 T MRI, standard radiography, morphologically, histologically, biochemically for their proteoglycans (PGs), collagen and DNA content	Both the MSCs- and pMSCs-groups exhibited significantly less reduction in disc height and lower Pfirrmann grades as compared to the control, but morphologic scores for the pMPC-injected discs were significantly lower. The PG content of the annulus Fibrosus injury site region 1 of pMSCs discs was significantly higher than MSCs and injury control AF1. At the AF1 and contralateral AF2 regions, the DNA content of pMSCs discs was significantly lower than injured control discs and MSCs-injected discs. Histologic and birefringent microscopy revealed increased structural organization and reduced degeneration in pMSC discs as compared to the MSCs and controls	Daly et al. (2018)
--	---	----------	--	-----------------	---	--	--------------------

sBM-MSCs may steadily improve radial and tibial nerve repair. Initially up to 3 months poor nerve regeneration and conduction upon MSCs implantation had been demonstrated. However, later at 6 months period myelinated nerve fibres had generated both proximally and distally. Further, nerve action potential had also improved (Casañas et al. 2014).

Amniotic fluid MSCs (AF-MSCs) may improve motor functions and increase large neuron preservation in spinal cord (Wang et al. 2015). The early gestation AF-MSCs in comparison to late stage gestation had better ability to preserve spinal cord architecture and large neurons in myelomeningocele (MMC). Lambs that received early gestation cells had normal ambulation while only limited distal motor function was demonstrated in animals that received late gestation MSCs (Brown et al. 2016). This questions if MSCs are able to maintain their neural specific properties with developmental advancement.

MSCs may offer therapeutics in intervertebral disc degeneration (Shamsul et al. 2014). Sheep intervertebral disc disease model treated with BM-MSCs had quantifiable improvement at 6 months period. Annulus fibrosis implantation may be better than nucleus pulposus transplantation (Freeman et al. 2016). Their disc regeneration potential may be promoted through pentosan polysulphate priming (Daly et al. 2018).

MSCs application in neurological ailments is in infancy and extensive research studies are desired for any conclusion.

7.7.7 Respiratory and Urinary Systems

Sheep has been used as an animal model for human respiratory ailments. Acute respiratory distress syndrome (ARDS) in these animals has been created through endotoxaemia. BM-MSCs or lung derived MSCs in combination with other support system had better managed the ARDS. The inflammation along with oedema had subsided post cell implantation (Ingenito et al. 2012; Asmussen et al. 2014; Kocyildirim et al. 2017) (Table 7.8).

MSCs have also been studied in acute renal injuries. The cells had ability to engraft although without any observable beneficial effect (Behr et al. 2007, 2009) (Table 7.8).

7.7.8 Reproductive System

As MSCs are able to trans-differentiate into germinal cells, these may be utilized for *in vivo* applications. One available study had demonstrated that transdifferentiated cells carry ability to home to seminiferous tubules of ram testis. Even these cells had formed colonies at the area (Ghasemzadeh-Hasankolaei et al. 2015) (Table 7.9). Initially, it is mandatory to develop differentiation media cocktail that ensures the typical germ cells properties of transdifferentiated cells. This may be followed by *in vivo* studies of these differentiated cells.

Table 7.8 In vivo preclinical experimental mesenchymal stem cell studies on respiratory and renal affections

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Emphysema	10 animals (control scaffold treated $n = 5$; cell treated $n = 5$)	4 weeks	Autologous lung MSCs + scaffold (1% fibrinogen + 20 $\mu\text{g}/$ ml fibronectin + 20 $\mu\text{g}/$ ml poly-L-lysine)	$5-10 \times 10^6$	Clinical toxicity, tissue mass (CT), lung physiology, scintigraphy, histology	Cells were well tolerated, increased perfusion and tissue mass as compared to control. Histology confirmed cell retention, increased cellularity and extracellular matrix in cell treated animals	Ingenito et al. (2012)
Acute respiratory distress syndrome (ARDS, bacterial pneumonia)	19 animals (control, PlasmaLyte A, $n = 8$; low dose MSCs, $n = 7$; high dose MSCs, $n = 4$)	24 h	Human BM-MSCs	$5 \times 10^6/\text{kg}$ (low dose); $10 \times 10^6/\text{kg}$ (high dose)	Post-mortem lung wet-to- dry weight ratio, broncho- alveolar lavage, serum biochemical parameters	Cells were well tolerated. $\text{PaO}_2/\text{FiO}_2$ ratio was significantly improved in cell treated groups compared to control. Median lung water content was significantly lower in high cell treated group than control	Asmussen et al. (2014)
Acute respiratory distress syndrome (Escherichia coli exotoxin induced)	11 animals (control, $n = 5$; extracorporeal membrane oxygenation (ECMO), $n = 3$; MSCs + ECMO, $n = 3$)	–	BM-MSCs	40×10^6	Blood gas results, radiography, histopathology	Better histopathologic changes with less inflammation in combined cell and ECMO treated group	Kocylidirim et al. (2017)

(continued)

Table 7.8 (continued)

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Renal ischaemic reperfusion injury (percutaneous transluminal placement of balloon catheter in renal artery)	24 animals [sham group (balloon catheterization performed but no ischaemic reperfusion injury (IRI), $n = 3$; control group (IRI induced without any cell treatment, $n = 3$); cell treated but no IRI induced, $n = 3$; IRI induced and cells injected 1 h. later, $n = 5$; IRI infused and cells infused 2 weeks afterwards. This group divided into two, one evaluated at 3 week, $n = 5$ and other at 5 week, $n = 5$]	3–5 weeks	Autologous MSCs	80×10^6	Identification of engrafted cell, immune-phenotype analysis	Renal (tubules and glomeruli) engraftment of MSCs. MSCs expressed tubular epithelial cell markers and podocyte phenotype. Significant increase in engraftment of MSCs was seen when injected immediately than at later period	Behr et al. (2007)
Renal ischaemic reperfusion injury (bilateral) (balloon catheter)	Sham group: control injected culture medium; cell treated group	7 days	BM-MSCs	50×10^6	Immuno-phenotypic analysis, renal function, histology and urine analysis	Transplanted cells were found in glomeruli but not tubules. Cell did not express glomerular cell markers (podocin and von Willebrand factor). Functional evaluated revealed no beneficial effects of implanted cells. MSCs failed to repair renal parenchyma and failed to modulate cell death and proliferation or cytokine release	Behr et al. (2009)

Table 7.9 In vivo preclinical experimental models of different types in sheep

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Intraperitoneal implantation of MSCs (76 days)	7 animals	2 weeks	Autologous amniotic fluid MSCs	1×10^5 to 1.8×10^7	PCR, western blotting, cytofluorometry, immunofluorescence	Liver, heart, placenta, umbilical cord, adrenal gland and muscle	Shaw et al. (2011)
Transverse subtotal ear resection at 123.4 ± 2.3 days (intact cartilage left to heal by second intention)	10 animals (2 wounds in each animal covered and uncovered)	7–14 days prior to term	GFP transfected autologous amniotic-MSCs	3.22– 4.32×10^6 (implanted in amniotic cavity)	Histomorphologic, collagen and hyaluronan content	Covered wounds carry significantly lower healing rate compared to non-covered. Significantly lower elastin levels in non-covered wounds. Insignificant difference in hyaluronic acid levels in wound types	Klien et al. (2011)
In vivo testicular homing and colony formation of in vitro MSCs derived germ-like cells	4 animals ($n = 2$ animals in each treatment group; 3 of the 4 testis were treated with treated cells and one non-injected control; 2 testis of lamb also as control. One testis treated with undifferentiated MSCs and other with only medium	2 months	In vitro MSCs derived germ-like cell through 14 day treatment of retinoic acid; 21 day treatment of retinoic acid; transforming growth factor- β	15– 20×10^6	Histological study	TGF- β transformed group exhibited highest efficiency of homing and colony formation followed by 21 day treatment of retinoic acid. 14 day treatment of retinoic acid exhibited lowest homing and colony formation	Ghasamzadeh-Hasankolaie et al. (2015)

7.8 Conclusion(s)

sMSCs isolation and characterization process is similar to that of other species. sMSCs characteristics are source dependant and are also affected by donor age and health status. A good amount of literature on sMSCs human translational studies on bone, skin, osteochondral and cardiovascular system could be observed. These cells had offered promising therapeutic effects although further extensive studies are desired for their actual utility. Additionally, inadequate MSCs studies on reproductive, nervous, respiratory and urinary system models have failed to provide any conclusion.

References

- Abdalmula A, Dooley LM, Kaufman C, Washington EA, House JV, Blacklaws BA, Ghosh P, Itescu S, Bailey SR, Kimpton WG (2017) Immunoselected STRO-3+ mesenchymal precursor cells reduce inflammation and improve clinical outcomes in a large animal model of monoarthritis. *Stem Cell Res Therapy* 8:22
- Adamzyk C, Kachel P, Hoss M, Gremse F, Modabber A, Hölzle F, Tolba R, Neuss S, Lethaus B (2016) Bone tissue engineering using polyetherketoneketone scaffolds combined with autologous mesenchymal stem cells in a sheep calvarial defect model. *J Craniomaxillofac Surg* 44(8):985–994
- Airey JA, Almeida-Porada G, Colletti EJ, Porada CD, Chamberlain J, Movsesian M, Sutko JL, Zanjani ED (2004) Human mesenchymal stem cells form Purkinje fibers in fetal sheep heart. *Circulation* 109:1401–1407
- Al Faqeh H, Nor Hamdan BM, Chen HC, Aminuddin BS, Ruszymah BH (2012) The potential of intra-articular injection of chondrogenic-induced bone marrow stem cells to retard the progression of osteoarthritis in a sheep model. *Exp Gerontol* 47:458–464
- Alexandre N, Ribeiro J, Gärtner A, Pereira T, Amorim I, Fragoso J, Lopes A, Fernandes J, Costa E, Santos-Silva A, Rodrigues M, Santos JD, Maurício AC, Luís AL (2014) Biocompatibility and hemocompatibility of polyvinyl alcohol hydrogel used for vascular grafting—in vitro and in vivo studies. *Biomed Mater Res* 102(12):4262–4275
- Anderson ML, Dhert WJ, De Bruijn JD, Dalmeijer RA, Leenders H, Van Blitterswijk CA, Verbout AJ (1999) Critical size defect in the goat's os ilium. A model to evaluate bone grafts and substitutes. *Clin Orthop Relat Res* 364:231–239
- Ando W, Heard BJ, Chung M, Nakamura N, Frank CB, Hart DA (2012) Sheep synovial membrane-derived mesenchymal progenitor cells retain the phenotype of the original tissue that was exposed to in-vivo inflammation: evidence for a suppressed chondrogenic differentiation potential of the cells. *Inflamm Res* 61:599–608
- Ardjomandi N, Duttonhoefer F, Xavier S, Oshima T, Kuenz A, Sauerbier S (2015) In vivo comparison of hard tissue regeneration with sheep mesenchymal stem cells processed with either the FICOLL method or the BMAC method. *J Craniomaxillofac Surg* 43(7):1177–1183
- Asmussen S, Ito H, Traber DL, Lee JW, Cox RA, Hawkins HK, McAuley DF, McKenna DH, Traber LD, Zhuo H, Wilson J, Herndon DN, Prough DS, Liu KD, Matthay MA, Enkhbaatar P (2014) Human mesenchymal stem cells reduce the severity of acute lung injury in a sheep model of bacterial pneumonia. *Thorax* 69(9):819–825
- Behr L, Hekmati M, Fromont G, Borenstein N, Noel LH, Lelievre-Pegorier M, Laborde K (2007) Intra renal arterial injection of autologous mesenchymal stem cells in an sheep model in the postischemic kidney. *Nephron Physiol* 107:65–76

- Behr L, Hekmati M, Houcinet K, Faussat A-M, Borenstein N, Noel L-H, Lelievre-Pegorier M, Laborde K (2009) Evaluation of the effect of autologous mesenchymal stem cell injection in a large-animal model of bilateral kidney ischaemia reperfusion injury. *Cell Prolif* 42:284–297
- Bel A, Messas E, Agbulut O, Richard P, Samuel JL, Bruneval P, Hagège AA, Menasché P (2003) Transplantation of autologous fresh bone marrow into infarcted myocardium: a word of caution. *Circulation* 108(Suppl 1):247–252
- Berner A, Reichert JC, Woodruff MA, Saifzadeh S, Morris AJ, Epari DR, Nerlich M, Schuetz MA, Hutmacher DW (2013) Autologous vs. allogenic mesenchymal progenitor cells for the reconstruction of critical sized segmental tibial bone defects in aged sheep. *Acta Biomater* 9(8):7874–7884
- Berner A, Henkel J, Woodruff MA, Steck R, Nerlich M, Schuetz MA, Hutmacher DW (2015) Delayed minimally invasive injection of allogenic bone marrow stromal cell sheets regenerates large bone defects in an sheep preclinical animal model. *Stem Cells Transl Med* 4:503–512
- Boos AM, Weigand A, Deschler G, Gerber T, Arkudas A, Kneser U, EHorch R, Beier JP (2014) Autologous serum improves bone formation in a primary stable silica-embedded nanohydroxyapatite bone substitute in combination with mesenchymal stem cells and rhBMP-2 in the sheep model. *Int J Nanomedicine* 9:5317–5339
- Bornes TD, Adesida AB, Jomha NM (2017) Articular cartilage repair with mesenchymal stem cells after chondrogenic priming: a pilot study. *Tissue Eng* 24:9–10. <https://doi.org/10.1089/ten.TEA.2017.0235>
- Brown EG, Keller BA, Lankford L, Pivetti CD, Hirose S, Farmer DL, Wang A (2016) Age does matter: a pilot comparison of placenta-derived stromal cells for in utero repair of myelomeningocele using a lamb model. *Fetal Diagn Ther* 39(3):179–185
- Burk J, Glauche SM, Brehm W, Crovace A, Francioso E, Hillmann A, Schubert S, Lacitignola L (2017) Characterisation and intracellular labelling of mesenchymal stromal cells derived from synovial fluid of horses and sheep. *Vet J* 222:1–8
- Caminal M, Fonseca C, Peris D, Moll X, Rabanal RM, Barrachina J, Codina D, García F, Cairó JJ, Gódia F, Pla A, Vives J (2014) Use of a chronic model of articular cartilage and meniscal injury for the assessment of long-term effects after autologous mesenchymal stromal cell treatment in sheep. *N Biotechnol* 31(5):492–498
- Caminal M, Moll X, Codina D, Rabanal RM, Morist A, Barrachina J, Garcia F, Pla A, Vives J (2014a) Transitory improvement of articular cartilage characteristics after implantation of polylactide:polyglycolic acid (PLGA) scaffolds seeded with autologous mesenchymal stromal cells in a sheep model of critical-sized chondral defect. *Biotechnol Lett* 36:2143–2153
- Caminal M, Vélez R, Rabanal RM, Vivas D, Batlle-Morera L, Aguirre M, Barquinero J, García J, Vives J (2017) A reproducible method for the isolation and expansion of sheep mesenchymal stromal cells from bone marrow for use in regenerative medicine preclinical studies. *J Tissue Eng Regen Med* 11(12):3408–3416
- Casañas J, de la Torre J, Solerc F, Garcíab F, Rodellard C, Pumarolac M, Climente J, Solerf R, Orozco L (2014) Peripheral nerve regeneration after experimental section in sheep radial and tibial nerves using synthetic nerve grafts, including expanded bone marrow mesenchymal cells: morphological and neurophysiological results. *Injury* 45(S4):S2–S6
- Ceccarelli G, Pozzo E, Scorletti F, Benedetti L, Cusella G, Ronzoni FL, Sahakyan V, Zambaiti E, Mimmi MC, Calcaterra V, Deprest J, Sampaolesi M, Pelizzo G (2015) Molecular signature of amniotic fluid derived stem cells in the fetal sheep model of myelomeningocele. *J Pediatr Surg* 50(9):1521–1527
- Chamberlain J, Yamagami T, Colletti E, Theise ND, Desai J, Frias A, Pixley J, Zanjani ED, Porada CD, Almeida-Porada G (2007) Efficient generation of human hepatocytes by the intrahepatic delivery of clonal human mesenchymal stem cells in fetal sheep. *Hepatology* 46(6):1935–1945
- Chandran S, Shenoy SJ, Babu SS, Nairm RP, Varma HK, John A (2017) Strontium Hydroxyapatite scaffolds engineered with stem cells aid osteointegration and osteogenesis in osteoporotic sheep model. *Colloids Surf B Biointerfaces* 163:346–354
- Chen J, Li Y, Wang L, Lu M, Zhang X, Chopp M (2001) Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. *J Neurol Sci* 189:49–57

- Chen F, Zhao C, Zhao Y, Li L, Liu S, Zhu Z, Guan W (2018) The biological characteristics of sheep umbilical cord mesenchymal stem cells. *Can J Vet Res* 82:216–224
- Cheng Y, Yi G, Conditt GB, Sheehy A, Kolodgie FD, Tellez A, Polyakov I, Gu A, Aboodi MS, Wallace-Bradley D, Schuster M, Martens T, Itescu S, Kaluza GL, Basu S, Virmani R, Granada JF, Sherman W (2013) Catheter-based endomyocardial delivery of mesenchymal precursor cells using 3D echo guidance improves cardiac function in a chronic myocardial injury sheep model. *Cell Transplant* 22(12):2299–2309
- Chevrier A, Nelea M, Hurtig MB, Hoemann CD, Buschmann MD (2009) Meniscus structure in human, sheep, and rabbit for animal models of meniscus repair. *J Orthop Res* 27(9):1197–1203
- Coleman SH, Ehteshami J, Kisiday JD, Warren RF, Turner AS (2011) The effects of mesenchymal stem cells on rotator cuff muscle in a chronic injury model in sheep. *American Academy of Orthopaedic Surgeons, San Diego*
- Colosimo A, Russo V, Mauro A, Curini V, Marchisio M, Bernabò N, Alfonsi M, Mattioli M, Barboni B (2013) Prolonged in vitro expansion partially affects phenotypic features and osteogenic potential of sheep amniotic fluid-derived mesenchymal stromal cells. *Cytotherapy* 15:930–950
- Crovace A, Lacitignola L, Francioso E, Rossi G (2008) Histology and immunohistochemistry study of sheep tendon grafted with cBMSCs and BMMNCs after collagenase-induced tendinitis. *Vet Comp Orthop Traumatol* 21(4):329–336
- Cuenca-López MD, Andrades JA, Gómez S, Zamora-Navas P, Guerado E, Rubio N, Blanco J, Becerra J (2014) Evaluation of posterolateral lumbar fusion in sheep using mineral scaffolds seeded with cultured bone marrow cells. *Int J Mol Sci* 15(12):23359–23376
- Cui P, He X, Pu Y, Zhang W, Zhang P, Li C, Guan W, Li X, Ma Y (2014) Biological characterization and pluripotent identification of sheep dermis-derived mesenchymal stem/progenitor cells. *Biomed Res Int* 2014:786234
- Czernik M, Fidanza A, Sardi M, Galli C, Brunetti D, Malatesta D, Della Salda L, Matsukawa K, Ptak GE, Loi P (2013) Differentiation potential and GFP labeling of sheep bone marrow-derived mesenchymal stem cells. *J Cell Biochem* 114:134–143
- Daly CD, Ghosh P, Zannettino ACW, Badal T, Shimmon R, Jenkin G, Oehme D, Jain K, Sher I, Vais A, Cohen C, Chandra RV, Goldschlager T (2018) Mesenchymal progenitor cells primed with pentosan polysulfate promote lumbar intervertebral disc regeneration in an sheep model of microdiscectomy. *Spine J* 18(3):491–506
- Dayan V, Sotelo V, Delfina V, Delgado N, Rodriguez C, Suanes C, Langhain M, Ferrando R, Armand K, Benech A, Touriño C (2016) Human mesenchymal stromal cells improve cardiac perfusion in an sheep immunocompetent animal model. *J Invest Surg* 29(4):1–8
- Delling U, Brehm W, Ludewig E, Winter K, Jülke H (2015a) Longitudinal evaluation of effects of intraarticular mesenchymal stromal cell administration for the treatment of osteoarthritis in a sheep model. *Cell Transplant* 24:2391–2407
- Delling U, Brehm W, Metzger M, Ludewig E, Winter K, Jülke H (2015b) In vivo tracking and fate of intra-articularly injected superparamagnetic iron oxide particle-labeled multipotent stromal cells in a sheep model of osteoarthritis. *Cell Transplant* 24:2379–2390
- Desando G, Giavaresi G, Cavallo C, Bartolotti I, Sartoni F, Aldini NN, Martini L, Parrilli A, Mariani E, Fini M, Grigolo B (2016) Autologous bone marrow concentrate in a sheep model of osteoarthritis: new perspectives for cartilage and meniscus repair. *Tissue Eng* 22(6):608–619
- Desantis S, Accogli G, Zizza S, Mastrodonato M, Blasi A, Francioso E, Rossi R, Crovace A, Resta L (2015) Ultrastructural study of cultured sheep bone marrow-derived mesenchymal stromal cells. *Ann Anat* 201:43–49
- DiVincenti LJ, Westcott R, Lee C (2014) Sheep (*Ovis aries*) as a Model for Cardiovascular Surgery and Management before, during, and after cardiopulmonary bypass. *J Am Assoc Lab Anim Sci* 53(5):439–448
- Dixon JA, Gorman RC, Stroud RE, Bouges S, Hirotsugu H, Gorman JH, Martens TP, Itescu S, Schuster MD, Plappert T, St John-Sutton MG, Spinale FG (2009) Mesenchymal cell transplantation and myocardial remodeling after myocardial infarction. *Circulation* 120(11):S220–S229
- Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause D, Deans R, Keating A, Prockop DJ, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal

- stromal cells. The International Society for Cellular Therapy position statement. *Cytherapy* 8(4):315–317
- Dooley LM, Abdalmula A, Washington EA, Kaufman C, Tudor EM, Ghosh P, Itescu S, Kimpton WG, Bailey SR (2015) Effect of mesenchymal precursor cells on the systemic inflammatory response and endothelial dysfunction in an sheep model of collagen-induced arthritis. *PLoS One* 10(5):e0124144
- Dowdell J, Erwin M, Choma T, Vaccaro A, Iatridis J, Cho SK (2017) Intervertebral disk degeneration and repair. *Neurosurgery* 80(3S):S46–S54
- Ersek A, Pixley JS, Goodrich AD, Porada CD, Almeida-Porada G, Thain DS, Zanjani ED, Fadel L, Viana BR, Feitosa MLT, Ercolin ACM, Roballo KCS, Casals JB, Pieri NC, Meirelles FV, Martins DS, Miglino MA, Ambrósio CE (2011) Protocols for obtainment and isolation of two mesenchymal stem cell sources in sheep. *Acta Cir Bras* 26:267–273
- Fadel L, VianaII BR, Feitosa MLT, ErcolinII ACM, RoballoII KCS, Casals JB, Pieri NCG, Meirelles FV, dos Santos MD, Miglino MA, Ambrósio CE (2011) Protocols for obtainment and isolation of two mesenchymal stem cell sources in sheep. *Acta Cir Bras* 26(4):267–273
- Feitosa MLT, Fadel L, Beltrão-Braga PCB, Wenceslau CV, Kerkis I, Kerkis A, Júnior EHB, Martins JFP, Martins DS, Miglino MA, Ambrósio CE (2010) Successful transplant of mesenchymal stem cells in induced osteonecrosis of the sheep femoral head. Preliminary results. *Acta Cir Bras* 25(5):416–422
- Feng C, Luo X, He N, Xia H, Lv X, Zhang X, Li D, Wang F, He J, Zhang L, Lin X, Lin L, Yin H, He J, Wang J, Cao W, Wang R, Zhou G, Wang W (2018) Efficacy and persistence of allogeneic adipose derived mesenchymal stem cells combined with hyaluronic acid in osteoarthritis after intra-articular injection in a sheep model. *Tissue Eng* 24(3–4):219–233
- Fernandes MBC, Guimarães JAM, Casadom PL, Cavalcantim AS, Gonçalves NN, Ambrósio CE, Rodrigues F, Pinto ACF, Miglino MA, Duarte MEL (2014) The effect of bone allografts combined with bone marrow stromal cells on the healing of segmental bone defects in a sheep model. *BMC Vet Res* 10:36
- Field JR, McGee M, Stanley R, Ruthenbeck G, Papadimitrakis T, Zannettino A, Gronthos S, Itescu S (2011) The efficacy of allogeneic mesenchymal precursor cells for the repair of an sheep tibial segmental defect. *Vet Comp Orthop Traumatol* 24:113–121
- Freeman BJ, Kuliwaba JS, Jones CF, Shu CC, Colloca CJ, Zarrinkalam MR, Mulaibrahimovic A, Gronthos S, Zannettino AC, Howell S (2016) Allogeneic mesenchymal precursor cells promote healing in postero-lateral annular lesions and improve indices of lumbar intervertebral disc degeneration in an sheep model. *Spine* 41(17):1331–1339
- Fuchs JR, Kaviani A, Oh JT, LaVan D, Udagawa T, Jennings RW, Wilson JM, Fauza DO (2004) Diaphragmatic reconstruction with autologous tendon engineered from mesenchymal amniocytes. *J Pediatr Surg* 39(6):834–838
- Gallego L, Perez-Basterrechea M, Garcia-Consuegra L, Alvarez-Viejo M, Megas J, Novoa A, Costilla S, Meana A, Junquera L (2015) Repair of segmental mandibular bone defects in sheep using bone marrow stromal cells and autologous serum scaffold: a pilot study. *J Clin Periodontol* 42:1143–1151
- Garcia D, Longo UG, Vaquero J, Forriol F, Loppini M, Khan WS, Denaro V (2015) Amniotic membrane transplant for articular cartilage repair: an experimental study in sheep. *Curr Stem Cell Res Therapy* 10:77–83
- Garcia-Gareta E, Hua J, Blunn GW (2015) Osseointegration of acellular and cellularized osteoconductive scaffolds: Is tissue engineering using mesenchymal stem cells necessary for implant fixation? *J Biomed Mater Res* 103A:1067–1076
- Ghasemzadeh-Hasankolaei M, Eslaminejad BM, Sedighi-Gilani M (2015) Derivation of male germ cells from ram bone marrow mesenchymal stem cells by three different methods and evaluation of their fate after transplantation into the testis. *In Vitro Cell Dev Biol Anim* 52(1):49–61
- Giannoni P, Mastrogiacomo M, Alini M, Pearce SG, Corsi A, Santolini F, Muraglia A, Bianco P, Cancedda R (2008) Regeneration of large bone defects in sheep using bone marrow stromal cells. *J Tissue Eng Regen Med* 2:253–262

- Goldschlager T, Ghosh P, Zannettino A, Gronthos S, Rosenfeld JV, Itescu S, Jenkin G (2010) Cervical motion preservation using mesenchymal progenitor cells and pentosan polysulfate, a novel chondrogenic agent: preliminary study in an sheep model. *Neurosurg Focus* 28(6):E4
- Goldschlager T, Jeffrey V, Rosenfeld JV, Ghosh P, Itescu S, Blecher C, McLean C, Jenkin G (2011) Cervical interbody fusion is enhanced by allogeneic mesenchymal precursor cells in an sheep model. *Spine* 36(8):615–623
- Gronthos S, Mrozik K, Shi S, Bartold PM (2006) Sheep periodontal ligament stem cells: isolation, characterization, and differentiation potential. *Calcif Tissue Int* 79(5):310–317
- Grzesiak J, Marycz K, Wrzeszcz K, Czogała J (2011) Isolation and morphological characterisation of sheep adipose-derived mesenchymal stem cells in culture. *Int J Stem Cells* 4(2):99–104
- Gugjoo MB, Amarpal (2018) Mesenchymal stem cell research in sheep: current status and future prospects. *Small Rum Res* 169:46–56. <https://doi.org/10.1016/j.smallrumres.2018.08.002>
- Gugjoo MB, Amarpal, Makhdoomi DM, Sharma GT (2019) Equine mesenchymal stem cells: properties, sources, characterization and potential therapeutic applications. *J Equine Vet Sci* 72:16–27. <https://doi.org/10.1016/j.jevs.2018.10.007>
- Gugjoo MB, Amarpal, Chandra V, Wani MY, Dhama K, Sharma GT (2018) Mesenchymal stem cell research in Veterinary Medicine. *Curr Stem Cell Res Therapy* 13(8):645–657. <https://doi.org/10.2174/1574888X13666180517074444>
- Gugjoo MB, Gayas MA, Ahmad RA, Handoo N (2018a) Mesenchymal stem cell studies in selected musculoskeletal tissues. In: Jalal H, Vinus, Gugjoo MB, Kawareti PK (eds) *Advances in veterinary sciences*. Akinik Publications, New Delhi, pp 51–77
- Gugjoo MB, Amarpal, Fazili MR, Shah RA, Mir MS, Sharma GT (2020a) Goat mesenchymal stem cell basic research and potential applications. *Small Rum Res* 183:106045
- Gugjoo MB, Amarpal, Ismail AA, Aithal HP, Kinjavdekar P, Kumar GS, Sharma GT (2020b) Allogeneic mesenchymal stem cells and growth factors in gel scaffold repair osteochondral defect in rabbit. *Regen Med* 15:2. <https://doi.org/10.2217/rme-2018-0138>
- Guo X, Wang C, Zhang Y, Xia R, Hu M, Duan C, Zhao Q, Dong L, Lu J, Qing S, Y. (2004) Repair of large articular cartilage defects with implants of autologous mesenchymal stem cells seeded into beta-tricalcium phosphate in a sheep model. *Tissue Eng* 10(11–12):1818–1829
- Gupta MC, Theerajunyaporn T, Maitra S, Schmidt MB, Holy CE, Kadiyala S, Bruder SP (2007) Efficacy of mesenchymal stem cell enriched grafts in an sheep posterolateral lumbar spine model. *Spine* 32(7):720–726
- Gutwald R, Haberstroh J, Kuschnierz J, Kister C, Lysek DA, Maglione M, Xavier SP, Oshima T, Schmelzeisen R, Sauerbier S (2010) Mesenchymal stem cells and inorganic bovine bone mineral in sinus augmentation: comparison with augmentation by autologous bone in adult sheep. *Br J Oral Maxillofac Surg* 48(4):285–290
- Hamamoto H, Gorman JH, Ryan LP, Hinmon R, Martens TP, Schuster MD, Plappert T, Kiupel M, St John-Sutton MG, Itescu S, Gorman RC (2009) Allogeneic mesenchymal precursor cell therapy to limit remodeling after myocardial infarction: the effect of cell dosage. *Ann Thorac Surg* 87(3):794–801
- Han J, Liu JY, Swartz DD, Andreadis ST (2010) Molecular and functional effects of organismal ageing on smooth muscle cells derived from bone marrow mesenchymal stem cells. *Cardiovasc Res* 87:147–155
- Heidari B, Shirazi A, Akhondi MM, Hassanpour H, Behzadi B, Naderi MM, Sarvari A, Borjian S (2013) Comparison of proliferative and multilineage differentiation potential of sheep mesenchymal stem cells derived from bone marrow, liver, and adipose tissue. *Avicenna J Med Biotechnol* 5(2):104–117
- Hindle P, Bailly J, Khan N, Biant LC, Simpson A, Hamish R, Péault B (2016) Perivascular mesenchymal stem cells in sheep: characterization and autologous transplantation in a model of articular cartilage repair. *Stem Cells Dev* 25(21):1659–1669
- Hnatiuk AP, Ong S-G, Olea FD, Locatelli P, Riegler J, Lee WH, Jen CH, De Lorenzi A, Gimenez CS, Laguens R, Wu JC, Crottogini A (2016) Allogeneic mesenchymal stromal cells overexpressing mutant human hypoxia-inducible factor 1- α (HIF1- α) in an sheep model of acute myocardial infarction. *J Am Heart Assoc* 5:e003714

- Hopper N, Wardale J, Brooks R, Power J, Rushton N, Henson F (2015) Peripheral blood mononuclear cells enhance cartilage repair in in vivo osteochondral defect model. *PLoS One* 10(8):e0133937
- Houtgraaf JH, de Jong R, Kazemi K, de Groot D, van der Spoel TI, Arslan F, Hoefer I, Pasterkamp G, Itescu S, Zijlstra F, Geleijnse ML, Serruys PW, Duckers HJ (2013) Intracoronary infusion of allogeneic mesenchymal precursor cells directly after experimental acute myocardial infarction reduces infarct size, abrogates adverse remodeling, and improves cardiac function. *Circ Res* 113(2):153–166
- Ingenito EP, Tsai L, Murthy S, Tyagi S, Mazan M, Hoffman A (2012) Autologous lung-derived mesenchymal stem cell transplantation in experimental emphysema. *Cell Transplant* 21(1):175–189
- Jahroomishirazi R, Bader A, Ebert S, Schmidt C, Sedaghati B, Schulz-Siegmund M, Zscharnack M (2014) Isolation and characterization of CD271 + stem cells derived from sheep dermal skin. *Cells Tissues Organs* 200:141–152
- Ji M, Bai C, Li L, Fan Y, Ma C, Li X, Guan W (2016) Biological characterization of sheep kidney-derived mesenchymal stem cells. *Exp Therap Med* 12:3963–3971
- Kabagambe SK, Chen YJ, Farmer DL (2017) Fetal surgery for myelomeningocele: a review of current clinical practice and translational research. *Minerva Pediatr* 69:59–65
- Khan MR, Chandrashekrana A, Smith RKW, Dudhia J (2016) Immunophenotypic characterization of sheep mesenchymal stem cells. *Cytometry A* 89(5):443–450
- Khan MR, Dudhia J, David FH, De Godoy R, Mehra V, Hughes G, Dakin SG, Carr AJ, Goodship AE, Smith RKW (2018) Bone marrow mesenchymal stem cells do not enhance intra-synovial tendon healing despite engraftment and homing to niches within the synovium. *Stem Cell Res Ther* 9:169
- Klein JD, Turner CGB, Steigman SA, Ahmed A, Zurakowski D, Eriksson A, Fauza DO (2011) Amniotic mesenchymal stem cells enhance normal fetal wound healing. *Stem Cells Dev* 20(6):969–976
- Kocyildirim E, Cárdenes N, Ting A, Cáceres E, Bermúdez C, Rojas M (2017) The use of GMP-produced bone marrow-derived stem cells in combination with extracorporeal membrane oxygenation in ARDS: An animal model. *ASAIO J* 63(3):324–332
- Kon E, Muraglia A, Corsi A, Bianco P, Marcacci M, Martin I, Boyde A, Ruspantini I, Chistolini P, Rocca M, Giardino R, Cancedda R, Quarto R (2000) Autologous bone marrow stromal cells loaded onto porous hydroxyapatite ceramic accelerate bone repair in critical-size defects of sheep long bones. *J Biomed Mater Res* 49(3):328–337
- Lacitignola L, Staffieri F, Rossi G, Francioso E, Crovace A (2014) Survival of bone marrow mesenchymal stem cells labelled with red fluorescent protein in an sheep model of collagenase-induced tendinitis. *Vet Comp Orthop Traumatol* 27(3):204–209
- Landa-Solis C, Olivos-Meza A, Olivos-Díaz B, Ibarra C (2016) Isolation and culture of mobilized mesenchymal stem cells in preclinical models for tissue engineering: basic science. *Adult Stem Cells: Recent Adv*. www.smgebooks.com
- Letouzey V, Tan KS, Deane JA, Ulrich D, Gurung S, Ong YR, Gargett CE (2015) Isolation and characterisation of mesenchymal stem/stromal cells in the sheep endometrium. *PLoS One* 10(5):e0127531
- Li D, Li M, Liu P, Zhang Y, Lu J, Lin J (2014) Tissue-engineered bone constructed in a bioreactor for repairing critical-sized bone defects in sheep. *Int Orthop* 38(11):2399–2406
- Liechty KW, Mackenzie TC, Shaaban AF, Radu A, Moseley AB, Deans R, Marshak DR, Flake AW (2000) Human mesenchymal stem cells engraft and demonstrate site specific differentiation after *in utero* transplantation in sheep. *Nat Med* 6(11):1282–1286
- Liu Z, Wang W, Gao J, Zhou H, Zhang Y (2014) Isolation, culture, and induced multiple differentiation of Mongolian sheep bone marrow-derived mesenchymal stem cells. *In Vitro Cell Dev Biol Anim* 50(5):464–474
- Locatelli P, Olea FD, Hnatiuk A, De Lorenzi A, Cerdá M, Giménez CS, Sepúlveda D, Laguens R, Crottogini A (2015) Mesenchymal stromal cells overexpressing vascular endothelial growth factor in sheep myocardial infarction. *Gene Ther* 22(6):449–457

- Lovati AB, Lopa S, Recordati C, Talò G, Turrise C, Bottagisio M, Losa M, Scanziani E, Moretti M (2016) In vivo bone formation within engineered hydroxyapatite scaffolds in a sheep model. *Calcif Tissue Int* 99(2):209–223
- Lv X, He J, Zhang X, Luo X, He N, Sun Z, Xia H, Liu V, Zhang L, Lin X, Lin L, Yin H, Jiang D, Cao W, Wang R, Zhou G, Wang W (2018) Comparative efficacy of autologous stromal vascular fraction and autologous adipose-derived mesenchymal stem cells combined with hyaluronic acid for the treatment of sheep osteoarthritis. *Cell Transplant* 27(7):1111–1125
- Lyahyai J, Mediano DR, Ranera B, Sanz A, Remacha AR, Bolea R, Zaragoza P, Rodellar C, Martín-Burriel I (2012) Isolation and characterization of sheep mesenchymal stem cells derived from peripheral blood. *BMC Vet Res* 8:169
- Lydon H, Getgood A, Henson FMD (2019) Healing of osteochondral defects via endochondral ossification in an ovine model. *Cartilage* 10(1):94–101
- Ma C, Liu C, Li X, Lu T, Bai C, Fan Y, Guan W, Guo Y (2017) Cryopreservation and multipotential characteristics evaluation of a novel type of mesenchymal stem cells derived from Small Tailed Han Sheep fetal lung tissue. *Cryobiology* 75:7–14
- Mackenzie TC, Flake AW (2001) Human mesenchymal stem cells persist, demonstrate site-specific multipotential differentiation, and are present in sites of wound healing and tissue regeneration after transplantation into fetal sheep. *Blood Cells Mol Dis* 27(3):601–604
- Manassero M, Viateau V, Deschepper M, Oudina K, Logeart-Avramoglou D, Petite H, Bensidhoum M (2013) Bone regeneration in sheep using acropora coral, a natural resorbable scaffold, and autologous mesenchymal stem cells. *Tissue Eng* 19(13–14):1554–1563
- Markides H, Newell KJ, Rudolf H, Ferreras LB, Dixon JE, Morris RH, Graves M, Kaggie J, Henson F, El Haj AJ (2019) Ex vivo MRI cell tracking of autologous mesenchymal stromal cells in an sheep osteochondral defect model. *Stem Cell Res Ther* 10:25
- Martinello T, Bronzini I, Perazzi A, Testoni S, De Benedictis GM, Negro A, Caporale G, Mascarello F, Iacopetti I, Patruno M (2013) Effects of in vivo applications of peripheral blood-derived mesenchymal stromal cells (PB-MSCs) and platelet-rich plasma (PRP) on experimentally injured deep digital flexor tendons of sheep. *J Orthop Res* 31(2):306–314
- Martinello T, Gomiero C, Perazzi A, Iacopetti I, Gemignani F, DeBenedictis GM, Ferro S, Zuin M, Martinez E, Brun P, Maccatrozzo L, Chiers K, Spaas JH, Patruno M (2018) Allogeneic mesenchymal stem cells improve the wound healing process of sheep skin. *BMC Vet Res* 14:202
- Mccarty R, Gronthos S, Zannettino AC, Foster BK, Xian CJ (2009) Characterisation and developmental potential of sheep bone marrow derived mesenchymal stem cells. *J Cell Physiol* 219:324–333
- McDonald CA, Oehme D, Pham Y, Kelly K, Itescu S, Gibbon A, Jenkin R (2015) Evaluation of the safety and tolerability of a high-dose intravenous infusion of allogeneic mesenchymal precursor cells. *Cytotherapy* 17(9):1178–1187
- Mediano DR, Sanz-Rubio D, Bolea R, Marín B, Vázquez FJ, Remacha AR, pez-Pérez O, Fernández-Borges N, Castilla J, Zaragoza P, Badiola JJ, Rodellar C, Martín-Burriel I (2015a) Characterization of mesenchymal stem cells in sheep naturally infected with scrapie. *J. General Virol* 96:3715–3726
- Mediano DR, Sanz-Rubio D, Ranera B, Bolea R, Martín-Burriel I (2015b) The potential of mesenchymal stem cell in prion research. *Zoonoses Public Health* 62(3):165–178
- Mendelson K, Aikawa E, Mettler BA, Sales V, Martin D, Mayer JE, Schoen FJ (2007) Healing and remodeling of bioengineered pulmonary artery patches implanted in sheep. *Cardiovasc Pathol* 16:277–282
- Mrugala D, Bony C, Neves N, Caillot L, Fabre S, Moukoko D, Jorgensen C, Noël D (2008) Phenotypic and functional characterisation of sheep mesenchymal stem cells: application to a cartilage defect model. *Ann Rheum Dis* 67(3):288–295
- Music E, Futrega K, Doran MR (2018) Sheep as a model for evaluating mesenchymal stem/stromal cell (MSC)-based chondral defect repair. *Osteoarthr Cartil* 26(6):730–740

- Niemeyer P, Schönberger TS, Hahn J, Kasten P, Fellenberg J, Suedkamp N, Mehlhorn AT, Milz S, Pearce S (2010) Xenogenic transplantation of human mesenchymal stem cells in a critical size defect of the sheep tibia for bone regeneration. *Tissue Eng* 16(1):33–43
- Ophelders DRMG, Wolfs TGAM, Jellema RK, Zwanenburg A, Andriessen P, Delhaas T, Ludwig A-K, Radtke S, Peters V, Janssen L, Giebel B, Kramer BW (2016) Mesenchymal stromal cell-derived extracellular vesicles protect the fetal brain after hypoxia-ischemia. *Stem Cells Transl Med* 5:754–763
- Pelagalli A, Nardelli A, Lucarelli E, Zannetti A, Brunetti A (2018) Autocrine signals increase sheep mesenchymal stem cells migration through Aquaporin-1 and CXCR4 overexpression. *J Cell Physiol* 233(8):6241–6249
- Pillai SM, Sereda NH, Hoffman ML, Valley EV, Crenshaw TD, Park Y-K, Lee J-Y, Zinn SA, Govoni KE (2016) Effects of poor maternal nutrition during gestation on bone development and mesenchymal stem cell activity in offspring. *PLoS One* 11(12):e0168382
- Przyborski SA, Hardy SA, Maltman DJ (2008) Mesenchymal stem cells as mediators of neural differentiation. *Curr Stem Cell Res Ther* 3:43–52
- Rabbani S, Soleimani M, Sahebjam M et al (2017) Effects of endothelial and mesenchymal stem cells on improving myocardial function in a sheep animal model. *J Tehran Heart Cent* 12(2):65–71
- Ragni E, Vigano M, Parazzi V, Montemurro T, Montelatici E, Lavazza C, Budelli S, Vecchini A, Rebulla P, Giordano R, Lazzari L (2013) Adipogenic potential in human mesenchymal stem cells strictly depends on adult or foetal tissue harvest. *Int J Biochem Cell Biol* 45:2456
- Rentsch C, Hess R, Rentsch B, Hofmann A, Manthey S, Scharnweber D, Biewener A, Zwipp H (2010) Sheep bone marrow mesenchymal stem cells: isolation and characterization of the cells and their osteogenic differentiation potential on embroidered and surface-modified polycaprolactone-co-lactide scaffolds. *In Vitro Cell Dev Biol Anim* 46:624–634
- Rhodes NP, Srivastava JK, Smith RF, Longinotti C (2004) Heterogeneity in proliferative potential of sheep mesenchymal stem cell colonies. *J Mater Sci Mater Med* 15(4):397–402
- Ribitsch I, Chang-Rodriguez S, Egerbacher M, Gabner S, Gueltekin S, Huber J, Schuster T, Jenner F (2017) Sheep placenta cotyledons: a noninvasive source of sheep mesenchymal stem cells. *Tissue Eng* 23(5):298–310
- Scharf A, Holmes S, Thoresen M, Mumaw J, Stumpf A, Peroni J (2015) Superparamagnetic iron oxide nanoparticles as a means to track mesenchymal stem cells in a large animal model of tendon injury. *Contrast Media Mol Imag* 10(5):388–397
- Shamsul BS, Tan KK, Chen HC, Aminuddin BS, Ruszymah BHI (2014) Posterolateral spinal fusion with osteogenesis induced BMSC seeded TCP/HA in a sheep model. *Tissue Cell* 46:152–158
- Shang Q, Wang Z, Liu W, Shi Y, Cui L, Cao Y (2001) Tissue-engineered bone repair of sheep cranial defects with autologous bone marrow stromal cells. *J Craniofac Surg* 12(6):586–593
- Shaw SW, Bollini S, Nader KA, Gastaldello A, Mehta V, Filippi E, Cananzi M, Gaspar HB, Qasim W, De Coppi P, David AL (2011) Autologous transplantation of amniotic fluid-derived mesenchymal stem cells into sheep fetuses. *Cell Transplant* 20(7):1015–1031
- Song F, Tang J, Geng R, Hu H, Zhu C, Cui W, Fan W (2014) Comparison of the efficacy of bone marrow mononuclear cells and bone mesenchymal stem cells in the treatment of osteoarthritis in a sheep model. *Int J Clin Exp Pathol* 7:1415–1426
- Sousa CP, Dias IR, Lopez-Peña M, Camassa JA, Lourenço PJ, Judas FM, Gomes ME, Reis RL (2015) Bone turnover markers for early detection of fracture healing disturbances: a review of the scientific literature. *An Acad Bras Cienc* 87:1049–1061
- Thangarajah T, Shahbazi S, Pendegrass CJ, Lambert S, Alexander S, Blunn GW (2016) Tendon reattachment to bone in a sheep tendon defect model of retraction using allogenic and xenogenic demineralised bone matrix incorporated with mesenchymal stem cells. *PLoS One* 11(9):e0161473
- Tian Y, Tao L, Zhao S, Tai D, Liu D, Liu P (2016) Isolation and morphological characterization of sheep amniotic fluid mesenchymal stem cells. *Exp Anim* 65(2):125–134

- Turner CG, Klein JD, Steigman SA, Ahmed A, Zurakowski D, Eriksson E, Fauza DO (2011) Preclinical regulatory validation of an engineered diaphragmatic tendon made with amniotic mesenchymal stem cells. *J Pediatr Surg* 46(1):57–61
- Vahedi P, Soleimanirad J, Roshangar L, Shafaei H, Jarolmasjed S, Charoudeh HN (2016) Advantages of sheep infrapatellar fat pad adipose tissue derived stem cells in tissue engineering. *Adv Pharm Bull* 6(1):105–110
- Van Der Donk S, Buma P, Aspenberg P, Schreurs BW (2001) Similarity of bone ingrowth in rats and goats: a bone chamber study. *Comp Med* 51:336–340
- Viateau V, Guillemain G, Bousson V, Oudina K, Hannouche D, Sedel L, Logeart-Avramoglou D, Petite H (2007) Long-bone critical-size defects treated with tissue-engineered grafts: a study on sheep. *J Orthop Res* 25(6):741–749
- Vivas D, Caminal M, Oliver-Vila I, Vives J (2018) Derivation of multipotent mesenchymal stromal cells from sheep bone marrow. *Curr Protoc Stem Cell Biol* 44(2B):9.1–9.22
- Wang A, Brown EG, Lankford L, Keller BA, Pivetti CD, Sitkin NA, Beattie MS, Bresnahan JC, Farmer DL (2015) Placental mesenchymal stromal cells rescue ambulation in sheep myelomeningocele. *Stem Cells Transl Med* 4:659–669
- Wheeler DL, Fredericks DC, Dryer RF, Bae HW (2016) Allogeneic mesenchymal precursor cells (MPCs) combined with an osteoconductive scaffold to promote lumbar interbody spine fusion in a sheep model. *Spinal J* 16:389–399
- Whitehouse M, Howells NR, Parry M, Austin E, Kafienah W, Brady K, Goodship AE, Eldridge JD, Blom AYW, Hollander AP (2017) Repair of torn avascular meniscal cartilage using undifferentiated autologous mesenchymal stem cells: from in vitro optimization to a first-in-human study. *Stem Cells Transl Med* 6(4):1237–1248
- Yr KJ, Evans RG, May CN (2018) A sheep model for studying the pathophysiology of septic acute kidney injury. *Methods Mol Biol* 1717:207–218
- Zhao Y, Zhang S, Zhou J, Wang J, Zhen M, Liu Y, Chen J, Qi Z (2010) The development of a tissue-engineered artery using decellularized scaffold and autologous sheep mesenchymal stem cells. *Biomaterials* 31(2):296–307
- Zhao Y, Li T, Wei X, Bianchi G, Hu J, Sanchez PG, Xu K, Zhang P, Pittenger MF, Wu ZJ, Griffith BP (2012) Mesenchymal stem cell transplantation improves regional cardiac remodeling following sheep infarction. *Stem Cells Transl Med* 1:685–695
- Zhu X, Liu Z, Deng W, Zhang Z, Liu Y, Wei L, Zhang Y, Zhou L, Wang Y (2017) Derivation and characterization of sheep bone marrow-derived mesenchymal stem cells induced with telomerase reverse transcriptase. *Saudi J Biol Sci* 24:519–525
- Zorzi AR, Amstalden EMI, Plepis AMG, Martins VCA, Ferretti M, Antonioli E, Duarte ASS, Luzo ACM, Miranda JB (2015) Effect of human adipose tissue mesenchymal stem cells on the regeneration of sheep articular cartilage. *Int J Mol Sci* 16:26813–26831
- Zscharnack M, Hepp P, Richter R, Aigner T, Schulz R, Somerson J, Josten C, Bader A, Marquass B (2010) Repair of chronic osteochondral defects using predifferentiated mesenchymal stem cells in a sheep model. *Am J Sports Med* 38(9):1857–1869

Goat Mesenchymal Stem Cell Basic Research and Potential Applications

8

M. B. Gugjoo, Amar Pal, M. R. Fazili, R. A. Shah, M. S. Mir,
and G. T. Sharma

Abstract

Innumerable goat MSC studies focussing on their culture properties in relation to their therapeutic properties have been conducted. The direct translation of ex vivo results into the in vivo application, however, remains distant. Goat MSCs (gMSCs) have been studied from various tissue sources, and their therapeutic evaluation in experimental models has been conducted mostly in relation to humans. Most of these experimental studies have favoured their applications. The current chapter details gMSCs sources, their characterization, and potential therapeutic applications.

M. B. Gugjoo (✉) · M. R. Fazili

Division of Veterinary Clinical Complex, FVSc & AH, SKUAST-Kashmir,
Jammu and Kashmir, India

A. Pal

Division of Surgery, Indian Veterinary Research Institute, Izatnagar, UP, India

R. A. Shah

Division of Animal Biotechnology, FVSc & AH, SKUAST-K,
Srinagar, Jammu and Kashmir, India

M. S. Mir

Directorate of Extension Education, SKUAST-K, Srinagar, Jammu and Kashmir, India
e-mail: masoodmir1@skuastkashmir.ac.in

G. T. Sharma

Division of Physiology & Climatology, Indian Veterinary Research Institute,
Izatnagar, UP, India

© The Editor(s) (if applicable) and The Author(s), under exclusive license to
Springer Nature Singapore Pte Ltd. 2020

M. B. Gugjoo, A. Pal (eds.), *Mesenchymal Stem Cell in Veterinary Sciences*,
https://doi.org/10.1007/978-981-15-6037-8_8

8.1 Introduction

There exists a considerable body of literature that has focussed on goat mesenchymal stem cells (gMSCs) properties to be translated to their therapeutic applications. An understanding of the basic cellular physiological properties, although currently very limited, may pave the way for their applications in veterinary medicine. Utilization of goat as translational animal model and application of gMSCs in these models has been aimed to provide further incites on stem cell applications in human medicine. The current chapter focuses on the gMSCs culture characteristics and their in vivo applications.

8.2 In Vitro Studies

8.2.1 Sources of gMSCs

In comparison to other species, gMSCs have been harvested from a limited number of tissue/organs. gMSCs from adult tissue organs and foetal membranes are enlisted in Table 8.1.

8.2.2 Characterization of gMSCs

gMSCs are characterized as per the standard criteria given for human and as followed by other species. gMSCs attach to the plastic surfaces and appear spindle-shaped fibroblasts. Short or long spindle size at various passages may be seen as has been reported for goat umbilical cord MSCs (gUC-MSCs). These cells may self-renew and grow as colonies for certain passages (Martins et al. 2017). The surface marker expression in general follows pattern as per standard recommendations (Table 8.1). However, weak expression of markers like CD90, CD166, and CD105 has also been reported in few studies (Knippenberg et al. 2005; Ghaneialvar et al. 2018). In addition to the surface markers, gMSCs express pluripotency markers (Table 8.1) and have been demonstrated to show plasticity and undergo extended differentiation into putative germ cells and myocyte-like cells (Tripathi et al. 2010; Li et al. 2017; Zhang et al. 2019).

8.2.3 Donor Tissue Source and gMSCs

Donor tissue determines the gMSCs initial concentration and the ability to proliferate and differentiate. Additionally, available culture environment too affects gMSCs characteristics. Bone tissue (ilium bone) in comparison to bone marrow may provide a higher yield (Akram et al. 2017). The presence of the periosteal layer (inner cambium) in bone chip may have led to the higher cell yield. gMSCs tend to proliferate for certain passages (p 20) beyond which these become

Table 8.1 Mesenchymal stem cell sources and characterization in goats

Positive markers	Negative markers	Differentiation potential	References
<i>Adipose tissue MSCs (AD-MSCs)</i>			
CD166 (30%), CD105 (35%)	–	Osteoblasts	Knippenberg et al. (2005)
CD166 (30%), CD105 (35%)	–	Osteoblasts	Knippenberg et al. (2006)
CD44, CD106, Vimentin	CD34, CD13	Adipogenic	Wang et al. (2018)
<i>Amniotic fluid MSCs (Am-MSCs)</i>			
CD-73, CD-90 and CD-105, Oct-4, Nanog, Sox-2, SSEA-1, SSEA-4	CD34	Adipogenic, chondrogenic, and osteogenic	Pratheesh et al. (2013)
CD73, CD90 and CD105 Pluripotency markers (Oct4, Sox2, Nanog, KLF, cMyc, FoxD3)	CD34	Adipogenic, chondrogenic, and osteogenic	Somal et al. (2016, 2017)
<i>Amniotic sac MSCs (AS-MSCs)</i>			
CD73, CD90 and CD105 Pluripotency markers (Oct4, Sox2, Nanog, KLF, cMyc, FoxD3)	CD34	Adipogenic, chondrogenic, and osteogenic	Somal et al. (2016, 2017)
<i>Bone marrow MSCs (BM-MSCs)</i>			
SH4, CD44	CD34, CD14	Adipogenic, chondrogenic, and osteogenic	Murphy et al. (2003)
CD44, CD29, CD90, CD166, OCT4, Nanog	CD45	Adipogenic, osteogenic	Zhang et al. (2006)
Oct-3/4, PCNA, Ck-Pan, vimentin and Nanog	–	–	da Silva Filho et al. (2014)
CD44	CD45	Adipogenic, chondrogenic, and osteogenic	Kwong et al. (2014)
CD29, CD90	CD34, CD45	Adipogenic, chondrogenic, and osteogenic	Elkhenany et al. (2015)
CD90, CD105 and CD73, OCT4	CD45	Adipogenic, chondrogenic, and osteogenic	Mohamad-Fauzi et al. (2015)
CD105, CD90	CD34, CD45	Adipogenic, chondrogenic, and osteogenic	Murphy et al. (2003)
–	–	Adipogenic, chondrogenic, and osteogenic	Burdzinska et al. (2017)
CD73, CD105, Stro-1	CD34	Adipogenic, chondrogenic, and osteogenic	Pratheesh et al. (2017)

(continued)

Table 8.1 (continued)

Positive markers	Negative markers	Differentiation potential	References
CD29, CD44	CD34	–	Xu et al. (2017)
CD44 and CD166, CD105, CD90 (weak expression)	CD34, CD45	–	Ghaneialvar et al. (2018)
<i>Cord blood MSCs (CB-MSCs)</i>			
CD73, CD90 and CD105 Pluripotency markers (Oct4, Sox2, Nanog, KLF, cMyc, FoxD3)	CD34	Adipogenic, chondrogenic, and osteogenic	Somal et al. (2016, 2017)
<i>Deciduous teeth MSCs (Dt-MSCs)</i>			
CD29, CD146	–	Osteogenic	Zhao et al. (2017)
<i>Endometrium MSCs (End-MSCs)</i>			
–	–	Adipogenic, chondrogenic, and osteogenic	Tamadon et al. (2017)
<i>Skeletal muscle MSCs (S-MSCs)</i>			
–	–	Osteocytes, adipocytes, and myogenic	Tripathi et al. (2010)
<i>Umbilical cord MSCs (UC-MSCs)</i>			
CD73, Thy-1, CD105	CD34	Adipogenic, osteogenic	Kumar et al. (2016)
CD90, CD105, CD44	CD34	Adipogenic, chondrogenic, and osteogenic	Martins et al. (2017)
CD90, CD105	CD34, CD44	Adipogenic, chondrogenic, and osteogenic	
<i>Wharton's jelly MSCs (WJ-MSCs)</i>			
CD-73, STRO-1, CD-105	CD34	Adipogenic, chondrogenic, and osteogenic	Pratheesh et al. (2014)
CD73, CD90 and CD105 Pluripotency markers (Oct4, Sox2, Nanog, KLF, cMyc, FoxD3)	CD34	Adipogenic, chondrogenic, and osteogenic	Somal et al. (2016, 2017)

AD-MSCs adipose-derived MSCs, *AF-MSCs* amniotic fluid-derived MSCs, *AS-MSCs* amniotic sac-derived MSCs, *BM-MSCs* bone marrow-derived MSCs, *CB-MSCs* cord blood-derived MSCs, *Dt-MSCs* deciduous teeth-derived MSCs, *End-MSCs* endometrium-derived MSCs, *Skeletal M-MSCs* skeletal muscle-derived MSCs, *UC-MSCs* umbilical cord-derived MSCs, *WJ-MSCs* Wharton's jelly-derived MSCs

senescent. With each passage, the population doubling time (PDT) tends to increase (Mohamad-Fauziet al. 2015). Average PDT of gUC-MSCs had increased from 33.49 h at passage 5 to 34.91 h at passage 12 (Qiu et al. 2012). gMSCs proliferation differences with tissue source also occur. The mean PDT of goat bone marrow (gBM)-, amniotic fluid (gAF)-, and Wharton's jelly (gWJ)-derived MSCs

had been 24.94 ± 2.67 h (Eslaminejad et al. 2009); 33.1 h (Pratheesh et al. 2013); 36.06 ± 1.2 h (Pratheesh et al. 2014), respectively, at specific passage. MSCs have also been harvested from goat foetal adnexa. The cells from particular adnexal tissues (gWJ-MSCs and goat cord blood-derived MSCs) tend to show faster proliferation as compared to others (gAF-MSCs and amniotic sac-derived MSCs) (Somal et al. 2016). Additionally, gWJ-MSCs had significantly higher expression of some of the surface and pluripotency markers as compared to MSCs from other sources. The expression of few pluripotency markers (Klf and cMyc), however, had remained comparable between gWJ-MSCs and goat amniotic fluid MSCs (gAF-MSCs) (Somal et al. 2017).

Self-renewal and differentiation properties of MSCs may be regulated by epigenetic modifications. MSCs derived from adipose tissues, bone marrow, and muscle may not carry identical promoter methylation profiles (Sorensen et al. 2010). The culture condition sensitivity of gMSCs might change their methylation pattern. Goat adipose tissue MSCs (gAD-MSCs) tend to undergo higher epigenetic changes as compared to goat bone marrow (BM)-MSCs (Wang et al. 2017). Likewise gAD-MSCs may have superior adipogenic but weaker osteogenic differentiation potential as compared to musculoskeletal sources like gBM-MSCs (Mohamad-Fauzi et al. 2015). The upregulation of osteogenic pathways in these cells had been variable; wap38 MAPK is upregulated in gBM-MSCs while p44/42 MAPK in gAD-MSCs (Elkhenany et al. 2016). gBM-MSCs tend to undergo low whole genome methylation as compared to fibroblasts as bone marrow requires cells that have high proliferation ability (Schoepet al. 2009).

8.2.4 Reproductive Cyclicity and gMSCs

Reproductive cyclicity too may affect MSCs proliferation. Anestral and cyclic endometrium-derived MSCs (End-MSCs) at passage 4 had 40.6 h and 53 h PDT, respectively (Tamadon et al. 2017). MSCs proliferation in anestrus may be promoted by the available higher concentration of growth factors like basic fibroblast growth factor (bFGF) and transforming growth factor- β (TGF- β) in endometrial tissue (Tamada et al. 2000; Tamadon et al. 2017).

8.2.5 Effect of Donor Age and Health Status on gMSCs

Donor age and type of ailment may also affect MSCs characteristics. MSCs harvested from an osteoporotic goat tend to proliferate slowly and show reduced osteogenic potential. Such a reduced differentiation potential may be enhanced through specific scaffold (β -TCP) incorporation (Cao et al. 2012). Ageing tends to decrease MSCs proliferation and differentiation potential. A single gMSCs study, however, had failed to demonstrate decrease in such properties with ageing (Vertenten et al. 2009).

8.3 Potential In Vivo Applications

Goat has been utilized as the translational model animal for human. As such gMSCs have been evaluated in various models of different conditions of bone and cartilage, among others for their therapeutic effects (Gugjoo et al. 2020). To avoid animal sufferings, substitutes like organ culture system are being developed. One of the studies had shown comparable results in organ culture system and in vivo conditions with respect to the gAD-MSCs spatial and temporal behaviour (Peeters et al. 2015). The current chapter, however, focuses on the in vivo therapeutic applications of gMSCs.

MSCs local implantation makes them available to the tissue directly in sufficient concentrations, while distant sources like intravenous routes may be required in large quantities for effective utilization. Additionally, intravenous application of gMSCs may activate disseminated intravascular coagulation (DIC) cascade and lead to hypercoagulable state, but the condition is transient (Liao et al. 2017). This may be due to the MSCs ability to express pro-coagulant factors, which increases as the cells are culture expanded (Tatsumi et al. 2013; Liao et al. 2017) and can be controlled by heparin therapy (Liao et al. 2017). The potential therapeutic applications of gMSCs are discussed below.

8.3.1 Cutaneous Wounds

Cutaneous healing encompasses complex system of organized biological and molecular events. These events may sometime become disorganized or fail to occur as seen in diabetes or chronic infections (Wu et al. 2007). Extensive tissue damages too fail to heal or may show delayed healing. This compromises the quality of life and also decreases production of the animal. MSCs tend to resolve and enhance the injured tissue healing (Phinney and Prockop 2007). gWJ-MSCs on local implantation tend to be viable up to day 12 and might promote epithelialization by as early as day 7. MSCs tend to promote and improve healing through their anti-inflammatory activities and secrete paracrine factors that promote granulation tissue formation with minimal to nil scar tissue formation (Table 8.2) (Azari et al. 2008,2011). MSCs applications in delayed/non-union wound may be conducted to confirm their utility in such obstinate cases.

8.3.2 Osteochondral Defects

Goat knee cartilage thickness (0.7–1.5 mm) approximates human knee (2.2–2.5 mm) cartilage, and as such it is being utilized as translational model animal for MSCs chondrogenic studies (Frisbie et al. 2006). Even antiangiogenesis factor-transfected MSCs have been studied for their therapeutic effects in avascular tissues including hyaline cartilage (Table 8.3) (Sun et al. 2009). The transfected MSCs were equally viable and had comparable chondrogenic potential to that of non-transfected gMSCs (Jeng et al. 2010).

Table 8.2 In vivo preclinical experimental mesenchymal stem cell studies on cutaneous wounds in goat

Model type	Number of animals	Study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Bilateral cutaneous wound on thoracic cavity (3 × 3 cm)	5 (right wound treated with MSCs; left wound control)	5 weeks	WJ-MSCs	9 × 10 ⁶	Morphometric evaluation, immunohistochemical staining (α-smooth muscle actin)	% wound contraction, epithelialization, and wound healing were significantly higher in cell-treated wounds as compared to control	Azari et al. (2008)
Cutaneous wound, 3 cm linear full-thickness skin incision	4 (4 wounds, 2 on each side of thoracic cavity, left wound treated with MSCs; right wounds with PBS)	7, 12 days	WJ-MSCs	3 × 10 ⁶	Histology	Transplanted cells were alive in wounds at day 12. At day 7 re-epithelialization was complete in cell-treated wounds while incomplete in control. Less inflammation and thinner granulation tissue with minimum scar tissue formation in cell-treated group	Azari et al. (2011)

Table 8.3 In vivo chondrogenic preclinical experimental mesenchymal stem cell studies in goat

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Full-thickness meniscal repair (0.75 mm)	32 (menisci repaired with two vertical sutures, $n = 8$; 0.5 mL exogenous fibrin clot, $n = 8$; fibrin clot + BM-MSCs, $n = 8$; left untreated, $n = 8$)	4 months	Autologous BM-MSCs, fibrin clot	20×10^6	Gross, tensile strength, and histology	Statistically non-significant differences in healing between suture repaired and fibrin clot repaired groups. Addition of MSCs did not enhance menisci repair	Port et al. (1996)
Chronic model of excision of the medial meniscus and resection of the anterior cruciate ligament	24 (6 animals cell treated/hyaluronan and 3 animals control for 12 weeks evaluation; 9 animals cell treated/hyaluronan and 6 animals for 26 weeks evaluation)	12 and 26 weeks	BM-MSCs + hyaluronan	10×10^6 loaded in hyaluronan	Histochemistry	Marked regeneration of the medial meniscus. Degeneration of the articular cartilage, osteophytic remodelling, and subchondral sclerosis were reduced in cell-treated group compared to control at 12 weeks. However, later the cell treated and control had severe osteoarthritis	Murphy et al. (2003)

Mandibular condyle osteochondral defect model	50 (12 in each group) (group I: NELL-1-modified BM-MSCs/PLGA, group II: BM-MSCs/PLGA; group III: PLGA alone; group IV: control)	3 mm diameter × 5 mm depth/6 weeks and 24 weeks	NELL-1 transfect autologous BM-MSCs and poly-lactic-co-polyglycolic acid scaffold	3 × 10 ⁶ cell seeded on PLGA scaffold	Macroscopic, histology, and immunohistochemistry, micro-CT	Group I showed rapid and vigorous healing leading to fibrocartilage formation at 6 weeks. At 24 weeks, complete repair of native articular cartilage and subchondral bone at 24 weeks. In group II: Repaired completely filled the defect with fibrocartilage at 24 weeks, but the cartilage was less well-organized group I. In groups III and IV, the defects were poorly repaired, and no cartilage in the group IV or only small portion of cartilage in the group III was formed	Zhu et al. (2011)
---	--	---	---	--	--	---	-------------------

(continued)

Table 8.3 (continued)

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Full-thickness chondral defect in medial femoral condyles	8 (16 knees) chondron and cell treatment vs. microfracture	5 mm/6 months	Chondron (chondrocytes in own matrix) and BM-MSCs	10% chondron/90% BM-MSC combination at a concentration of 1×10^6 cells/mL	Macroscopic and microscopic scoring, biochemical analysis, and histological and immunohistochemical analyses	Combination of BM-MSCs and chondrons leads to significantly better microscopic, macroscopic, and biochemical cartilage regeneration compared to microfracture treatment	Bekkers et al. (2013)
Osteochondral defects created in medial condyles and trochlear grooves	8 (group I: Scaffold seeded with cultured AD-MSCs; group II: Scaffold seeded with SVF cells; group III: Acellular scaffold)	5 mm $\times$ 3 mm/1 month and 4 months	Stromal vascular fraction (SVF), AD-MSCs along with collagen type I/III scaffold	5×10^6 (SVF) and 5×10^5 (AD-MSCs) seeded on scaffold	Macroscopy, immunohistochemistry, biomechanical analysis, micro-CT analysis, and biochemistry	Cell-treated groups had more extensive collagen type II, hyaline-like cartilage, and higher elastic moduli, and their glycosaminoglycan content in the cartilaginous layer that approached native tissue values. In control, lesser regenerative effect was seen. No difference in healing was seen between SVF-treated and AD-MSC-treated animals	Jurgens et al. (2013)

Chronic full-thickness chondral defect in medial femoral condyles	18 (36 defects) group I: Bone marrow stimulation and BM-MSCs; group II: Bone marrow stimulation; group III: Control	5 mm/6 months	BM-MSCs	1 × 10 ⁷ cells after 2 weeks after bone marrow stimulation for three consecutive weeks	Macroscopic, histology, biochemical assays (glycosaminoglycans), and gene expressions (aggrecan, collagen II and Sox9)	Hyaline-like tissue with higher glycosaminoglycans and chondrogenic gene expression in group I compared to group II that had fibrocartilage. Lowest healing in control	Nam et al. (2013)
Osteochondral defect (bilateral, 6 mm diameter and 12 mm depth)	n-12 (TEO cultured in double-chambered bioreactor without mechanical stimulation of stirring, n = 4; stirring, n = 4; no stirring, n = 4; no TEO implantation, control, n = 4)	12 and 24 weeks	Autologous BM-MSCs, β-TCP	–	Histology and immunohistochemistry, gross morphology	Reparative effects of TEO cultured in bioreactor were better than control, and mechanical stimulation could further improve the effects	Pei et al. (2014)

(continued)

Table 8.3 (continued)

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Full-thickness femoral condyle cartilage defects	6 (microfracture and cell/scaffold groups)	6.5 mm diameter/6 and 9 months	Human WJ-MSCs seeded in an acellular cartilage extracellular matrix (ACECM)-oriented scaffold	1 × 10 ⁶ cells seeded on ACECM	Analysis of inflammatory response, magnetic resonance imaging, gross morphology, histology, immunohistochemical and immunofluorescent staining, biomechanical testing, and biochemical quantitative analyses	No significant differences between the two groups in immuno-inflammatory parameters. MRI demonstrated higher-quality cartilage and complete subchondral bone at defect sites in the cell-treated group at 9 months. Histological revealed extracellular cartilage, cartilage lacuna, and collagen type II levels were higher in cell-treated group compared to the microfracture, while the cell-treated group exhibited a higher elasticity modulus	Zhang et al. (2018)

Various sources like bone marrow- (Murphy et al. 2003; Bekkers et al. 2013; Nam et al. 2013), adipose tissue- (Jurgens et al. 2013), and Wharton's Jelly- (Zhang et al. 2018) derived MSCs have been evaluated in osteochondral defect models. The cells along with various scaffolds like hyaluronan, collagen I/III, natural cartilage ingredients, and poly lactic co-glycolic acid (PLGA) have been implanted to prevent loss of cells through adverse effects of local environment. An overall improvement in osteochondral defect healing have been demonstrated to occur with MSCs and scaffold implantations (Table 8.3). The improved healing has been characterized by enhanced secretion of cartilage-specific matrices like collagen type II and glycosaminoglycan. The regenerated hyaline-like cartilage had biomechanical factors almost comparable to native tissue (Jurgens et al. 2013). These cells tend to retard degeneration of the articular cartilage and prevent osteophyte formation and sclerosis of subchondral bone (Murphy et al. 2003). Even mechanical stimulation of these cells seeded in scaffolds may further enhance their repair potential (Pei et al. 2014).

The femoral condyle defect-healing potential of MSCs (BM-MSCs or xenogenic WJ-MSCs) seeded over cartilage extracellular matrix may be better than commonly used surgical techniques (microfracture and bone marrow stimulation). The healing had been evaluated utilizing International Cartilage Repair Society (ICRS) and O'Driscoll scores, in addition to the expression profile of cartilage-specific genes (Bekkers et al. 2013; Nam et al. 2013; Zhang et al. 2018). However, no differences in inflammatory response at the defect site had been demonstrated among the cellular implantation- and surgical techniques-treated animals (Zhang et al. 2018). One of the studies had failed to demonstrate any significant improvement in meniscal repair with gBM-MSCs and fibrin clot (Port et al. 1996).

Transfected MSCs may further enhance osteochondral defect healing (Zhu et al. 2011; Wei et al. 2019). Among various factors, transfection of gBM-MSCs by NELL-like molecule-1 (Nell-1) seeded on poly lactic co-glycolic acid (PLGA) had repaired goat mandibular condyle defects by fibrocartilage at 6 weeks and by 24 weeks with native articular cartilage. Simple BM-MSCs and PLGA assembly had repaired the defect by fibrocartilage even at 24 weeks (Zhu et al. 2011). Nell-1 is a novel growth factor that specifically target cells committed to osteochondral lineage. Further studies in the area are desired.

8.3.3 Bone Defects

Bone tissue engineering is carried out extensively in humans (Costa-Pinto et al. 2011). Goat is increasingly being used as human orthopaedic research animal model (Proffen et al. 2012). Goat and human long bones have approximate size and macrostructure, in addition to the biomechanics and biochemistry (Anderson et al. 1999; Van Der Donk et al. 2001; Sousa et al. 2015). Furthermore, goat osteoporosis effectively mimics human condition (Cao et al. 2012).

Various goat bone defect models have been created to study MSCs therapeutic potential (Table 8.4). Variable critical size defects have been created that varied

Table 8.4 In vivo preclinical experimental mesenchymal stem cell studies on osteogenesis in goat

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Tibial bone defects (2.6 cm)	26 (BCB + transfected cells, $n = 9$; β -gal-transfected cells + BCB, $n = 6$; BCB + simple cells, $n = 8$; BCB, $n = 3$)	8 and 26 weeks	Biphasic calcined bone (BCB), BMP-2-transfected BM-MSCs, simple BM-MSCs	2×10^8	Histology, biomechanical examination	5/8 defects were completely healed, while 3/8 had partial healed in BCB + transfected cell-treated group. In other experimental groups, slight or no healing was reported	Dai et al. (2005)
Cranial suture distraction (coronal) (0.4 mm/day for 8 days)	10 (MSCs, $n = 5$; PBS, $n = 5$)	4 weeks	MSCs	5×10^9	SEM and histology	The new bone formation at the edge of suture was superior in cell-treated group than control	Shen et al. (2006)
Bilateral early stage osteonecrosis of femoral head (ligation of lateral medial circumflex arteries and delivery of liquid nitrogen to femoral head, 6-mm defect filled with biomaterials)	23 (right and left femoral defect-treated BMP-2- and β -gal-transfected cells, respectively, $n = 20$; control, $n = 3$)	16 weeks	Bone morphogenetic protein-2 (BMP-2)- or β -galactosidase (β -gal)-transfected BM-MSCs, β -tricalcium phosphate (β -TCP)	1×10^7	Radiography, bone mineral density, histology, and biomechanical testing	The femoral heads in BMP-2-transfected cell-treated group had normal density and surface, while those in β -gal-treated cell group had low density and irregular surface. Histologically, lamellar bone was formed in BMP-2 cell-treated group, while in β -gal cell-treated group residual fibrous tissue was formed. The new bone formed in BMP-2 group had significantly higher bone as compared to β -gal. The maximum compressive strength and Young's modulus of repaired tissue in BMP-2 treated cell group were similar to normal bone and significantly higher as compared to β -gal cell-treated group. In non-treated group, femoral heads collapsed at 16 weeks	Tang et al. (2007)

Segmental femoral defect (2 cm, nail stabilized)	6 (HASi, $n = 4$; tissue-engineered HASi, $n = 2$)	4 months	BM-MSCs, triphasic ceramic-coated hydroxyapatite (HASi)	1×10^5 cm ² of HASi	Radiography, micro-CT, histology, histomorphometry, scanning electron microscopy, and inductive couple plasma spectrometry	Good osteointegration and osteoconduction were observed in TE HASi and HASi. Tissue-engineered HASi showed better and faster performance evident by lamellar bone organization of newly formed bone with the degradation of material. In HASi only group, immature interwoven bony bridges still intermingled with scattered small remnants of material	Nair et al. (2009)
Bilateral segmental tibial defect (30 mm)	6 (β -TCP seeded BM-MSCs cultured in dynamic perfusion reactor implanted in left tibia; β -TCP seeded BM-MSCs cultured static implanted in right tibia)	24 weeks	Autologous BM-MSCs	2×10^6 mL	Radiography, histology and micro-CT	Significant osteogenic capacity of experimental group compared to control. Segmental bone defect repair by dynamic perfusion reactor culture MSCs outweigh static mode	Wang et al. (2010)
Tibial unicortical defect (6 mm) (four defects per tibia)	8 (4 defects in each tibia; PLA, PLA + cell; PLA + cells + β -TCP; control)	2, 4, 8, and 12 weeks	BM-MSCs, β -TCP, methacrylate-encapped poly(D,L-lactide-Co- ϵ -caprolactone)	–	Radiography, histology, histomorphology, and immunohistochemistry	Cell survival and proliferation in polymer-substituted bone defects was demonstrated. The incorporation of β -TCP was associated with less expansion and growth of MSCs	Vertenten et al. (2009)
Non-critical-sized unicortical tibial defect model (bilateral, 4 holes, 6 mm)	8 (autologous BM-MSCs, Pluronic® F127 hydrogel; tubular sintered hydroxyapatite)	2 and 4 weeks, 6 and 8 weeks	Autologous BM-MSCs, Pluronic® F127 hydrogel, tubular sintered hydroxyapatite	4×10^5 cultured on carriers	Clinical, radiology, and histology	Faster bone repair in defects filled with hydrogel plus cultiSpher-S carriers in comparison to control. No new bone formation was originating from hydroxyapatite carriers. However, the hydrogels stimulated the natural repair process	Lippens et al. (2010)

(continued)

Table 8.4 (continued)

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Segmental tibial defect (25 mm)	32 (nHACP/CF + cells, $n = 8$; autograft, $n = 8$; nHACP/CF, $n = 8$; control, $n = 8$)	8 weeks	Autologous BM-MSCs, nano-hydroxyapatite/collagen/poly (L-lactic acid), PLLA/chitin fibres (nHACP/CF)	2×10^6	Radiography, histology, and biomechanics	Cell along with scaffold and autograft groups had defect repaired by 8 weeks	Liu et al. (2010)
Medial femoral condyle defect of osteoporotic defect (oestrogen deficiency induced, 0.8-cm diameter and 8-mm deep cylindrical defects)	16 (sham, $n = 6$; β -TCP treated, $n = 10$; BM-MSCs/ β -TCP, $n = 16$)	16 weeks after defect creation	Autologous BM-MSCs (oestrogen deficient donor), β -TCP	–	Radiography, CT, micro-CT and histomorphometry	MSCs/ β -TCP treated animals had successful repair of bone defects	Cao et al. (2012)
Maxillary sinus augmentation (bilateral)	9 (cells/CPC, $n = 6$; CPC alone, $n = 6$; autogenous graft, $n = 6$)	3 months	BM-MSCs, calcium phosphate cement(CPC)	2×10^7	Micro-CT, histology and histomorphometry	Group treated with cells/CPC promoted early bone formation and mineralization and maximally maintain volume and height of augmented maxillary sinus as compared to other groups. Osteointegration was significantly higher in cells/CPC treated group as compared to others	Zou et al. (2012)

Critical sized femur defects (bilateral, 20 mm)	12 (right defects treated with SAP/DBM/BMA, $n = 12$; left with DBM/BMA, $n = 12$)	8 and 16 weeks	Bone marrow aspirate (BMA), nano-scale self-assembly peptide (SAP) modified demineralized bone matrix (DBM)	–	Radiography, CT and 3D image processing techniques	Volume of new bone formed in SAP/DBM/BMA assembly implanted bone defects was significantly higher as compared to DBM/MSCs	Li et al. (2015)
Iliac crest defect (4 defects bilaterally)	10 (one defect received either autologous bone graft or was left empty ($n = 5$). In the other 3 locations, the experimental groups ($n = 10$) were tested: (1) biphasic calcium phosphate (BCP) control, (2) BCP with alginate, pBMP-2 and allogeneic MSCs and (3) BCP with alginate and pBMP-2	3–12 weeks	Allogeneic BM-MSCs, bicalcium phosphate (BCP)	2×10^6	Histomorphometry, fluorescent microscopy, immunohistochemistry	Fluorochrome incorporation evaluated at 3, 6 and 9 weeks and histomorphometry at 12 weeks after implantation revealed clear differences between the groups, with pBMP-2 combined with MSCs being the most effective. The BMP-2 was demonstrated in a variety of bone-residing cells through immunohistochemistry. Further analysis indicated that multinucleated giant cells might have an important role in transgene expression. The model appeared robust, showed no neighbouring effects and demonstrated effectiveness of combined cell and gene therapy	Loozen et al. (2015)
Distraction osteogenesis (mandibular)	12 (MSCs, $n = 6$; control, $n = 6$)	30 days	BM-MSCs	1.5×10^6 (two injections at day 10 and day 20 after surgery)	Energy dispersive X-ray, scanning electron microscopy (SEM) and histology	An increased calcification and significant trabecular bone size and limited osteoid formation in cell-treated group as compared to control	El Hadidi et al. (2016)

(continued)

Table 8.4 (continued)

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Maxillary sinus floor elevation	18 (<i>n</i> = 6 each group); group A (SGDs-CPC compound), group B (CPC alone), and group C (autogenous bone obtained from an iliac crest)	3 months	Deciduous teeth MSCs (DT-MSCs), calcium phosphate cement (CPC)		Computed tomography (CT), histology, and histomorphometric analysis	At 3 months, SGDs-CPC compound had promoted bone formation and mineralization. The areas of new bone formation in elevated sinuses were DT-MSCs/CPC group, which was significantly higher than the CPC-alone group or in the autogenous bone group. Immunohistochemical staining revealed that GFP and OCN were both expressed in the new bone tissue, which suggested that the implanted DT-MSCs might have contributed to new bone formation on the elevated sinus floor. DT-MSCs can promote new bone formation and maturation in the goat maxillary sinus, and the tissue-engineered bone composite of SGDs and CPC might be a potential substitute for existing maxillary sinus floor elevation methods	Zhao et al. (2017)

among studies even on the same bone type. Critical size defect for ilium had been 17 mm (Anderson et al. 1999), femoral defect 20 mm (Li et al. 2015), and tibial defects 25 mm (Liu et al. 2010) and 30 mm (Wang et al. 2010). Various bone tissue-engineered constructs along with MSCs have been employed in goats for critical size defects of mandible, femur, and tibia (Table 8.4). Mostly BM-MSCs have been utilized along with various kinds of scaffolds (Table 8.4). The cellular scaffolds had led to the early and improved bone repair both in quality and quantity as compared to the acellular scaffolds. The lamellar bone organization and its osteo-integration in the cell-treated defects had been better than those treated without cells. In vitro cell-scaffold compatibility does not guarantee in vivo regenerative effects (Vertenten et al. 2009). Thus, it is important to confirm in vivo applicability of MSCs and/or scaffolds.

gBM-MSCs genetic engineering with human bone morphogenetic protein (hBMP) gene (Tang et al. 2007; Loozen et al. 2015) and beta-galactosidase (gal)-gene (Tang et al. 2007) have been demonstrated to further promote healing of bone. The healed bone tissue formed had higher volume with better mechanical strength as compared to that with normal MSCs. BMP-2 transduced gBM-MSCs, however, may be more pro-healing as compared to BM-MSCs transfected with beta-galactosidase (Tang et al. 2007). Bone remains under stress that may determine the healing outcome. On that principle, dynamically cultured MSCs and/scaffolds and distraction osteogenesis had favoured bone healing (Shen et al. 2006; Cao et al. 2012; Gardel et al. 2013).

Bone tissue-engineered cellular scaffolds have favoured early and better bone regeneration. To achieve evidence-based medicine and for definitive applications of these constructs, more uniform and extensive studies are desired.

8.3.4 Intervertebral Disc Disease

Goats carry larger size of lumbar intervertebral discs (~5 mm disc height) and have disc mechanical properties and biochemical composition resembling to that of human. This makes goats more suitable animal model for human (Table 8.5) (Beckstein et al. 2008). Goat preliminary ex vivo studies on MSCs and/scaffold have shown favourable role in intervertebral disc diseases (Gullbrand et al. 2017). Few in vivo studies conducted have been pro to the utilization of MSCs along with scaffolds. The healing may be observed as early as 3 weeks of time (Xu et al. 2017). Among various factors, basement membrane molecules too determine nucleus pulposus chondrogenesis and cartilage regeneration (Toh et al. 2013).

8.3.5 Periodontal Tissue

The cellular grafting of periodontal defects may further promote periodontal tissue regeneration against the normal grafting. Among various tissue-regenerated structures are cementum, bone, and periodontal ligament (Table 8.5) (Marei et al. 2009). The preliminary reports demand in-depth studies for any definitive outcome.

Table 8.5 In vivo preclinical experimental mesenchymal stem cell studies on different conditions in goat

Model type	Number of animals	Study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Annulus fibrosus defect (30 discs, T1-L5, 1 × 1 cm)	$n = 6$ (30 discs in each group) Sham group, injury group, and treatment group	3, 6 and 12 weeks	BM-MSCs, platelet-rich plasma, gelatine sponge	–	HE and mason trichrome staining, Alcian blue periodic acid Schiff and collagen II staining	Repaired annulus fibrosus in cell-treated group had cell density and morphology of cells closer to sham group. Chondrocytes participated in repair process and collagen II progressively increased	Xu et al. (2017)
Canine tooth extraction (bilateral, titanium fixture)	$n = 5$ (control: Poly (DL-Lactide-co-glycolide), $n = 5$; treatment: BM-MSCs, poly (DL-Lactide-co-glycolide)	10 days and 1 month	Autologous BM-MSCs, poly (DL-Lactide-co-glycolide)	9.4×10^6 per scaffold	Radiography, histology, histophotometric, and scanning electron microscopy	Experimental side showed periodontal-like tissue with newly formed bone at 10 days and 1 month. Control showed connective tissue regeneration at 10 days but not at 1 month	Marei et al. (2009)
Bronchopleural fistula (upper tracheal lobectomy)	9 (BM-MSCs, $n = 4$; bronchoscopic fibrin glue, $n = 5$)	28 day	BM-MSCs, bronchoscopic fibrin glue	2×10^6 / mL (5 mL injected)	Histopathology, CT, and MRI	Bronchopleural closure occurred by extraluminal fibroblast proliferation and collagenous matrix development in cell-treated group. In the other group, the fistula was still present. Even 40% (2/5) animals died	Perella et al. (2014)

8.3.6 Cardiovascular System

Goat has also been used to study the effect of MSCs in cardiovascular affections relevant to the humans. Autologous gBM-MSCs and/or scaffold in coronary artery ligation model has been evaluated. The assembly could prevent left ventricular chamber dilatation and had improved myocardial perfusion and contraction (Liao et al. 2006). It is a preliminary study that demands further evaluation.

8.3.7 Bronchopleural Fistula

Lung cancer remains one of the leading causes of death (Wakelee et al. 2007). Surgical resection in limited lung disease is preferable option but tends to develop bronchopleural fistula (BPF) causing higher mortality (Sonobe et al. 2000). In an induced bronchopleural fistula, implantation of the gBM-MSCs had led to the healing of fistula in about a month (Table 8.5) (Petrella et al. 2014), demanding further studies.

8.3.8 Reproduction

Nuclear Transfer (NT) technology is used to develop clones. The current efficiency remains low, and one of the important limiting factors is the nucleus of donor cell type (Kwong et al. 2014). gMSCs as donor karyoplast appear promising to improve such efficiency as these cells can be passaged for extended periods (Ren et al. 2014). Ex vivo studies have demonstrated gBM-MSCs as donor karyoplast to produce cloned caprine embryos. These cells had better developed up to the hatched blastocyst stage than that with ear fibroblasts. Compared to somatic cells, better proliferation rate, growth capacity, transfection efficiency and convergence, and cleavage had been demonstrated with MSCs (Kwong et al. 2014; Ren et al. 2014). Contrarily, one of the studies had failed to show any difference in fusion and cleavage rates between gBM-MSCs and somatic cells (Kwong et al. 2014).

Apart from the NT, MSCs have been evaluated for their effect on the growth and activation of pre-antral follicles. A significant reduction in the percentage of morphologically normal follicles cultured along with BM-MSCs had been demonstrated as compared to the fresh control. However, a significantly higher rate of morphologically normal pre-antral follicles had been reported when cultured in BM-MSCs as compared to those cultured normally. The follicular diameter, however, had remained comparable in all the follicles irrespective of the culture conditions. In conclusion, BM-MSCs had no influence on follicular or oocyte development but had promoted survival of pre-antral follicles (Arrivabene Neves et al. 2019).

8.3.9 Mastitis

Stem cells have been harnessed from mammary gland and as such are considered as potential targets to improve milk production (Martignani et al. 2009; Capuco et al.

2012; Martignani et al. 2014). MSCs due to their characteristic features have potential to regenerate mammary tissue. A single MSCs study on goat chronic mastitis had demonstrated their potential to reduce fibrosis and in maintaining normal tissue architecture and ability to secrete milk (Costa et al. 2019).

8.3.10 Urinary System

Goats may act as a model for stress urinary incontinence disorder of human (Burdzinska et al. 2017) and as such applicability of gMSCs in such ailments may be evaluated.

8.4 Conclusions

gMSCs have been harvested from various sources including foetal membranes. MSCs characteristic properties like proliferation and differentiation potential have been evaluated in relation to tissue sources. The cells have been characterized as per the standard protocols. Bone marrow, adipose tissue, and foetal adnexa-derived MSCs have been mainly evaluated under experimental studies. A wide array of gMSCs research mainly focussed on human translational studies has been conducted. Preliminary therapeutic literature on gMSCs has variably favoured their in vivo applications. Different biomaterials like cellularized scaffolds and genetically engineered cells together with growth factors have also been evaluated. Currently, the studies remain inconclusive.

References

- Akram T, Hakim M, Mir BA, Hussain SS, Shabir N, Andrabi M, Dar PA, Ganaia NA, Shabir N (2017) Comparative efficiency of goat mesenchymal stem cell isolation from bone marrow and bone chip. *Small Rum Res* 153:87–94
- Anderson ML, Dhert WJ, De Bruijn JD, Dalmeijer RA, Leenders H, Van Blitterswijk CA, Verbout AJ (1999) Critical size defect in the goat's os ilium. A model to evaluate bone grafts and substitutes. *Clinical Orthop Rel Res* 364:231–239
- Arrivabene Neves C, dos Santos Silva L, Sousa de Carvalho CE, Carvalho MS, Sarmiento JLR, Cavalcante TV, Arrivabene M, Arrivabene Neves T, de Sousa Bezerra MÉ, Gomes Júnior AL, Gomes da Silva CM, Martins de Carvalho MA (2019) Culture of goat preantral follicles in situ associated with mesenchymal stem cell from bone marrow. *Zygote* 28:65–71
- Azari O, Babaei H, Molaei MM, Nematollahi-Mahani SN, Layasi T (2008) The use of Wharton's jelly-derived mesenchymal stem cells to accelerate second-intention cutaneous wound healing in goat. *Ir J Vet Surg* 3(3):15–26
- Azari O, Babaei H, Derakhshanfar A, Nematollahi-Mahani SN, Poursahebi R, Moshrefi M (2011) Effects of transplanted mesenchymal stem cells isolated from Wharton's jelly of caprine umbilical cord on cutaneous wound healing; histopathological evaluation. *Vet Res Commun* 35(4):211–222
- Beckstein JC, Sen S, Schaer TP, Vresilovic EJ, Elliott DM (2008) Comparison of animal discs used in disc research to human lumbar disc: axial compression mechanics and glycosaminoglycan content. *Spine* 33:E166–E173

- Bekkers JE, Tsuchida AI, van Rijen MH, Vonk LA, Dhert WJ, Creemers LB, Saris DB (2013) Single-stage cell-based cartilage regeneration using a combination of chondrons and mesenchymal stromal cells: comparison with microfracture. *Am J Sports Med* 41(9):2158–2166
- Burdzinska A, Dybowski B, Zarychta-Wisniewska W, Kulesza A, Zagodzón R, Gajewski Z, Paczek L (2017) The anatomy of caprine female urethra and characteristics of muscle and bone marrow derived caprine cells for autologous cell therapy testing. *Anat Rec* 300(3):577–588
- Cao L, Liu G, Gan Y, Fan Q, Yang F, Zhang X, Tang T, Dai K (2012) The use of autologous enriched bone marrow MSCs to enhance osteoporotic bone defect repair in long-term estrogen deficient goats. *Biomaterials* 33(20):5076–5084
- Capuco AV, Choudhary RK, Daniels KM, Li RW, Evock-Clover CM (2012) Bovine mammary stem cells: cell biology meets production agriculture. *Animal* 6(3):382–393
- Costa CRM, Feitosa MLT, Rocha AR, Bezerra DO, Leite YKC, Argolo Neto NM et al (2019) Adipose stem cells in reparative goat mastitis mammary gland. *PLoS One* 14(10):e0223751
- Costa-Pinto AR, Reis RL, Neves NM (2011) Scaffolds based bone tissue engineering: the role of chitosan. *Tissue Eng Part B Rev* 17:331–347
- da Silva Filho OF, Argôlo Neto NM, de Carvalho MAM, de Carvalho YK, das Neves Diniz A, da Silva Moura L, Ambrósio CE, Monteiro JM, de Almeida HM, Miglino XMA, de Jesus Rosa Pereira A, Macedo JKV, da Rocha AR, Feitos MLT, Ribeiro Alves F (2014) Isolation and characterization of mesenchymal progenitors derived from the bone marrow of goats native from northeastern Brazil. *Acta Cir Bras* 29(8):478–484
- Dai KR, Xu XL, Tang TT, Zhu ZA, Yu CF, Lou JR, Zhang XL (2005) Repairing of goat Tibial bone defects with BMP-2 gene-modified tissue-engineered bone. *Calcif Tissue Int* 77:55–61
- El Hadidi YN, El Kassaby M, El Fatah Ahmed SA, Khamis N (2016) The effect of mesenchymal stem cells application on the distracted bone microstructure. (An experimental study). *J Oral Maxillofac Surg* 74(7):1463.e1–1463.e11
- Elkhenany H, Amelse L, Lafont A, Bourdo S, Caldwell M, Neilsen N, Dervishi E, Derek O, Biris AS, Anderson D, Dhar M (2015) Graphene supports in vitro proliferation and osteogenic differentiation of goat adult mesenchymal stem cells: potential for bone tissue engineering. *J Appl Toxicol* 35(4):367–374
- Elkhenany H, Amelse L, Caldwell M, Abdelwahed R, Dhar M (2016) Impact of the source and serial passaging of goat mesenchymal stem cells on osteogenic differentiation potential: implications for bone tissue engineering. *J Anim Sci Biotechnol* 7:16
- Eslaminejad MB, Nazarian H, Falahi F, Taghiyar L, Daneshzadeh MT (2009) Ex vivo expansion and differentiation of mesenchymal stem cells from goat bone marrow. *Ir J Basic Med Sci* 12(2):70–79
- Frisbie DD, Cross MW, McIlwraith CW (2006) A comparative study of articular cartilage thickness in the stifle of animal species used in human pre-clinical studies compared to articular cartilage thickness in the human. *Vet Comp Orthop Traumatol* 19(3):142–146
- Gardel LS, Correia-Gomes C, Serra LA, Gomes ME, Reis RL (2013) A novel bidirectional continuous perfusion bioreactor for the culture of large-sized bone tissue-engineered constructs. *J Biomed Mat Res Part B* 101B:1377–1386
- Ghaneialvar H, Soltani L, Rahmani HR, Lotfi AS, Soleimani M (2018) Characterization and classification of mesenchymal stem cells in several species using surface markers for cell therapy purposes. *Ind J Clin Biochem* 33(1):46–52
- Gugjoo, MB, Amarpal, Fazili MR, Shah RA, Mir MS, Sharma GT (2020). Goat mesenchymal stem cell basic research and potential applications. *Small Rum Res.* 183:106045.
- Gullbrand SE, Schaer TP, Agarwal P, Bendigo JR, Dodge GR, Chen W, Elliott DM, Mauck RL, Malhotra NR, Smith LJ (2017) Translation of an injectable triple-interpenetrating-network hydrogel for intervertebral disc regeneration in a goat model. *Acta Biomater* 15(60):201–209
- Jeng L, Olsen BR, Spector M (2010) Engineering Endostatin-producing cartilaginous constructs for cartilage repair using nonviral transfection of chondrocyte-seeded and Mesenchymal-stem-cell-seeded collagen scaffolds. *Tissue Eng Part A* 16(10):3011–3021
- Jurgens WJFM, Kroeze RJ, Zandieh-Doulabi B, van Dijk A, Renders GAP, Smit T, van Milligen FJ, Ritt MJ, Helder MN (2013) One-step surgical procedure for the treatment of Osteochondral

- defects with adipose-derived stem cells in a Caprine knee defect: a pilot study. *BioRes Open Access* 2(4):315–325
- Knippenberg M, Helder M, Doulabi BZ, Semeins CM, Wuisman PIJM, Klein-Nulend J (2005) Adipose tissue-derived Mesenchymal stem cells acquire bone cell-like responsiveness to fluid shear stress on Osteogenic stimulation. *Tissue Eng* 11(11/12):1780–1787
- Knippenberg M, Helder M, Doulabi BZ, Semeins CM, Wuisman PIJM, Klein-Nulend J (2006) Osteogenesis versus chondrogenesis by BMP-2 and BMP-7 in adipose stem cells. *Biochem Biophys Res Commun* 342:902–908
- Kumar K, Agarwal P, Das K, Milli B, Madhusoodan AP, Kumar A, Bag S (2016) Isolation and characterization of mesenchymal stem cells from caprine umbilical cord tissue matrix. *Tissue Cell* 48(6):653–658
- Kwong PJ, Nam HY, Wan Khadijah WE, Kamarul T, Abdullah RB (2014) Comparison of in vitro developmental competence of cloned Caprine embryos using donor Karyoplasts from adult bone marrow mesenchymal stem cells vs ear fibroblast cells. *Reprod Domest Anim* 49:249–253
- Li Z, Hou T, Moyuan D, Luo F, Wu X, Xing J, Chang Z, Xu J (2015) The Osteogenic efficacy of goat bone marrow-enriched self-assembly peptide/demineralized bone matrix *in vitro* and *in vivo*. *Tissue Eng* 21(7–8):1398
- Li PZ, Yan GY, Han L, Pang J, Zhong BS, Zhang GM, Wang F, Zhang YL (2017) Overexpression of STRA8, BOULE, and DAZL genes promotes goat bone marrow-derived mesenchymal stem cells in vitro trans-differentiation toward putative male germ cells. *Reprod Sci* 24(2):300–312
- Liao B, Deng L, Wang F (2006) Effects of bone marrow mesenchymal stem cells enriched by small intestinal submucosal films on cardiac function and compensatory circulation after myocardial infarction in goats. *Zhongguo Xue Fu Chong Jian Wai Ke Za Zhi* 20(12):1248–1252
- Liao L, Shi B, Chang H, Su X, Zhang L, Bi C, Shuai Y, Du X, Deng Z, Jin Y (2017) Heparin improves BMSC cell therapy: anticoagulant treatment by heparin improves the safety and therapeutic effect of bone marrow-derived mesenchymal stem cell cytotrophy. *Theranostics* 7(1):106–116
- Lippens E, Vertenten G, Gironès J, Declercq H, Saunders J, Luyten J, Duchateau L, Schacht E, Vlamincx L, Gasthuys F, Cornelissen M (2010) Evaluation of bone regeneration with an injectable, in situ polymerizable Pluronic F127 hydrogel derivative combined with autologous mesenchymal stem cells in a goat tibia defect model. *Tissue Eng Part A* 16(2):617–627
- Liu X, Li X, Fan Y, Zhang G, Li D, Dong W, Sha Z, Yu X, Feng Q, Cui F, Watari F (2010) Repairing goat tibia segmental bone defect using scaffold cultured with mesenchymal stem cells. *J Biomed Mat Res Part B Appl Biomater* 94:44–52
- Loozen LD, van der Helm YJM, Oner FC, Dhert WJA, Kruij MC, Alblas J (2015) Bone morphogenetic protein-2 nonviral gene therapy in a goat iliac crest model for bone formation. *Tissue Eng* 21(9):10
- Marei MK, Saad MM, El-Ashwah AM, El-Backly RM, Al-Khodary MA (2009) Experimental formation of periodontal structure around titanium implants utilizing bone marrow mesenchymal stem cells: a pilot study. *J Oral Implantol* 35(3):106–129
- Martignani E, Eirew P, Eaves C, Baratta M (2009) Functional identification of bovine mammary epithelial stem/progenitor cells. *Vet Res Commun* 33:101–103. <https://doi.org/10.1007/s11259-009-9254-z>
- Martignani E, Cravero D, Miretti S, Accornero P, Baratta M (2014) Bovine mammary stem cells: new perspective for dairy science. *Vet Q* 34:52–58
- Martins GR, Marinho RC, Bezerra-Junior RQ, Câmara LMC, Albuquerque-Pinto LC, Teixeira MFS (2017) Isolation, culture and characterization of multipotent mesenchymal stem cells from goat umbilical cord blood. *Pesquisa Vet Bras* 37(6):643–649
- Mohamad-Fauzi N, Ross PJ, Maga EA, Murray JD (2015) Impact of source tissue and ex vivo expansion on the characterization of goat mesenchymal stem cells. *J Anim Sci Biotechnol* 6:1
- Murphy JM, Fink DJ, Hunziker EB, Barry FP (2003) Stem cell therapy in a Caprine model of osteoarthritis. *Arthritis Rheumatol* 48(12):3464–3474

- Nair MB, Varma HK, Menon KV, Shenoy SJ, John A (2009) Tissue regeneration and repair of goat segmental femur defect with bioactive triphasic ceramic-coated hydroxyapatite scaffold. *Biomed Mat Res* 91A:855–865
- Nam HY, Karunanithi P, Poh Loo WC, Naveen SV, Chen HC, Hussin P, Chan L, Kamarul T (2013) The effects of staged intra-articular injection of cultured autologous mesenchymal stromal cells on the repair of damaged cartilage: a pilot study in caprine model. *Arthr Res Ther* 15:R129
- Peeters M, van Rijn S, Vergroesen P-PA, Paul CPL, Noske DP, Vandertop WP, Wurdinger T, Helder MN (2015) Bioluminescence-mediated longitudinal monitoring of adipose-derived stem cells in a large mammal *ex vivo* organ culture. *Sci Rep* 5:13960
- Pei Y, Fan J-J, Zhang X-Q, Zhang Z-Y, Yu M (2014) Repairing the Osteochondral defect in goat with the tissue-engineered Osteochondral graft Preconstructed in a double-chamber stirring bioreactor. *BioMed Res Int* 219203:11
- Petrella F, Toffalorio F, Brizzola S, Martino De Pas T, Rizzo S, Barberis M, Pelicci P, Spaggiari L, Acocella F (2014) Stem cell transplantation effectively occludes Bronchopleural fistula in an animal model. *Ann Thorac Surg* 97:480–483
- Phinney DG, Prockop DJ (2007) Concise review: Mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair—current view. *Stem Cells* 25:2896–2902
- Port J, Jackson DW, Lee TQ, Simon TM (1996) Meniscal repair supplemented with exogenous fibrin clot and autogenous cultured marrow cells in the goat model. *Am J Sports Med* 24(4):547–555
- Pratheesh MD, Gade NE, Katiyar AN, Dubey PK, Sharma B, Saikumar G, Amarpal, Sharma GT (2013) Isolation, culture and characterization of caprine mesenchymal stem cells derived from amniotic fluid. *Res Vet Sci* 94(2):313–319
- Pratheesh MD, Gade NE, Dubey PK, Nath A, Sivanarayanan TB, Madhu DN, Sharma B, Amarpal, Saikumar G, Sharma GT (2014) Molecular characterization and xenogenic application of Wharton's jelly derived caprine mesenchymal stem cells. *Vet Res Commun* 38(2):139–148
- Pratheesh MD, Gade NE, Nath A, Dubey PK, Sivanarayanan TB, Madhu DN, Sreekumar TR, Amarpal, Saikumar G, Sharma GT (2017) Evaluation of persistence and distribution of intra-dermally administered PKH26 labelled goat bone marrow derived mesenchymal stem cells in cutaneous wound healing model. *Cytotechnology* 69(6):841–849
- Proffen BL, McElfresh M, Fleming BC, Murray MM (2012) A comparative anatomical study of the human knee and six animal species. *Knee* 19:493–499
- Qiu P, Bai Y, Liu C, He X, Cao H, Li M, Zhu H, Hua J (2012) A dose-dependent function of follicular fluid on the proliferation and differentiation of umbilical cord mesenchymal stem cells (MSCs) of goat. *Histochem Cell Biol* 138:593–603
- Ren Y, Wu H, Ma Y, Yuan J, Liang H, Liu D (2014) Potential of adipose-derived Mesenchymal stem cells and skeletal muscle-derived satellite cells for somatic cell nuclear transfer mediated Transgenesis in Arbas cashmere goats. *PLoS One* 9(4):e93583
- Schop D, Janssen FW, van Rijn LDS, Fernandes H, Bloem RM, de Bruijn JD, van Dijkhuizen-Radersma R (2009) Growth, metabolism, and growth inhibitors of Mesenchymal stem cells. *Tissue Eng* 15(8):1877–1886
- Shen Q-R, Zou S-J, Chen U-X, Xou H-X, Hu J (2006) Effect of Mesenchymal stem cells transplantation on cranial suture distraction osteogenesis in growing goats. *West China J Stomatol* 24(2):153–155
- Somal A, Bhat IA, Baiju I, Pandey S, Panda BSK, Thakur N, Sarkar M, Chandra V, Saikumar G, Sharma GT (2016) A comparative study of growth kinetics, in vitro differentiation potential and molecular characterization of fetal adnexa derived Caprine Mesenchymal stem cells. *PLoS One* 11(6):e0156821
- Somal A, Bhat IA, Baiju I, Singh AP, Panda BSK, Desingu PA, Pandey S, Bharti MK, Pal A, Saikumar G, Chandra V, Sharma GT (2017) Impact of cryopreservation on caprine fetal adnexa derived stem cells and its evaluation for growth kinetics, phenotypic characterization and wound healing potential in xenogenic rat model. *J Cell Physiol* 232(8):2186–2200

- Sonobe M, Nakagawa M, Ichinose M, Ikegami N, Nagasawa M, Shindo T (2000) Analysis of risk factors in bronchopleural fistula after pulmonary resection for primary lung cancer. *Eur J Cardiothorac Surg* 18:519–523
- Sorensen AL, Jacobsen BM, Reiner AH, Andersen IS, Collas P (2010) Promoter DNA methylation patterns of differentiated cells are largely programmed at the progenitor stage. *Mol Biol Cell* 21:2066–2077
- Sousa CP, Dias IR, Lopez-Peña M, Camassa JA, Lourenço PJ, Judas FM, Gomes ME, Reis RL (2015) Bone turnover markers for early detection of fracture healing disturbances: a review of the scientific literature. *An Acad Bras Cienc* 87:1049–1061
- Sun X-D, Jeng L, Bolliet C, Olsen BR, Spector M (2009) Non-viral endostatin plasmid transfection of mesenchymal stem cells via collagen scaffolds. *Biomaterials* 30:1222–1231
- Tamada H, Yoh C, Inaba T, Takano H, Kawate N, Sawada T (2000) Epidermal growth factor (EGF) in the goat uterus: immunohistochemical localization of EGF and EGF receptor and effect of EGF on uterine activity in vivo. *Theriogenol* 54(1):159–169
- Tamadon A, Mehrabani D, Zarezadeh Y, Rahmanifar F, Dianatpour M, Zare S (2017) Caprine endometrial Mesenchymal stromal stem cell: multilineage potential, characterization, and growth kinetics in breeding and anestrus stages. *Vet Med Int* 2017:5052801
- Tang TT, Lu B, Yue B, Xie XH, Xie YZ, Dai KR, Lu JX, Lou JR (2007) Treatment of osteonecrosis of the femoral head with hBMP-2-gene-modified tissue-engineered bone in goats. *J Bone Joint Surg Br* 89(1):127–129
- Tatsumi K, Ohashi K, Matsubara Y, Kohori A, Ohno T, Kakidachi H, Horii A, Kanegae K, Utoh R, Iwata T, Okano T (2013) Tissue factor triggers procoagulation in transplanted mesenchymal stem cells leading to thromboembolism. *Biochem Biophys Res Commun* 431:203–209
- Toh WS, Foldager CB, Olsen BR, Spector M (2013) Basement membrane molecule expression attendant to chondrogenesis by nucleus Pulposus cells and Mesenchymal stem cells. *J Orthop Res* 31:1136–1143
- Tripathi AK, Ramani UV, Ahir VB, Rank DN, Joshi CG (2010) A modified enrichment protocol for adult caprine skeletal muscle stem cell. *Cytotechnology* 62:483–488
- Van Der Donk S, Buma P, Aspenberg P, Schreurs BW (2001) Similarity of bone in growth in rats and goats: a bone chamber study. *Comp Med* 51:336–340
- Vertenten G, Lippens E, Gironès J, Gorski T, Declercq H, Saunders J, Van den Broeck W, Chiers K, Duchateau L, Schacht E, Cornelissen M, Gasthuys F, Vlaminck L (2009) Evaluation of an injectable, photopolymerizable, and three-dimensional scaffold based on methacrylate-endcapped poly(D,L-lactide-co-epsilon-caprolactone) combined with autologous mesenchymal stem cells in a goat tibial unicortical defect model. *Tissue Eng Part A* 15(7):1501–1511
- Wakelee HA, Chang ET, Gomez SL, Keegan TH, Feskanich D, Clarke CA, Holmberg L, Yong LC, Kolonel LN, Gould MK, West DW (2007) Lung cancer incidence in never smokers. *J Clin Oncol* 25:472–478
- Wang C, Wang Z, Li A, Bai F, Lu J, Xu S, Li D (2010) Repair of segmental bone-defect of goat's tibia using a dynamic perfusion culture tissue engineering bone. *J Biomed Mat Res A* 92:1145–1153
- Wang X, Wang Z, Wang Q, Wang H, Liang H, Liu D (2017) Epigenetic modification differences between fetal fibroblast cells and mesenchymal stem cells of the Arbas cashmere goat. *Res Vet Sci* 114:363–369
- Wang Q, Wang X, Lai D, Deng J, Hou Z, Liang H, Liu D (2018) BIX-01294 promotes the differentiation of adipose mesenchymal stem cells into adipocytes and neural cells in Arbas cashmere goats. *Res Vet Sci* 119:9–18
- Wei X, Liu B, Liu G, Yang F, Cao F, Dou X (2019) Mesenchymal stem cell-loaded porous tantalum integrated with biomimetic 3D collagen-based scaffold to repair large osteochondral defects in goats. *Stem Cell Res Ther* 10:72
- Wu Y, Chen L, Scott PG, Tredget EE (2007) Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells* 25:2648–2654

- Xu X, Hu J, Lu H (2017) Histological observation of a gelatin sponge transplant loaded with bone marrow-derived mesenchymal stem cells combined with platelet-rich plasma in repairing an annulus defect. *PLoS One* 12(2):e0171500
- Zhang Y, Liu S, Guo W, Wang M, Hao C, Gao S, Zhang X, Li X, Chen M, Jing X, Wang Z, Peng J, Lu S, Guo Q (2018) Human umbilical cord Wharton's jelly mesenchymal stem cells combined with an acellular cartilage extracellular matrix scaffold improve cartilage repair compared with microfracture in a Caprine model. *Osteoarthritis Cartilage* 30077(18):S1063–S4584
- Zhang, X.L., Jiang, X.Q., 2006. Osteoblastic differentiation of goat bone marrow stromal cells after AdBMP-2 mediated transduction in vitro. *Shanghai Kou Qiang Yi Xue* 15(6):610–3.
- Zhang YL, Li PZ, Pang J, Wan YJ, Zhang GM, Fan YX, Wang ZY, Tao NH (2019) Induction of goat bone marrow mesenchymal stem cells into putative male germ cells using mRNA for STRA8, BOULE and DAZL. *Cytotechnology* 71(2):563–572
- Zhao W, Lu JY, Hao YM, Cao CH, Zou DR (2017) Maxillary sinus floor elevation with a tissue-engineered bone composite of deciduous tooth stem cells and calcium phosphate cement in goats. *J Tissue Eng Regen Med* 11(1):66–76
- Zhu S, Zhang B, Man C, Ma Y, Hu J (2011) NELL like molecule-1 modified bone marrow mesenchymal stem cells/poly-lactic-co-glycolic acid composite improves repair of large osteochondral defects in mandibular condyle. *Osteoarthritis Cartilage* 19:743–750
- Zou D, Guo L, Lu J, Zhang X, Wei J, Liu C, Zhang Z, Jiang X (2012) Engineering of bone using porous calcium phosphate cement and bone marrow stromal cells for maxillary sinus augmentation with simultaneous implant placement in goats. *Tissue Eng* 18(13,14):1464–1478



Cattle/Buffalo Mesenchymal Stem Cell Basic Research and Potential Applications

9

M. B. Gugjoo, Amar Pal, M. R. Fazili, R. A. Shah,
and G. T. Sharma

Abstract

Cattle/buffalo has an important position in the economics of the livestock industry, worldwide. These animals are main contributors of the meat and milk, and any relevant ailment like reproductive inefficiency and mastitis ipso facto pose a major challenge to the veterinarian. To address these health issues and other relevant conditions, stem cells especially mesenchymal stem cells are being evaluated. MSCs in large ruminants have been harvested from various tissue including fetal membranes and are well characterized. Slim assimilation of knowledge in their basic physiological properties limits their definitive applications. MSCs applications in large ruminants have mostly been evaluated for production-related traits. In this chapter potential applications of cattle and buffalo MSCs, in addition to their characteristics, have been discussed.

M. B. Gugjoo (✉) · M. R. Fazili

Division of Veterinary Clinical Complex, FVSc & AH, SKUAST-Kashmir,
Jammu and Kashmir, India

A. Pal

Division of Surgery, Indian Veterinary Research Institute, Izatnagar, UP, India

R. A. Shah

Division of Animal Biotechnology, FVSc & AH, SKUAST-K,
Srinagar, Jammu and Kashmir, India

G. T. Sharma

Division of Physiology and Climatology, Indian Veterinary Research Institute,
Izatnagar, UP, India

© The Editor(s) (if applicable) and The Author(s), under exclusive license to
Springer Nature Singapore Pte Ltd. 2020

M. B. Gugjoo, A. Pal (eds.), *Mesenchymal Stem Cell in Veterinary Sciences*,
https://doi.org/10.1007/978-981-15-6037-8_9

9.1 Introduction

Among various domestic animals, cattle/buffalo has a crucial position in the economics of the livestock industry, worldwide. These animals mainly contribute through meat and milk production, in addition to hides and other biological materials (Jean and Anderson 2014; Aghamohammadi et al. 2018; Das et al. 2018). Medical conditions like endometritis, mastitis, lameness, fracture, and others can badly affect their production and reproductive efficiency (Izquierdo et al. 2017). With the biotechnological advancement, cattle- or buffalo-MSCs (c-/buf-MSCs) are being studied for improved production, reproduction, and/or genetic improvement (Shibiru et al. 2017). Additionally, bovines can offer suitable translational experimental animal model for human medicine (Bosnakovski et al. 2004). c-/buf-MSCs potential applications are being evaluated mostly in relation to reproduction and/or production. The current chapter throws light on the c-/buf-MSCs characteristics and their potential applications.

9.2 c-/buf-MSCs Sources and Their Characterization

Various sources have been utilized to harvest c-/buf-MSCs. These cells are being evaluated and characterized as per the standard criteria laid down for humans. Cattle- and buffalo-MSCs characterization markers including differentiation potential have been detailed in Tables 9.1 and 9.2, respectively (Gugjoo et al. 2018). Plastic adherent c-/buf-MSCs exhibit fibroblastic morphology. All these studies have not demonstrated all the laid down characterization principles (Donofrio et al. 2008). Even some of the studies have failed to report typical surface marker expression of these MSCs as shown in Table 9.1. In addition to the trilineage, the cells have been studied for their extended differentiation potential including germ cell-, hepatocyte-, islet cell-, myocyte-, and neurocyte-like cells (Cardoso et al. 2012a; Lu et al. 2014a, b, c; Xiong et al. 2014; Peng et al. 2017; Deng et al. 2018).

9.2.1 c-/buf-MSCs Morphology

Plastic adherent c-/buf-MSCs show variable morphologies. These cells may exhibit variable morphologies in early passages followed by mostly fibroblastic in extended passages as has been reported for cattle amniotic fluid MSCs (cAF-MSCs). The early passage morphology includes fibroblastic, and flat and circular type (Corradetti et al. 2013) or fusiform (Yang et al. 2016). These fibroblasts tend to show higher mean population doubling potential and are better able to migrate and home to distant tissues. The higher migration potential and homing was confirmed by their ability to show greater CD44 expression (Rossi et al. 2014). Buffalo amniotic membrane MSCs (bufAm-MSCs) too had shown mixed cell morphologies in early passages, which had changed to mostly fibroblastic subsequently (Dev et al. 2012). The time of cell plastic adherence may also determine their ability to appear fibroblasts.

Table 9.1 Cattle mesenchymal stem cell sources and their characterization

Positive markers	Negative markers	Differentiation	References
<i>Adipose tissue MSCs (AD-MSCs)</i>			
Vimentin, CD49d, CD13	CD34	Adipogenic	Ren et al. (2010)
β -Integrin, CD44, and CD73	–	Adipogenic, osteogenic	Lu et al. (2014c)
CD29, CD73, CD90, CD105	CD34, CD45	Adipogenic, chondrogenic, osteogenic	da Silva et al. (2016a)
CD70, CD90	CD34, CD45	Adipogenic	Cebo (2017)
CD29, CD44, Vimentin	CD34, MHC-II	Adipogenic, chondrogenic, osteogenic	Queiroz et al. (n.d.)
CD73, Oct4 and Nanog	CD34	–	Cahuascano et al. (2019)
<i>Amniotic fluid MSCs (AF-MSCs)</i>			
CD29, CD44, CD73, CD166, MHC-I, Oct-4, C-Myc	CD14, CD34, CD45, CD90, MHC-II	Adipogenic, chondrogenic, neurogenic	Corradetti et al. (2013)
CD44, CD73, CD166, Oct-4	CD34, CD45	Adipogenic, osteogenic, neurogenic	Gao et al. (2014)
CD90, CD105, CD34	CD14, CD73, CD34, Oct-4, Sox2, Cytokeratin, E-Cadherin	Adipogenic, osteogenic, chondrogenic	Rossi et al. (2014)
CD29, CD73, CD90, CD105	CD34, CD44, CD45	Adipogenic, chondrogenic, osteogenic	da Silva et al. (2016a)
β -Integrin, CD44, CD73, CD166, Oct-4	CD34, CD45	Adipogenic, osteogenic, chondrogenic	Chang et al. (2018)
<i>Amniotic membrane MSCs (Am-MSCs)</i>			
CD29, CD44, CD73, CD105, CD166, MHC-I, Oct-4, C-Myc	CD14, CD34, CD45, MHC-II	Adipogenic, chondrogenic, neurogenic	Corradetti et al. (2013)
Nanog, Oct-4, Sox-2	–	Osteogenic	Mann et al. (2013)
CD44, Nanog, Oct-4	MHC-II	Adipogenic, chondrogenic, osteogenic	Campos et al. (2017)
<i>Bone marrow MSCs (BM-MSCs)</i>			
CD29, CD44, and CD166	CD14, CD31, CD34, CD45, CD90, CD105, CD140a, and CD117	Adipogenic, osteogenic, neuroectodermal	Kato et al. (2004)
CD73, CD90, CD105	CD34, CD45	Adipogenic, chondrogenic, osteogenic	Cortes et al. (2013)
CD29, CD73, Oct-4, Nanog	CD34, CD45	Hepatogenic, neurogenic	Dueñas et al. (2014)

(continued)

Table 9.1 (continued)

Positive markers	Negative markers	Differentiation	References
CD29, CD44, CD73	CD31, CD34, CD45	Osteoblasts, adipocytes, hepatic, and islet-like cells	Lu et al. (2014a)
CD29, CD90, CD44, CD105, Oct-4, Sox2 Nanog	CD45	Adipogenic, chondrogenic, osteogenic	Lee et al. (2015)
CD73, Oct4 and Nanog	CD34	–	Cahuascano et al. (2019)
<i>Dermal MSCs (D-MSCs)</i>			
CD29, CD44, CD73, CD90	CD34, CD106, CD166, Oct-4	Adipogenic, osteogenic, neurogenic	Sun et al. (2014)
<i>Endometrium MSCs (End-MSCs)</i>			
CD44, CD117, STAT-3, Oct-4, Sox-2	Nanog	Chondrogenic, osteogenic	Cabezas et al. (2014)
CD44, CD29, Vimentin	CD34 (low expression), MHC-II	Adipogenic, osteogenic	de Moraes et al. (2016)
OCT4, SOX2, CD44, c-KIT	NANOG	Adipogenic, osteogenic, chondrogenic	Lara et al. (2017a, b)
<i>Liver MSCs (Li-MSCs)</i>			
CD29, CD44, CD73, CD90, CD106, CD166	CD34, CD45, BLA-DR	Adipogenic, osteogenic, chondrogenic	Lu et al. (2014b)
<i>Lung MSCs (L-MSCs)</i>			
CD29, CD44, CD73, CD166	CD34, CD45, BOLA-DR α	Osteogenic, neural lineages	Hu et al. (2015)
<i>Placental MSCs (Pl-MSCs)</i>			
CD166, CD73, β -integrin, Oct-4	CD45	Islet like cells (insulin, glucagon, pax-4, Nkx6.1, pax-6, Fox)	Peng et al. (2017)
<i>Tendon MSCs (T-MSCs)</i>			
CD44, Tenascin, Collagen I, Collagen III	Collagen II	Adipogenic, chondrogenic, osteogenic	Yang et al. (2016)
<i>Umbilical cord MSCs (UC-MSCs)</i>			
Oct-4, CD73	–	Adipogenic, chondrogenic, osteogenic	Raoufi et al. (2011)
CD29, CD44, CD73, CD90, CD166	CD34, CD45, BLA-DR	Adipogenic, osteogenic, hepatocyte-, Islet cell-, neurocyte-like cells,	Xiong et al. (2014)
CD44, Nanog, Oct-4	MHC-II	Adipogenic, chondrogenic, osteogenic	Campos et al. (2017)
<i>Wharton's jelly MSCs (WJ-MSCs)</i>			
CD29, CD73, CD90, CD105, POUF51, ITSN1	CD34, CD45	Adipogenic, chondrogenic, osteogenic, neural-like cells	Cardoso et al. (2012b)

(continued)

Table 9.1 (continued)

Positive markers	Negative markers	Differentiation	References
CD105, CD29, CD73, CD90	–	Adipogenic, chondrogenic, osteogenic, astrocyte	Cardoso et al. (2017)
CD29, CD73, CD90, CD105	CD34, CD44, CD45	Adipogenic, chondrogenic, osteogenic	da Silva et al. (2016a)
<i>Yolk sac MSCs (YS-MSCs)</i>			
CD90, CD105, and CD79	CD45, CD44, and CD79	Adipogenic, chondrogenic, osteogenic	Mançanares et al. (2015)

Table 9.2 Buffalo mesenchymal stem cell sources and their characterization

Positive markers	Negative markers	Differentiation	References
<i>Adipose tissue MSCs</i>			
Oct-4, CD44	–	Adipogenic, chondrogenic, osteogenic	Hepsibha et al. (2011)
CD105, CD90, CD73	CD34, CD45, CD79A	Adipogenic, osteogenic, chondrogenic	Sampaio et al. (2015)
<i>Amniotic membrane MSCs</i>			
Oct-4, Sox-2, Nanog	–	Neurogenic	Dev et al. (2012)
CD29, CD44, CD105, OCT4, SOX2, NANOG, TERT	CD34	Adipogenic, chondrogenic, osteogenic	Ghosh et al. (2015)
CD29, CD44, Nanog, Oct-4, Sox-2	CD34	Adipogenic, chondrogenic, osteogenic	Sadeesh et al. (2016)
CD44, CD90, CD105, CD73, CD29, CD166, OCT4, SOX2, NANOG, REX-1, SSEA-1, SSEA-4 and TRA-1-81, Cytokeratin 18 (epithelial)	CD34, CD45	Adipogenic, osteogenic, chondrogenic, and neural lineages (FABP4, Ost, ACAN, COL2A1, Nestin, and β III-tubulin)	Deng et al. (2018)
<i>Bone marrow MSCs</i>			
CD73, CD90, CD105, Nanog, Oct-4, STRO-1, Sox2	CD31, CD34	Adipogenic, chondrogenic, osteogenic	Gade et al. (2013)
<i>Umbilical cord MSCs</i>			
OCT4, NANOG and SOX2	–	Adipogenic, osteogenic	Singh et al. (2013)
<i>Wharton's jelly MSCs</i>			
CD-73, CD-105 and CD-90, Stro-1, Oct-4, Nanog, Sox-2	CD34	Adipogenic, chondrogenic, osteogenic	Sreekumar et al. (2014)

AD-MSCs adipose tissue MSCs, *BM-MSCs* bone marrow MSCs, *d-MSCs* dermis MSCs, *Am-MSCs* amniotic membrane MSCs, *UC-MSCs* umbilical cord MSCs, *WJ-MSCs* Wharton's jelly MSCs

Earlier the cells attach to plastic, earlier the fibroblastic cells appear as has been demonstrated for early gestation-derived bufAm-MSCs (Deng et al. 2018)

9.2.2 Culture Characteristics

Cattle and buffalo MSCs tend to have similar culture characteristics. Only difference may remain in their ability to survive for longer periods. Buffalo adipose tissue (AD)-MSCs had been proliferated for more passages in comparison to cattle (cAD)-MSCs, yielding higher cell concentration in the former sources (Sampaio et al. 2015). Such differences may arise with respect to tissue sources, their physiological state, and age of donor.

9.2.3 Extended Passaging and MSCs Proliferation

MSCs in general fail to survive indefinitely. Their colony-forming unit (CFU) potential along the path of extended passaging decreases (Lu et al. 2014a, b, c). MSCs may show differences in the proliferation potential and self-renewal properties based on the sources. Cattle bone marrow MSCs (cBM-MSCs) had shown CFU potential as $63.6 \pm 7.57\%$ at passage 3, $59.0 \pm 4.38\%$ at passage 9, $32.6 \pm 3.88\%$ at passage 15, and $19.5 \pm 3.27\%$ at passage 25 (Lu et al. 2014a, b, c). Likewise, population doubling time (PDT) of bufAm-MSCs had remained 43.9 ± 20 h. at passage 3, 45.9 ± 30 h. at passage 6, 46.7 ± 30 h. at passage 9, and 60.0 ± 50 h. at passage 20 (Deng et al. 2018). However, cattle bone marrow MSCs (cBM-MSCs) population doubling had been maintained up to passage 7 (Cortes et al. 2013).

MSCs proliferation potential and viability may be maintained up to as low as passage 10 (Corradetti et al. 2013) to the maximum of passage 60 (Cardoso et al. 2012b). Cattle tendon-derived MSCs (cT-MSCs), dermal MSCs (cDer-MSCs), and lung MSCs (cL-MSCs) had been maintained up to passages 42, 19, and 25 and afterward these cells had undergone senescence (Sun et al. 2014; Hu et al. 2015; Yang et al. 2016). Morphologically senescent cells appear vacuolated and show karyopyknosis while growth kinetics show much reduced or no growth at all.

Comparison of cAF-MSCs and cAm-MSCs had shown higher proliferation capacity of former cell lines up to the passage 10. Thereafter both cell lines had reduced viability (Corradetti et al. 2013). Another study on cAF-MSCs however, had been able to successfully culture them up to passage 34 (Gao et al. 2014). Similarly, cattle Wharton's jelly (cWJ)-MSCs and cattle bone marrow (cBM)-MSCs had comparable proliferation up to passage 10 after which both the cell lines had become senescent (Cortes et al. 2013; Xiong et al. 2014). Thus, MSCs proliferation is mainly affected by tissue source and culture environment. To keep the record of cellular activity, evaluation of karyotypic features is desired (Colleoni et al. 2005).

9.2.4 Culture Modification and MSCs Characteristics

MSCs may also be cultured in serum-free media. cWJ-MSCs cultured in serum-free media had remained viable and had stable karyotype up to passage 60. Under 3-D culture system, cMSCs may undergo morphological changes and tend to differentiate (Cardoso et al. 2012a, b). These systems offer uniform culture environment and thus can potentially give rise to a uniform batch of cells. However, further studies are required to standardize these culture techniques.

9.2.5 Gestational Phase and cMSCs Proliferation

cAF-MSCs had shown differential proliferation potential as per the gestational phase being higher in first trimester in comparison to other stages (Rossi et al. 2014). Estral cyclicity had non-significant effect on cattle endometrium (cEnd)-MSCs as the cells at different stages had almost comparable cellular properties up to passage 3 (de Moraes et al. 2016). The late luteal phase MSCs, however, may be more amenable to mesodermal lineages (Cabezas et al. 2014).

9.2.6 Effect of Age and Health Status on MSCs

MSCs proliferation potential tends to decrease with age. cBM-MSCs from young donor had demonstrated vigorous proliferation in comparison to the adult source (Jiwu et al. 2008). Health status also affects the MSCs culture characteristics as infections like endometritis had reduced proliferation potential of cEnd-MSCs (Lara et al. 2017a, b), while tumor growth (adenomyotic uterus) had enhanced their pluripotency expression profile (Nanog and Oct-4) (Łupicka et al. 2015).

9.3 Potential Applications of c-/buf-MSCs

c-/buf-MSCs are seen as potential candidates to improve production and reduce clinical problems in animals. Therapeutic applications of MSCs in large ruminants have lagged far behind as compared to equine or pet animals. It is due to the lack of interest in high-end therapeutics for such species as these animals are mainly reared for economic benefits. In vivo research is preferably conducted in lab animals and in advanced stage large animals are employed. The large animals being ontogenically closer to humans are considered to provide better translational models for them. Considering the importance of large ruminants in relation to production purpose, currently MSCs applications ipso facto have been prioritized in the field (Gugjoo et al. 2018). However, the potential goes beyond as these animals have clinical relevance to veterinary and human medicine. It, therefore, becomes imperative to carry out therapeutic studies on c-/buf-MSCs. Below are described the potential applications of c-/buf-MSCs.

9.3.1 Reproductive System

9.3.1.1 MSCs as Donor in Nuclear Transfer Technique

Nuclear transfer technique (NTT) is aimed at improving the reproductive efficiency though the current success rate is very low (0–5%). Donor cell plays a key role in the success. In comparison to fibroblasts, MSCs as donor cell have shown better fusion rate but little success in cleavage rate. The overall blastocyst formation efficiency has remained good (da Silva et al. 2016b; Nazari et al. 2016; Sadeesh et al. 2016). MSCs fusion rate may vary with the donor tissue source as cAF-MSCs had shown better fusion rate almost 30–35% higher than the cAD-MSCs (85.89% vs. 50.06%). However, fusion rate of MSCs from different sources had remained higher than fibroblasts (44.85%). In contrast the cleavage rate had been better for fibroblasts, and an overall comparable percent of calves had been born (da Silva et al. 2016a, b).

Apart from MSCs source, oocyte quality too plays an important role in the success of nuclear transfer (NT). cBM-MSCs form only 7% blastocysts to preserved oocyte and 39% blastocysts to fresh oocytes (Kato et al. 2004), showing that recipient oocyte too has bearing on development of blastocysts. The cleavage rate of bufAm-MSCs had been lower than that of in vitro fertilized (IVF) embryos, though comparable percent of blastocysts were formed (Sadeesh et al. 2016).

Cell size may affect fusion rate as better fusion rate with larger cAF-MSCs/cWJ-MSCs had been demonstrated in comparison to smaller cAD-MSCs/ fibroblasts. The larger size of cell favors better contact with the zona pellucida and/or cell membrane of oocyte (da Silva et al. 2016a, b). However, larger-sized cAF-MSCs may lead to large offspring syndrome (larger-sized offspring). This larger-sized offspring may not survive long due to dystocia and/or fetal abnormalities that tend to develop with limited available space in dam uterus (da Silva et al. 2016a, b). Furthermore, short survival of offspring may also develop due to the epigenetically dysregulated expression of imprinted genes (Farin et al. 2006; Opiela et al. 2017).

Contrary to these reports, cBM-MSCs had failed to show any significant difference in the developmental rates at any stage in comparison to the fibroblasts (Colleoni et al. 2005). Thus, further studies are desired to evaluate the possible options to improve the success rate of the fusion and cleavage with the MSCs.

9.3.1.2 MSCs as Supplementation Medium

MSCs may provide secretory factors that can replace granulosa cells. Granulosa cells supplement embryo culture media for their growth. cAD-MSCs ($10^3/10^4$ cells/mL) supplementation to in vitro fertilized embryos had led to their better blastocyst formation rates. The higher cell concentration (10^4 cells/mL) and their conditioned medium were more effective than lower (10^3 cells/mL) ones (Miranda et al. 2016).

9.3.1.3 MSCs and Genital Affections

The cells may be effective in male/female genital affections due to their trophic actions including immunomodulatory actions. Amniotic fluid MSCs injection into

the ovary had induced estrus in bilateral ovarian dystrophic cattle. Fifty percent of animals (4 out of 8 animals) had come into heat. Out of those four animals, 50% (2 out of 4 animals) had ovulated, formed corpus luteum (CL), and attained pregnancy. It could be inferred that cAF-MSCs injection may bring animals into heat and subsequently CL formation can occur but may also be affected by other factors (Chang et al. 2018). Endometritis reduce cEnd-MSCs proliferation potential (Lara et al. 2017a, b), and as such extrinsic implantation of MSCs may be effective way to control endometritis due to their immunomodulatory or anti-inflammatory properties. The cellular implantation may be made through per vaginal route where cells may migrate and home to endometrium as has been demonstrated in horse (Mambelli et al. 2013; Falomo et al. 2015).

MSCs may be utilized to improve and/ or maintain elite bull germplasm. In vitro cMSCs differentiated into germ cell-like cells may be implanted into the testicles. It has been demonstrated that ram testis had supported germ cell-like cells growth and survival. The cells had even migrated and homed to the basement membrane of seminiferous tubules. These cells had formed spermatogonia-like cell colonies similar to testicular native spermatogonia (Ghasemzadeh-Hasankolaei et al. 2015). In lieu cBM-MSCs trans-differentiated to germ cell-like cells may be evaluated for their utility.

9.3.2 Oxidative Stress

In livestock sector, elite animals are preferred for their higher production potential, which creates an environment of stress (Jóźwik et al. 2012). Stress makes these tissue cells more amenable to early aging. MSCs exhibit dynamic antioxidant action through endogenous production of hemeoxygenase-1 (HO-1) and glutathione and restricting cellular production of reactive oxygen species (Vanella et al. 2012; Calió et al. 2014; Ayatollahi et al. 2014). These features also attribute to MSCs tolerance toward the oxidative stress (Brandl et al. 2011). Due to these features, MSCs may prevent telomere shortening in cells preventing their senescence (Estrada et al. 2013).

9.3.3 Bovine Mastitis

Mastitis that causes decrease in milk production has lot more associated problems as it makes human health amenable to diseases through bad quality milk and through antibiotic resistance. Currently, no ideal treatment is available to combat this problem in productive cattle. Mammary stem cells are being isolated and characterized from mammary gland of bovines in addition to other species (Shackleton et al. 2006; Martignani et al. 2009; Martignani et al. 2014). Isolation of different stem cell types from mammary gland is currently under process (Thomas et al. 2011; Rauner and Barash 2012; Martignani et al. 2014).

MSCs can prove useful in two ways: on one way the cells may secrete specific proteins and on other way their antimicrobial effects may prevent mastitis. Co-culture of

mammary epithelial cells and cUC-MSCs had enhanced quantity of β -casein and κ casein, in addition to the expression of other proteins in mammary epithelial cells. The possible pathway followed in the mammary epithelial cells might have been JAK/STAT as IGF-1 had inhibited their expression profile (Zhao et al. 2017).

Fetal cBM-MSCs and cAD-MSCs conditioned medium carries antibacterial activity. The conditioned medium had decreased about 30% of the growth of *Staphylococcus aureus* under in vitro conditions (Cahuascano et al. 2019). Transfection of c-/buf-MSCs with bovine lactoferricin (LFcinB) gene may endow them with the properties to express antibacterial peptide LfcinB (Sharma et al. 2017). Such LFcinB-cloned MSCs if injected into the mammary gland may prove fruitful through their higher antibacterial properties especially against *Staphylococcus aureus* and *Escherichia coli* (Sharma et al. 2017). Thus, extensive studies are desired to confirm the feasibility and efficacy of using these transfected MSCs in clinical settings.

9.3.4 Cultured Meat Production

Due to growing human population demands and impediments to animal-rearing (greenhouse gases emission) and slaughter policies (ethics), lab-grown meat is being aimed. Cultured meat like burger and meat balls have been produced in 2013 and 2016, respectively (Post 2012; Arshad et al. 2017). The main focus remains to produce cost-effective meat that has acceptable taste and appearance. MSCs that can trans-differentiate into muscle cell-like cells and adipocytes may be cultured on edible scaffold (Post 2012; Bhat et al. 2015; Arshad et al. 2017) and, thus, need further studies.

9.3.5 Wound Healing

Cattle/ buffalo teat fistula and fibrosis have huge economic impact through loss of milk and potential to develop mastitis. MSCs applications in such conditions are desired. In our own unpublished study, allogeneic MSCs applications in post-surgical repair of teat fistula promote early healing of wounds. Thus, MSCs applications appear promising in such cases. The possible beneficial effects may be through MSCs differentiation into various types of epithelial cells (Wu et al. 2007; Borena et al. 2009) and/or through vasculogenesis (Borena et al. 2009).

9.3.6 Bovine Spongiform Encephalopathy (BSE) and Nerve Injuries

BM-MSCs/ peripheral blood (PB)-MSCs neurogenic potential is affected in scrapie (Mediano et al. 2015), although marrow infectivity in BSE remains undiagnosed on

the spleen, lymph nodes and brain assays (Sohn et al. 2009). Thus, there is need to confirm if the said potential is also reduced in BSE and whether these cells can be used to treat infected animals.

Stem cells may trans-differentiate into the neural cell-like cells or secrete trophic factors (Hokari et al. 2008; Saito et al. 2008; Cardoso et al. 2012a; Xiong et al. 2014), though the direct neuronal differentiation is questionable (Hokari et al. 2008; Wu et al. 2003; Dobkin et al. 2006). Commonly affected peripheral nerves like radial, obturator, and sciatic nerves make large ruminants lame and/or recumbent. Thus, MSCs studies may be undertaken to evaluate their therapeutic potential in these peripheral nerve injuries.

9.3.7 Degenerative Joint Diseases (DJD) and Laminitis

MSCs are extensively studied for their therapeutic effects in osteoarthritis. Cattle chronic osteoarthritis (OA) or degenerative joint disease (DJD) tends to cause male infertility (impotentia coeundi) in bulls (Heinola et al. 2013; Wolfe 2018). MSCs may be effective in OA; however, currently the therapy has not been able to provide desired results and may be due to the inhibitory effects of locally available inflammatory mediators. The inflammatory environment tends to enhance expression of adhesion molecule preventing cell migration and decrease their secretion of matrix (Zayed et al. 2016; Reesink et al. 2017).

Cattle and horse laminitis carry almost similar pathogenesis (Nilsson 1982) and as MSCs have been demonstrated to be play effective role in equine acute laminitis (Dryden et al. 2013; Morrison et al. 2014), studies in cattle may also be undertaken.

9.3.8 Miscellaneous Conditions

c-/buf-MSCs potentially trans-differentiate into myocyte-, osteocyte-, tenocyte-, hepatocyte-, and islet cell-like cells and as such may be employed to treat associated ailments. Additionally, MSCs may also be evaluated for their antiviral effect (Cardoso et al. 2012a, b).

9.4 Conclusions

Cattle and buffalo MSCs have been harvested from numerous sources and are well characterized. MSCs properties may vary with respect to the source and may be affected with respect to the age and health status. Literature on cattle/ buffalo MSCs applications mainly focuses on their production and reproduction-related traits. The results of these studies however are currently inconclusive. MSCs potential applications go beyond these reproductive and/ production-related traits and may be studied in such areas as well.

References

- Aghamohammadi M, Haine D, Kelton DF, Barkema HW, Hogeveen H, Keefe GP, Dufour S (2018) Herd-level mastitis-associated costs on Canadian dairy farms. *Front Vet Sci* 5:100
- Arshad MS, Javed M, Sohaib M, Saeed F, Imran A, Amjad Z (2017) Tissue engineering approaches to develop cultured meat from cells: a mini review. *Cogent Food Agric* 3:1320814
- Ayatollahi M, Hesami Z, Jamshidzadeh A, Gramizadeh B (2014) Antioxidant effects of bone marrow mesenchymal stem cell against carbon tetrachloride-induced oxidative damage in rat livers. *Int J Organ Transplant Med* 5:166–173
- Bhat ZF, Kumar S, Fayaz H (2015) *In vitro* meat production: challenges and benefits over conventional meat production. *J Integr Agric* 14(2):241–248
- Borena BM, Pawde AM, Pal A, Aithal HP, Kinjavdekar P, Singh R, Kumar D (2009) Autologous bone marrow-derived cells for healing excisional dermal wounds of rabbits. *Vet Rec* 165(19):563–568
- Bosnakovski D, Mizuno M, Kim G, Ishiguro T, Okumura M, Iwanaga T, Kadosawa T, Fujinaga T (2004) Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells in pellet cultural system. *Exp Hematol* 32(5):502–509
- Brandl A, Meyer M, Bechmann V, Nerlich M, Angele P (2011) Oxidative stress induces senescence in human mesenchymal stem cells. *Exp Cell Res* 317:1541–1547
- Cabezas J, Lara E, Pacha P, Rojas D, Veraguas D, Saravia F, Rodríguez-Alvarez L, Castro FO (2014) The endometrium of cycling cows contains populations of putative mesenchymal progenitor cells. *Reprod Domest Anim* 49(4):550–559
- Cahuascanco B, Bahamonde J, Huaman O, Jervis M, Cortez J, Palomino J, Escobar A, Retama P, Torres CG, Peralta OA (2019) Bovine fetal mesenchymal stem cells exert antiproliferative effect against mastitis causing pathogen *Staphylococcus aureus*. *Vet Res* 50:25
- Calíó ML, Marinho DS, Ko GM, Ribeiro RR, Carbonel AF, Oyama LMM, Ormanji T, Guirao P, Calíó PL, Reis LA, de Jesus Simões M, Lisbôa-Nascimento T, Ferreira AT, Bertoncini CR (2014) Transplantation of bone marrow mesenchymal stem cells decreases oxidative stress, apoptosis, and hippocampal damage in brain of a spontaneous stroke model. *Free Radic Biol Med* 70:141–154
- Campos LL, Landim-Alvarenga FC, Ikeda TL, Monteiro BA, Maia L, Freitas-Dell'Aqua CP, De Vita B (2017) Isolation, culture, characterization and cryopreservation of stem cells derived from amniotic mesenchymal layer and umbilical cord tissue of bovine fetuses. *Pesqui Vet Bras* 37(3):278–286
- Cardoso TC, Ferrari HF, Garcia AF, Novais JB, Silva-Frade C, Ferrarezi MC, Andrade AL, Gamiero R (2012a) Isolation and characterization of Wharton's jelly-derived multipotent mesenchymal stromal cells obtained from bovine umbilical cord and maintained in defined serum free three-dimensional system. *BMC Biotechnol* 12:18
- Cardoso TC, Novais JB, Antello TF, Silva-Frade C, Ferrarezi MC, Ferrari HF, Gameiro R, Flores EF (2012b) Susceptibility of neuron-like cells derived from bovine Wharton's jelly to bovine herpesvirus type 5 infections. *BMC Vet Res* 8:242
- Cardoso TC, Okamura LH, Baptistella JC, Gameiro R, Ferreira HL, Marinho M, Flores EF (2017) Isolation, characterization and immunomodulatory-associated gene transcription of Wharton's jelly-derived multipotent mesenchymal stromal cells at different trimesters of cow pregnancy. *Cell Tissue Res* 367(2):243–256
- Cebo D (2017) Characterization of bovine adipose-derived stem cells. *Int J Sci Technol Res* 6(5):16–18
- Chang L-B, Peng S-Y, Chou C-J, Chen Y-J, Shiu J-S, Tu P-A, Gao S-X, Chen Y-C, Lin T-K, Wu S-C (2018) Therapeutic potential of amniotic fluid stem cells to treat bilateral ovarian dystrophy in dairy cows in a subtropical region. *Reprod Domest Anim* 53(4):1024–1024
- Colleoni S, Donofrio G, Lagutina I, Duchi R, Galli C, Lazzari G (2005) Establishment, differentiation, electroporation, viral transduction, and nuclear transfer of bovine and porcine mesenchymal stem cells. *Cloning Stem Cells* 7(3):154–166

- Corradetti B, Meucci A, Bizzaro D, Cremonesi F, Lange Consiglio A (2013) Mesenchymal stem cells from amnion and amniotic fluid in the bovine. *Reproduction* 145:391–400
- Cortes Y, Ojeda M, Araya D, Dueñas F, Fernández MS, Peralta OA (2013) Isolation and multi-lineage differentiation of bone marrow mesenchymal stem cells from abattoir-derived bovine fetuses. *BMC Vet Res* 9:133
- da Silva CG, Martins CF, Cardoso TC, da Cunha ER, Bessler HC, MacManus CM, Pivato I, Bao SN (2016a) Isolation and characterization of Mesenchymal stem cells derived from bovine Wharton's jelly and their potential use in cloning by nuclear transfer. *Ciência Rural* 46(10):1830–1837
- da Silva CG, Martins CF, Cardoso TC, da Cunha ER, Bessler HC, Martins GHL, Pivato I, Bao SN (2016b) Production of bovine embryos and calves cloned by nuclear transfer using Mesenchymal stem cells from amniotic fluid and adipose tissue. *Cell Reprogram* 18(2):127–135
- Das D, Panda SK, Jena B, Sahoo AK (2018) Economic impact of subclinical and clinical mastitis in Odisha. *India Int J Curr Microbiol App Sci* 7(3):3651–3654
- de Moraes CN, Maia L, Dias MC, Dell'Aqua CP, da Mota LS, Chapwanya A, Landim-Alvarenga FD, Oba E (2016) Bovine endometrial cells: a source of mesenchymal stem/progenitor cells. *Cell Biol Int* 40(12):1332–1339
- Deng Y, Huang G, Zou L, Nong T, Yang X, Cui J, Wei Y, Yang S, Shi D (2018) Isolation and characterization of buffalo (*bubalus bubalis*) amniotic mesenchymal stem cells derived from amnion from the first trimester pregnancy. *J Vet Med Sci* 80(4):710–719
- Dev K, Giri S, Kumar A, Yadav A, Singh B, Gautam S (2012) Derivation, characterization and differentiation of buffalo (*Bubalus bubalis*) amniotic fluid derived stem cells. *Reprod Domest Anim* 47(5):704–711
- Dobkin BH, Curt A, Guest J (2006) Cellular transplants in China: observational study from the largest human experiment in chronic spinal cord injury. *Neurorehabil Neural Repair* 20(1):5–13
- Donofrio G, Franceschi V, Capocefalo A, Cavarani S, Sheldom IM (2008) Bovine endometrial stromal cells display osteogenic properties. *Reprod Biol Endocrinol* 6:65
- Dryden VC, Morrison S, Bras R, Morrell SA (2013) Using stem cells in clinical cases. *J Equine Vet* 33(10):872–873
- Dueñas F, Becerra V, Cortes Y, Vidal S, Sáenz L, Palomino J, De Los Reyes M, Peralta OA (2014) Hepatogenic and neurogenic differentiation of bone marrow mesenchymal stem cells from abattoir-derived bovine fetuses. *BMC Vet Res* 10:154
- Estrada JC, Torres Y, Benguría A, Dopazo A, Roche E, Carrera-Quintanar L, Pérez RA, Enríquez JA, Torres R, Ramírez JC, Samper E, Bernad A (2013) Human mesenchymal stem cell-replicative senescence and oxidative stress are closely linked to aneuploidy. *Cell Death Discov* 4:e691
- Falomo ME, Ferroni L, Tocco I, Gardin C, Zavan B (2015) Immunomodulatory role of adipose-derived stem cells on equine endometriosis. *BioMed Res Int* 2015:1
- Farin PW, Piedrahita JA, Farin CE (2006) Errors in development of fetuses and placentas from in vitro produced bovine embryos. *Theriogenology* 65:178–191
- Gade NE, Pratheesh MD, Nath A, Dubey PK, AmarPal, Sharma B, Saikumar G, Sharma GT (2013) Molecular and cellular characterization of buffalo bone marrow-derived mesenchymal stem cells. *Reprod Domest Anim* 48(3):358–367
- Gao Y, Zhu Z, Zhao Y, Hua J, Ma Y, Guan W (2014) Multi-lineage potential research of bovine amniotic fluid mesenchymal stem cells. *Int J Mol Sci* 15:3698–3710
- Ghasemzadeh-Hasankolaei M, Baghaban Eslaminejad M, Sedighi-Gilani M (2015) Derivation of male germ cells from ram bone marrow mesenchymal stem cells by three different methods and evaluation of their fate after transplantation into the testis. *Vitro Cell Dev Biol Anim* 52(1):49–61
- Ghosh K, Kumar R, Singh J, Gahlawat SK, Kumar D, Selokar NL, Yadav SP, Gulati BR, Yadav PS (2015) Buffalo (*Bubalus bubalis*) term amniotic-membrane-derived cells exhibited mesenchymal stem cells characteristics in vitro. *Vitro Cell Dev Biol Anim* 51(9):915–921
- Gugjoo MB, Amarpal, Fazili MR, Shah RA, Sharma GT (2018) Mesenchymal stem cell: Basic research and potential applications in cattle and buffalo. *J Cell Physiol* 234(6):8618–8635

- Heinola T, Grau JC, Virkki L, Kontinen A, Raulo SM, Sukura A, Kontinen YT (2013) Bovine chronic osteoarthritis causes minimal change in synovial fluid. *J Comp Pathol* 148(Suppl.4):335–344
- Hepsibha P, Meenambigai TV, Mangalagowri A, Palanisamy A, Stalin A, Nithya S, Kumanan K (2011) Multipotent differentiation potential of buffalo adipose tissue derived mesenchymal stem cells. *Asian J Anim Vet Adv* 6:772–788
- Hokari M, Kuroda S, Shichinohe H, Yano S, Hida K, Iwasaki Y (2008) Bone marrow stromal cells protect and repair damaged neurons through multiple mechanisms. *J Neurosci Res* 86:1024–1035
- Hu P, Pu Y, Li X, Zhu Z, Zhao Y, Guan W, Ma Y (2015) Isolation, *in vitro* culture and identification of a new type of mesenchymal stem cell derived from fetal bovine lung tissues. *Mol Med Rep* 12:3331–3338
- Izquierdo AC, Guerra Liera JE, Cervantes RE, Inzunza Castro JF, Abel Villa Mancera E, Crispín RH, Juárez Mosqueda ML, Vázquez AG, Pérez JO, Aparicio PS, Rodríguez Denis BE (2017) Production of milk and bovine mastitis. *J Adv Dairy Res* 5:174
- Jean GS, Anderson DE (2014) Decision analysis for fracture management in cattle. *Vet Clin North Am Food Anim Pract* 30(1):1–10
- Jiwu F, Xuejin B, Jing Z (2008) A comparative study of calf and adult bone marrow mesenchymal stem cell in vitro. *Sci Agric Sin* 41(7):2128–2135
- Jóźwik A, Krzyżewski J, Strzałkowska N, Poławska E, Bagnicka E, Wierzbicka A, Niemczuk K, Lipińska P, Horbańczuk JO (2012) Relations between the oxidative status, mastitis, milk quality, and disorders of reproductive functions in dairy cows—a review. *Anim Sci Paper Rep* 30:297–307
- Kato Y, Imabayashi H, Mori T, Tani T, Taniguchi M, Higashi M, Matsumoto M, Umezawa A, Tsunoda Y (2004) Nuclear transfer of adult bone marrow mesenchymal stem cells: developmental totipotency of tissue-specific stem cells from an adult mammal. *Biol Reprod* 70(2):415–418
- Lara E, Rivera N, Rojas D, Rodríguez-Alvarez LL, Castro FO (2017a) Characterization of mesenchymal stem cells in bovine endometrium during follicular phase of oestrous cycle. *Reprod Domest Anim* 52(5):707–714
- Lara E, Velásquez A, Cabezas J, Rivera N, Pacha P, Rodríguez-Alvarez L, Saravia F, Castro FO (2017b) Endometritis and *in vitro* PGE2 challenge modify properties of cattle endometrial mesenchymal stem cells and their transcriptomic profile. *Stem Cells Int*. 2017:1
- Lee SH, Cha S-H, Kim C-L, Lillehoj HS, Song J-Y, Lee K-W (2015) Enhanced adipogenic differentiation of bovine bone marrow derived mesenchymal stem cells. *J Appl Anim Res* 43(1):15–21
- Lu T, Huang Y, Wang H, Ma Y, Guan W (2014a) Multi-lineage potential research of bone marrow-derived stromal cells (BMSCs) from cattle. *Appl Biochem Biotechnol* 172:21–35
- Lu T, Hu P, Su X, Li C, Ma Y, Guan W (2014b) Isolation and characterization of mesenchymal stem cells derived from fetal bovine liver. *Cell Tissue Bank* 15(3):439–450
- Lu T, Xiong H, Wang K, Wang S, Ma Y, Guan W (2014c) Isolation and characterization of adipose-derived mesenchymal stem cells (ADSCs) from cattle. *Appl Biochem Biotechnol* 174(2):719–728
- Łupicka M, Socha B, Szczepańska A, Korzekwa A (2015) Expression of pluripotency markers in the bovine uterus with adenomyosis. *Reprod Biol Endocrinol* 13:110
- Mambelli LI, Winter GH, Kerkis A, Malschitzky E, Mattos RC, Kerkis I (2013) A novel strategy of mesenchymal stem cells delivery in the uterus of mares with endometrosis. *Theriogenology* 79(5):744–750
- Mañanares CA, Oliveira VC, Oliveira LJ, Carvalho AF, Sampaio RV, Mañanares AC, Souza AF, Perecin F, Meirelles FV, Miglino MA, Ambrósio CE (2015) Isolation and characterization of mesenchymal stem cells from the yolk sacs of bovine embryos. *Theriogenology* 84(6):887–898
- Mann A, Yadav RP, Singh J, Kumar D, Singh B, Yadav PS (2013) Culture, characterization and differentiation of cells from buffalo (*Bubalus bubalis*) amnion. *Cytotechnology* 65(1):23–30
- Martignani E, Eirew P, Eaves C, Baratta M (2009) Functional identification of bovine mammary epithelial stem/progenitor cells. *Vet Res Commun* 33:101–103

- Martignani E, Cravero D, Miretti S, Accornero P, Baratta M (2014) Bovine mammary stem cells: new perspective for dairy science. *Vet Q* 34:52–58
- Mediano DR, Sanz-Rubio D, Bolea R, Marín B, Vázquez FJ, Remacha AR, López-Pérez Ó, Fernández-Borges N, Castilla J, Zaragoza P, Badiola JJ, Rodellar C, Martín-Burriel I (2015) Characterization of mesenchymal stem cells in sheep naturally infected with scrapie. *J Gen Virol* 96:3715–3726
- Miranda MS, Nascimento HS, Costa MP, Costa NN, Brito KN, Lopes CT, Santos SS, Cordeiro MS, Ohashi OM (2016) Increasing of blastocyst rate and gene expression in co-culture of bovine embryos with adult adipose tissue-derived mesenchymal stem cells. *J Assist Reprod Genet* 33(10):1395–1403
- Morrison S, Dryden VC, Bras R, Morrell S (2014) How to use stem cells in clinical laminitis cases. How to choose regenerative medicine and therapies. In: American association of equine practitioners: proceedings, vol 60. American Association of Equine Practitioners, Lexington, pp 499–503
- Nazari H, Shirazi A, Shams-Esfandabadi N, Afzali A, Ahmadi E (2016) The effect of amniotic membrane stem cells as donor nucleus on gene expression in reconstructed bovine oocytes. *Int J Dev Biol* 60:95–102
- Nilsson SA (1982) Laminitis in cattle in comparison with equine laminitis. In: Forth international symposium on disorders of ruminant digit, Paris
- Opiela J, Samiec M, Romanek J (2017) In vitro development and cytological quality of inter-species (porcine/bovine) cloned embryos are affected by trichostatin A-dependent epigenomic modulation of adult mesenchymal stem cells. *Theriogenology* 97:27e33
- Peng S-Y, Chou C-W, Kuo Y-H, Shen P-C, Shaw S-WS (2017) Potential differentiation of islet like cells from pregnant cow derived placental stem cells. *Taiwan J Obstet Gynecol* 56:306–311
- Post MJ (2012) Cultured meat from stem cells: challenges and prospects. *Meat Sci* 92(3):297–301
- Queiroz CM, Moraes CN, Maia L, Kandim-Alvaranga FC, Ferreira JCP (n.d.) Isolation, culture and characterization of mesenchymal stem cells from adipose tissue. *Vet Zootec*
- Raoufi MF, Tajik P, Dehghan MM, Eini F, Barin A (2011) Isolation and differentiation of mesenchymal stem cells from bovine umbilical cord blood. *Reprod Domest Anim* 46:95–99
- Rauner G, Barash I (2012) Cell hierarchy and lineage commitment in the bovine mammary gland. *PLoS One* 7:e30113
- Reesink HL, Sutton RM, Shurer CR, Peterson RP, Tan JS, Jin S, Paszek MJ, Nixon AJ (2017) Galectin-1 and galectin-3 expression in equine mesenchymal stromal cells (MSCs), synovial fibroblasts and chondrocytes, and the effect of inflammation on MSC motility. *Stem Cell Res Ther* 8:243
- Ren Y, Wu H, Ma Y, Cang M, Wang R, Liu D (2010) Isolation, cultivation and identification of adipose-derived stem cell in bovines. *Chin J Biotechnol* 26(12):1645–1651
- Rossi B, Merlo B, Colleoni S, Iacono E, Tazzari PL, Ricci F, Lazzari G, Galli C (2014) Isolation and in vitro characterization of bovine amniotic fluid derived stem cells at different trimesters of pregnancy. *Stem Cell Rev Rep* 10(5):712–724
- Sadeesh EM, Shah F, Yadav PS (2016) Differential developmental competence and gene expression patterns in buffalo (*Bubalus bubalis*) nuclear transfer embryos reconstructed with fetal fibroblasts and amnion mesenchymal stem cells. *Cytotechnology* 68(5):1827–1848
- Saito F, Nakatani T, Iwase M, Maeda Y, Hirakawa A, Murao Y, Suzuki Y, Onodera R, Fukushima M, Ide C (2008) Spinal cord injury treatment with intrathecal autologous bone marrow stromal cell transplantation: the first clinical trial case report. *J Trauma* 64:53–59
- Sampaio RV, Chiaratti MR, Santos DCN, Bressan FF, Sangalli JR, Sá ALA, Silva TVG, Costa NN, Cordeiro MS, Santos SSD, Ambrosio CE, Adona PR, Meirelles FV, Miranda MS, Ohashi OM (2015) Generation of bovine (*Bos indicus*) and buffalo (*Bubalus bubalis*) adipose tissue derived stem cells: isolation, characterization, and multipotentiality. *Genet Mol Res* 14(1):53–62
- Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML, Wu L, Lindeman GJ, Visvader JE (2006) Generation of a functional mammary gland from a single stem cell. *Nature* 439:84–88

- Sharma N, Huynh DL, Kim SW, Ghosh M, Sodhi SS, Singh AK, Kim NE et al (2017) A PiggyBac mediated approach for lactoferricin gene transfer in bovine mammary epithelial stem cells for management of bovine mastitis. *Oncotarget* 8(61):104272
- Shibiru B, Abriham K, Ashebr A (2017) Review on reproductive biotechnology and its role in dairy cattle production and health. *Reprod Opin* 9(3):60–70
- Singh J, Mann A, Kumar D, Duhan JS, Yadav PS (2013) Cultured buffalo umbilical cord matrix cells exhibit characteristics of multipotent mesenchymal stem cells. *In Vitro Cell Dev Biol Anim* 49:408–416
- Sohn HJ, Lee YH, Green RB, Spencer YI, Hawkins SAC, Stack MJ, Konold T, Wells GAH, Matthews D, Cho IS, Joo YS (2009) Bone marrow infectivity in cattle exposed to the bovine spongiform encephalopathy agent. *Vet Rec* 164:272–274
- Sreekumar TR, Ansari MM, Chandra VG, Sharma T (2014) Isolation and characterization of buffalo Wharton's jelly derived mesenchymal stem cells. *J Stem Cell Res Ther* 4:207
- Sun T, Yu C, Gao Y, Zhao C, Hua J, Cai L, Guan W, Ma Y (2014) Establishment and biological characterization of a dermal mesenchymal stem cells line from bovine. *Biosci Rep* 34(2):139–146. <https://doi.org/10.1042/BSR20130095>
- Thomas E, Zeps N, Cregan M, Hartmann P, Martin T (2011) 14-3-3sigma (sigma) regulates proliferation and differentiation of multipotent p63-positive cells isolated from human breast milk. *Cell Cycle* 10:278–284
- Vanella L, Sanford C Jr, Kim DH, Abraham NG, Ebraheim N (2012) Oxidative stress and heme oxygenase-1 regulated human mesenchymal stem cells differentiation. *Int J Hyperth* 2012:1
- Wolfe DF (2018) Abnormalities of the bull—occurrence, diagnosis and treatment of abnormalities of the bull, including structural soundness. *Animal* 12:s148–s157
- Wu S, Suzuki Y, Ejiri Y, Toru N, Hongliang B, Masaaki K, Kazuya K, Ohta M, Chou H, Ide C (2003) Bone marrow stromal cells enhance differentiation of cocultured neurosphere cells and promote regeneration of injured spinal cord. *J Neurosci Res* 72:343–351
- Wu Y, Chen L, Scott PG, Tredget E (2007) Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells* 25:2648–2659
- Xiong H, Bai C, Wu S, Gao Y, Lu T, Hu Q, Guan W, Ma Y (2014) Biological characterization of mesenchymal stem cells from bovine umbilical cord. *Anim Cell Syst* 1(1):59–67
- Yang J, Zhao Q, Wang K, Liu H, Ma C, Huang H, Liu Y (2016) Isolation and biological characterization of tendon-derived stem cells from fetal bovine. *Vitro Cell Dev Biol Anim* 52(8):846–856
- Zayed MN, Schumacher J, Misk N, Dhar MS (2016) Effects of pro-inflammatory cytokines on chondrogenesis of equine mesenchymal stromal cells derived from bone marrow or synovial fluid. *Vet J* 217:26–32
- Zhao L, Wang Z, Zhang J, Yang J, Gao XF, Wu B, Zhao G, Bao S, Hu S, Liu P, Li X (2017) Characterization of the single-cell derived bovine induced pluripotent stem cells. *Tissue Cell* 49(5):521–527

Cat Mesenchymal Stem Cell Characteristics and Potential Applications

10

M. B. Gugjoo and Amar Pal

Abstract

Cat mesenchymal stem cells (fMSCs) have been culture expanded and characterized from various tissue sources, though mainly from adipose tissues. The cells have been evaluated for their therapeutic effects in various experimental models and clinical trials relevant to veterinary and human practice. The studies have potentially favored MSCs applications; however, limited available studies currently fail to provide any conclusive result(s). The current chapter discusses fMSCs characteristics and their potential applications.

10.1 Introduction

Cat is increasingly being kept as a companion animal, and as such their clinical practice is increasing by each passing day. Regenerative medicine involving MSCs is gradually being employed in feline practice. Cat and human genome are highly linked, and as such cat MSCs experimental/clinical studies may provide proof of therapeutics for human medicine (Munoz et al. 2012), in addition to the therapeutics in veterinary practice.

M. B. Gugjoo (✉)

Division of Veterinary Clinical Complex, FVSc & AH, SKUAST-Kashmir,
Jammu and Kashmir, India

A. Pal

Division of Surgery, Indian Veterinary Research Institute, Izatnagar, UP, India

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2020

M. B. Gugjoo, A. Pal (eds.), *Mesenchymal Stem Cell in Veterinary Sciences*,
https://doi.org/10.1007/978-981-15-6037-8_10

10.2 fMSCs In Vitro Characteristics

An initial report on feline mesenchymal stem cells was reported by Martin and co-workers in 2002 that had isolated and characterized the cells from bone marrow (Martin et al. 2002). Since then many reports have been published on their isolation and culture expansion. The cells have been isolated from various although limited tissue types as detailed in Table 10.1. Most of the studies have focused on the adipose tissue derived MSCs. Cat MSCs (fMSCs) are being characterized based on the standard criteria laid down by the International Society for Cellular Therapy (ISCT). fMSCs are plastic adherent with spindle-shaped fibroblast-like appearance (Quimby et al. 2011; Arzi et al. 2015; Sato et al. 2016; Quimby and Borjesson 2018). These cells variably express various surface markers and lack hematopoietic markers. Among various sources, some of the studies had shown MSCs expression of the hematopoietic (CD34) and other markers like MHC-II, while others had reported MSCs failure to express CD90 (Iacono et al. 2012; Mumaw et al. 2015; Vidane et al. 2016). The cells show trilineage differentiation (adipogenic, chondrogenic, and osteogenic lineages) (Table 10.1).

The cells have been culture expanded in growth medium containing the fetal bovine serum (FBS). A study that utilized combination of FBS (10%) and autologous plasma had shown similar fPB-MSCs characteristics as that of cells being cultured under FBS. Currently, studies on FBS alternatives to culture expand fMSCs is lacking. fMSCs tend to undergo early senescence with extensive passaging. Cat peripheral blood (fPB)-MSCs had steadily expanded up to 5–6 passages, but almost all the cells had stopped proliferation at passages 7–9. Among 15 cultures, only 01 culture had proliferated up to passage 10. The cellular proliferation, however, had remained unaffected by age, sex, and breed (Sato et al. 2016). The population doubling levels of cat adipose tissue (fAD)-MSCs demonstrated at different passages were 2.08 ± 0.02 (P1), 3.95 ± 0.07 (P2), 5.88 ± 0.15 (P3), 7.14 ± 0.15 (P4), 8.00 ± 0.16 (P5), and 8.78 ± 0.19 (P6). From P6 onward, minimal increase in doubling levels had occurred. As such the population doubling time had significantly increased to an average of 95.7 h. at P4 and 141.3 h. at P5 from 62.1 h. at P3 (Kim et al. 2017). Similarly, another study had shown that fAD-MSCs population doubling time at passage 5 had increased significantly. The decreased expression of pluripotency markers and of surface markers was also seen after extended culture, significantly at P5. The musculoskeletal (especially osteogenic and chondrogenic) differentiation potential too had reduced with progressive culturing in these cells (Lee et al. 2018). fAD-MSCs at P2 had been characterized by their normal metaphase and karyotype (Villatoro et al. 2018).

To improve cellular proliferation and self-renewal, culture modifications are being worked out. Culture addition of recombinant acid ceramidase (rAC) had improved growth of cat bone marrow (fBM)-MSCs by twofold at 1 week. Even addition of rAC to chondrogenic medium had improved cellular chondrogenic potential, synergistic with TGF- β 1 (Simonaro et al. 2013). rAC might have reduced the cell stress that arises with the harvesting procedure and during their ex vivo expansion. The possible beneficial effect of the rAC treatment in chondrogenic

Table 10.1 Cat mesenchymal stem cell sources and characterization

Positive markers	Negative markers	Differentiation	References
<i>Adipose tissue MSCs (AD-MSCs)</i>			
CD44, CD90, CD105	CD4, MHC-II (low expression)	Adipogenic, chondrogenic, and osteogenic	Webb et al. (2011)
CD44, CD90	CD4, MHC-II	Adipogenic, chondrogenic, and osteogenic	Quimby et al. (2013)
CD44, CD90, CD105	CD14, CD34, CD45	Adipogenic, chondrogenic, osteogenic, and myogenic	Kono et al. (2014)
CD105, CD44, CD90	CD18, MHC-II	–	Arzi et al. (2015)
CD90, CD105, CD146, Oct-4, Nanog, Sox-2, and Klf4	CD73, CD14, CD45, CD271, HLA-DR, Sox-2	Adipogenic, chondrogenic, osteogenic, and neurogenic	Gómez et al. (2015)
CD29, CD44, CD73, CD90, CD105, CD45	MHCII, CD79 α , CD34	Adipogenic, chondrogenic, and osteogenic	Mumaw et al. (2015)
CD90, CD105, CD44	MHC-II	Adipogenic, chondrogenic, and osteogenic	Parys et al. (2016)
CD29, CD44, CD90, CD105, CD166, and MHC-I, Oct3/4, Nanog, and SSEA-4	CD14, CD34, CD45, CD73, Sox2	Adipogenic, chondrogenic, and osteogenic	Kim et al. (2017)
CD29, CD44, CD73, CD90, MHC-I, Stro-1	CD34, CD45, MHC-II	Adipogenic, chondrogenic, and osteogenic	Villatoro et al. (2018)
CD44, CD90, CD105	MHC-II	Adipogenic, chondrogenic, and osteogenic	
<i>Amniotic membrane MSCs (Am-MSCs)</i>			
CD90, CD44, CD105	CD34, CD14, CD45, CD73	Adipogenic, chondrogenic, and osteogenic	Iacono et al. (2012)
CD73, CD90	CD34, CD45, CD79	Adipogenic, chondrogenic, and osteogenic	Vidane et al. (2014)
CD73 and CD90, CD105 (low expression), OCT4, SOX2.	CD30, CD45	–	Cardoso et al. (2016)
CD73 (89.5%) and CD90 (91.9%), CD79 (29.1%), CD34 (47%),	CD45	–	Vidane et al. (2016)

(continued)

Table 10.1 (continued)

Positive markers	Negative markers	Differentiation	References
<i>Bone marrow MSCs (BM-MSCs)</i>			
CD44, CD90, CD105	CD4, MHC-II	Adipogenic, chondrogenic, and osteogenic	Quimby et al. (2011)
CD44, CD90, CD105	CD4, MHC-II (low expression)	Adipogenic, chondrogenic, and osteogenic	Webb et al. (2011)
CD29, CD44, CD105, Stro-1	CD14, CD34, CD45, MHC-II	Adipogenic, osteogenic, and neurogenic	Munoz et al. (2012)
CD29, CD44, CD105, CD45 (30%), MHC-II (39%)	CD73, CD90, CD79 α , and CD34,	Adipogenic, chondrogenic, and osteogenic	Mumaw et al. (2015)
<i>Peripheral blood MSCs (PB-MSCs)</i>			
CD44, CD90	CD4, MHC-II	Adipogenic, chondrogenic, and osteogenic	Sato et al. (2016)

AD-MSCs adipose tissue MSCs, *Am-MSCs* amniotic membrane MSCs, *BM-MSCs* bone marrow MSCs, *PB-MSCs* peripheral blood MSCs

differentiation process may occur through better TGF- β pathway signaling, in addition to the effects of sphingolipid changes on chondrogenic differentiation. Further, stress induced with serum-free chondro-differentiation of MSCs may also be controlled (Simonaro et al. 2013).

The cells isolated from various tissues and at variable locations may have variable culture characteristics. fBM-MSCs and fAD-MSCs had similar surface markers expression, but fAD-MSCs had proliferated significantly faster as compared to BM-MSCs. Thus, fBM-MSC and fAD-MSC were phenotypically similar, but higher intrinsic proliferation rate had made fAD-MSC culture proliferation easier (Webb et al. 2011). In relation to variable donor tissue locations, abdominal fAD-MSCs harbored higher percentage of cells expressing positive surface markers (CD90 and CD105) than those of subcutaneous fAD-MSCs. Although comparable pluripotency marker (Oct-4 and Klf4) expression had been reported, except for Nanog that had been highly expressed in subcutaneous fAD-MSCs. The cells had shown an equivalent in vitro multilineage differentiation including the neuron-like cells (Gómez et al. 2015).

10.2.1 MSCs from Infected Donor

fAD-MSCs can contract latent Feline Foamy Virus (FFV, retrovirus) infection that induces formation of syncytial cell and subsequent apoptosis in them. FFV in cultured cells had led to their morphologic and proliferative alterations. FFV-infected fAD-MSC lines cultured in growth media had formed multinucleated giant cells and had topped proliferation at passages 3–5. Incorporation of antiviral tenofovir

(reverse transcriptase inhibitor) at 5 or 10 mM had prevented such alterations in morphology and the proliferative capacity of FFV-infected fAD-MSCs. The effect on differentiation potential, however, remains to be evaluated (Arzi et al. 2015).

10.3 MSCs Applications

MSCs culture expansion is aimed to achieve enough concentration for their *in vivo* applications. MSCs have been applied either through intravenous and intraperitoneal or through local routes. All these routes have been found to be safe and without any potential adverse effects. For better concentration of MSCs in cerebrospinal fluid (CSF), intraventricular route of implantation was demonstrated to be superior as compared to the subdural and brain parenchyma route (Glage et al. 2011). The mild reactions in the form of local lymph node swelling and transient lethargy and inactivity had been encountered (Webb and Webb 2015; Trzil et al. 2016; Parys et al. 2016). The intravenous applications of MSCs have usually been followed by coagulation cascade being prevented by heparin. Below are the *in vivo* fMSCs experimental (Table 10.2) and clinical (Table 10.3) studies.

10.3.1 Chronic Enteropathy

Feline chronic enteropathy especially idiopathic inflammatory bowel disease (IBD) requires lifelong medication including immunosuppressive and chemotherapeutic drugs. IBD compromises gastrointestinal (GI) mucosal immunity and tolerance to intestinal antigens (Janeczko et al. 2008; Trepanier 2009). MSCs due to their immunomodulatory features are considered to play the therapeutic role.

In a feline chronic enteropathy of 02 months study, two intravenous doses of allogeneic AD-MSCs had improved the clinical symptoms based on owners report. Five out of seven cases (in blinded study) had significant improvement in clinical signs, while those in placebo (normal saline) treated cases had no improvement and/or symptoms worsened with time. Owner (aware about the treatment) had mixed response. One had reported marked improvement in condition; other conveyed no change while third case follow-up report had been unavailable (Webb and Webb 2015). Thus, further studies are desired to actually confirm MSCs efficacy in these ailments.

10.3.2 Chronic Kidney Disease

Kidney ailments are one of the common clinical conditions with life-threatening potential in domestic cats (Lawson et al. 2015). Currently, an effective and specific treatment to restore the affected kidney to normalcy is unavailable (Vidane et al. 2016).

Table 10.2 In vivo preclinical experimental mesenchymal stem cell studies in cats

Model type	Number of animals	Study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Chronic allergic asthma (Bermuda grass allergen induced)	9 (MSCs, $n = 5$; placebo PBS, $n = 4$)	8 months and 12 months	Allogeneic, AD-MSCs	1.44×10^7 MSCs/ infusion (bimonthly intravenous injected 6 doses)	Bronchoalveolar lavage fluid cytology, pulmonary mechanics and clinical scoring to assess airway hyper-responsiveness AHR; and thoracic computed tomography (CT)	No differences between treatment groups or over time with respect to airway eosinophilia or AHR have been seen. Significantly lower lung attenuation and bronchial wall thickening scores were noted in CT images of MSC-treated animals compared to placebo-treated cats at month 8 of the study, although the effect was not sustained at month 12. No differences were noted between groups in the immunologic assays	Trzil et al. (2014)
Allergic asthma (Bermuda grass allergen induced)	15 (MSCs treated, 5; placebo-treated 4; healthy cat, 6)	9 months after treatment	Allogeneic AD-MSCs	2×10^6 , 4×10^6 , 4.7×10^6 , 1×10^7 and 1×10^7 in each cat (at 0, 14, 28, 98, 130 days)	Airway eosinophilia, pulmonary mechanics, thoracic computed tomography, and several immunologic assays	At early points (days 12, 26, 47, 108, and 133), airway eosinophil percentage was not affected by MSC administration. By month 9, eosinophil percentages in all MSC-treated cats decreased to normal reference intervals. Diminished airway hyper-responsiveness was noted in all MSC-treated compared with placebo-treated cats at day 133. Lung attenuation and bronchial wall thickening scores were significantly reduced in MSC-treated vs. untreated asthmatic cats, consistent with decreased airway remodeling at month 9	Trzil et al. (2016)

Ischemic kidney injury (IKI)	18 (AD-MSCs, $n = 5$; BM-MSCs, $n = 5$; fibroblasts, $n = 5$; reference, $n = 3$)	6 days	Allogeneic AD-MSCs, BM-MSCs, fibroblasts	4×10^6 (intravenous after 1 h of injury per animal in each group)	Serum creatinine and blood urea nitrogen concentrations were measured. Urine-specific gravity, urine protein to urine creatinine ratio, and glomerular filtration rate. International Renal Interest Society (IRIS) Grade	Time, but not treatment, had a significant effect on renal function. No difference was noted in % of cats with IRIS AKI. Significantly fewer mitotic figures were observed in ischemic kidneys that received bone marrow-derived MSCs vs. fibroblasts. No differences in smooth muscle actin staining were noted. This study did not support the use of allogeneic MSCs in AKI in the regimen described here. Type of renal injury, MSC dose, allogeneicity, duration, and route or timing of administration could influence the efficacy of MSCs	Rosselli et al. (2016)
Cardiomyopathy (L-isoproterenol induced for 10 days)	22 (cardiac stem cells + atrial biopsy, $n = 7$; atrial biopsy + sham, $n = 5$; bone marrow + BM-MSCs, $n = 4$; bone marrow aspiration + sham, $n = 6$)	66 days after induction of cardiomyopathy	Autologous cardiac stem cells (CSCs), MSCs	1×10^6 (CSCs), 1×10^6 (MSCs); intracoronary injected	Echocardiography, invasive hemodynamics, and immunohistochemistry	Fractional shortening significantly improved with both CSC and MSC as compared to shams. MSC was superior to CSC in improving left ventricle end-diastolic (LVED) volume and ejection fraction. LVED pressure was less in MSC as compared to CSC and sham. LV BrdU + myocytes were higher in MSC than CSC and sham. CSC and MSC therapies improve cardiac function and attenuate pathological remodeling. However, MSC appear to confer additional benefit	Taghavi et al. (2015)

Table 10.3 In vivo clinical mesenchymal stem cell studies in cats

Clinical case	Number of animals	Study period	Biomaterial used	Cell dose/assembly	Evaluation criteria	Overall result	References
Chronic enteropathy	14: 7 treated with allogeneic MSCs (blinded study); 4 placebo; 3 treated with allogeneic MSCs (nonblind study)	2 weeks and 2 months	Allogeneic AD-MSCs	2×10^6 /kg b wt (two doses repeated after 2 weeks)	Clinical evaluation based on questionnaire	Owners of 5/7 IMSC-treated cats reported significant improvement or complete resolution of clinical signs, while the owner of the remaining two cats reported modest but persistent improvement. Owners of placebo-treated cats reported no change or worsening of clinical signs. Of the owners not blinded to the treatment, one reported marked improvement, one reported no change, and one was lost to follow-up	Webb and Webb (2015)
Chronic kidney disease (CKD)	6 (control healthy, $n = 2$; CKD, $n = 4$)	7 and 30 days	Autologous AD-MSCs, BM-MSCs	Unilateral intrarenal injection	Minimum database and glomerular filtration rate (GFR) via nuclear scintigraphy were determined pre-injection, at 7 days and at 30 days post-injection	Intrarenal injection did not induce immediate or long-term adverse effects. Two cats with CKD that received aMSC experienced modest improvement in GFR and a mild decrease in serum creatinine concentration	Quimby et al. (2011)
Chronic kidney disease (CKD)	16 (pilot study 1: 6; pilot study 2: 5; pilot study 3: 5)	0, 2, 4, 6, and 8 weeks	Allogeneic AD-MSCs	2×10^6 (cryopreserved cells), 4×10^6 (cryopreserved cells), 4×10^6 (cryopreserved adipose tissue)	Serum biochemistry, complete blood count, urinalysis, urine protein, glomerular filtration rate, and urinary cytokine concentrations	Administration of cryopreserved aMSCs was associated with significant adverse effects and no discernible clinically relevant improvement in renal functional parameters. Administration of aMSCs cultured from cryopreserved adipose was not associated with adverse effects but was also not associated with improvement in renal functional parameters	Quimby et al. (2013)

Chronic kidney disease (CKD) (randomized, placebo-controlled)	8 MSCs treated, $n = 6$; placebo control, $n = 2$	0 and 8 weeks	Allogeneic AD-MSCs	2×10^6 per kg b wt (3 doses at 2, 4, and 6 weeks)	Complete blood counts, chemistry and urinalysis were performed at weeks 0, 2, 4, 6, and 8. Glomerular filtration rate (GFR) via nuclear scintigraphy and urine protein:creatinine ratio (UPC)	Six cats received three doses of allogeneic MSC culture expanded from cryopreserved adipose without adverse effects. No significant change in serum creatinine, blood urea nitrogen, potassium, phosphorus, GFR by nuclear scintigraphy, UPC, or packed cell volume was seen in cats treated with MSCs. While administration of MSC culture expanded from cryopreserved adipose was not associated with adverse effects, significant improvement in renal function was not observed immediately after administration. Long-term follow-up is necessary to determine whether MSC administration affects disease progression in cats with CKD	Quimby et al. (2016)
Chronic kidney disease (CKD)	10 (1 healthy cat, intrarenal infusion of cells; 9 CKD cats intravenous infused cells)	60 days after initial injection	Allogeneic amniotic membrane MSCs	2×10^6 (CKD cats two doses 21 days apart), healthy cat (5×10^5 bilateral intrarenal infusion)	Ultrasonography and lab analysis	CDK cats treated with MSCs registered a significant improvement of renal function (decrease in serum creatinine and urine protein concentrations and increase in urine-specific gravity). The kidney architecture and morphology did not change following the treatment. The feline AMSCs have a renoprotective effect and improve renal function in cats with naturally occurring CKD, stabilizing the clinical condition and disease progression	Vidane et al. (2016)

Table 10.3 (continued)

Clinical case	Number of animals	Study period	Biomaterial used	Cell dose/assembly	Evaluation criteria	Overall result	References
Feline chronic gingivostomatitis	9 cats	6 and 24 weeks	Autologous AD-MSCs	20×10^6 (2 doses 30 days apart, intravenous)	Histology and immunohistochemistry, blood immune cell subsets, serum protein, and cytokine levels	7/9 cats completed the study. Five cats responded to treatment by either complete clinical remission ($n = 3$) or substantial clinical improvement ($n = 2$). Two cats were nonresponders. Cats that responded to treatment also exhibited systemic immunomodulation demonstrated by decreased numbers of circulating CD8+ T cells, a normalization of the CD4/CD8 ratio, decreased neutrophil counts, and interferon- γ and interleukin (IL)- 1β concentration, and a temporary increase in serum IL-6 and tumor necrosis factor- α concentration. No clinical recurrence has occurred following complete clinical remission (follow-up of 6–24 months)	Arzi et al. (2016)
Feline eosinophilic keratitis (FEK) (unilateral)	5	1, 3, 6, and 11 months	Allogeneic AD-MSCs	2×10^6 (2 doses 2 months apart subconjunctivally)	Ocular surface integrity, cytology, PCR, and biochemistry	Clinical signs showed a significant change during the follow-up with resolution of the corneal and conjunctiva lesions, and there were no signs of regression or worsening	Villatoro et al. (2018)
L1-L5 compressive fracture (treated by hemilaminectomy)	1	3 months	Autologous BM-MSCs, collagen I gel, and physiotherapy	7×10^8	Clinical evaluation	Seven days after surgery and cell transplantation, the examination revealed a progressive recovery of the panniculus reflexes and of the responses to superficial and deep pain stimuli despite the low proprioceptive and hyperreflexic ataxic hind limbs. Physiotherapy protocols were applied for clinical rehabilitation after surgery. The cat's first steps, 3-min weight-bearing, and intestine and urinary bladder partial reestablishment were observed 75 days post-surgery	Penha et al. (2012)

In an ischemic kidney injury model, comparative efficacy of allogeneic AD-MSCs vs. BM-MSCs vs. fibroblasts had shown that restoration in renal function occurs only with time in either case and as such MSCs application in ischemic kidney injury was not supported. It can be inferred that further studies that consider all the criteria like renal injury type, cell dose and source, and route or timing of administration should be considered in order to draw any conclusion (Rosselli et al. 2016).

In a clinical chronic kidney disease (CKD) study, intravenous infusion of amniotic membrane MSCs had shown improved clinical condition although without any change in the kidney architecture. The intrarenal implantation of MSCs in CKD cats had failed to yield desired result at 1 month post-injection (Quimby et al. 2011). Intra-renal infusion of the cells may be associated with the anesthetic requirement and has associated stress response in a healthy cat. The intravenous injection of MSCs for CKD may be preferable route (Vidane et al. 2016). In another CKD clinical study, intravenous implantation of MSCs had failed to improve the clinical signs. Administration of cryopreserved cells, however, had elicited the adverse reaction, while cells harvested from cryopreserved adipose tissue were devoid of such adverse reactions in the cases (Quimby et al. 2013). Same authors in another study had demonstrated no immediate improvement with allogeneic MSCs. However, it was concluded that improvement that may occur later in the follow-up requiring long duration studies (Quimby et al. 2016).

10.3.3 Cat Asthma

Feline asthma exhibits airway eosinophilia, airway hyper-responsiveness (AHR) and finally remodeling (Norris Reinero et al. 2004). Current therapeutics in such cases involves glucocorticoids and bronchodilators, but these drugs fail to provide consistent results (Murdoch and Lloyd 2010; Freishtat et al. 2010; Cocayne et al. 2011; Durrani et al. 2011). To control such ailment and provide relief to the patients, advanced therapeutic is desired.

In an induced chronic asthma model (Bermuda grass allergen) of cat, allogeneic AD-MSCs and placebo-treated cases had similar AHR and eosinophilia. At 8 months, lung attenuation and bronchial wall thickening were significantly lowered in MSC-treated animals in comparison to placebo-treated cats. The effect, however, was not sustained at month 12 (Trzil et al. 2014). In another similar model, asthmatic cats injected with multiple doses of allogeneic AD-MSCs had shown an improvement in clinical response (lowered lung attenuation bronchial wall thickening). The improvement was not demonstrated earlier (up to 133 days), but later on at 9 months much improved clinical response had been demonstrated. It was concluded that MSCs tend to show delayed effect and as such delayed response may be observed (Trzil et al. 2016). However, to confirm sustenance of these improved results further investigations are required.

10.3.4 Chronic Gingivostomatitis

Feline chronic gingivostomatitis (FCGS) is an idiopathic, oral inflammatory disease that affects 0.7%–10% of cat population (Arzi et al. 2010; Alpsoy et al. 2015; Jennings et al. 2015). It is generally treated by extraction of all the teeth. Even about 30% of cats do not respond to full mouth extraction demanding continuous medication (Jennings et al. 2015). Thus, MSCs due to their immunomodulatory and/anti-inflammatory role are being evaluated for FCGS.

In a feline chronic gingivostomatitis study, intravenous implantation of two doses of MSCs had improved the clinical symptoms in more than 50% (5 out of 7) of the cases. Among responders some had shown clinical remission (3/5), while others (2/5) had significant clinical improvement. In responsive cases the systemic immunomodulation was seen (Arzi et al. 2016).

10.3.5 Feline Eosinophilic Keratitis (FEK)

In FEK cornea undergoes superficial vascularization, yellow-white plaques, stromal infiltration, and edema (Allgoewer et al. 2001). The etiological agent is currently unknown, but disease appears to be immune mediated (Maggs 2008) and as such anti-inflammatory or immunosuppressants are being prescribed for its control (Dean and Meunier 2013). Since these compromise body's immunity and lead to the other ailments (Villatoro et al. 2015), other treatment options are being evaluated. Topical cyclosporine to some extent appears useful but leads to corneal irritation and chemosis (Allgoewer et al. 2001). MSCs due to their immunomodulation and/anti-inflammatory properties appear suitable therapeutic option.

In a clinical feline eosinophilic keratitis (FEK) study, two doses of allogeneic MSCs applied subconjunctivally had led to the significant improvement in its symptoms. The clinical signs like corneal and conjunctiva lesions were resolved without any sign of regression or worsening (Villatoro et al. 2018).

10.3.6 Neurological Ailments

fMSCs have been demonstrated to increase the tyrosine hydroxylase expression of PC12 cell line being used for neurological studies (Jin et al. 2007). This could possibly be through MSCs secreted nerve growth factor (NGF) and brain-derived nerve factor (BDNF) in combination with dopamine produced by PC12 cells (Zhou et al. 1994; Li et al. 1997; Zhou et al. 1998). Red fluorescent protein (RFP)-transfected cat umbilical cord blood (UCB)-MSCs under neurogenic differentiation medium were differentiated into neuron-like cells. Immunocytochemistry had demonstrated that differentiated cells were β III-tubulin-positive (6.85%), neurofilament light (NF-L)-positive (3.37%), and neurofilament medium (NF-M)-positive (7.04%) (Jin et al. 2008). Thus, UCB-MSCs can act as an alternative source.

In a cat lumbar compressive fracture (surgically rectified by hemilaminectomy), implantation of MSCs along with physiotherapy had improved the clinical symptoms. The initial observable positive responses were demonstrated at day 7 with the return of panniculus reflex. After 75 days, a partial improvement in weight bearing along with intestine and urinary bladder tone was recorded (Penha et al. 2012). These positive results demand further extensive investigations of MSCs application in such conditions (Table 10.2).

10.3.7 Cardiomyopathy

In cats, hypertrophic cardiomyopathy is common. In an isoproterenol induced hypertrophic model, intracoronary injection of cardiac stem cells (CSCs) and BM-MSCs had improved fractional shortening in comparison to sham. MSCs had led to superior improvement in left ventricle end-diastolic volume and ejection fraction as compared to CSCs (Taghavi et al. 2015). Such a preliminary study shows promising results with the use of MSCs though further extensive studies are desired for any conclusion.

10.4 Conclusions

fMSCs have been harvested from various sources mainly from adipose tissues. The cells have been culture expanded and characterized as per the ISCT. The cells exhibit limited self-renewal properties in currently available media as their viability decreases and become more senescent at early culture passages (p6). fAD-MSCs are more prone to FFV that leads to their morphologic and proliferative alterations. The cellular applications have been made in various experimental models and or clinical conditions. The results of these studies have variably been pro-therapeutic. In case of kidney failure, however, little to no success with fMSCs has been demonstrated. These studies are very limited with lack of sufficient data to be considered sufficient for any conclusion(s).

References

- Allgoewer I, Schäffer EH, Stockhaus C, Vögtlin A (2001) Feline eosinophilic conjunctivitis. *Vet Ophthalmol* 4:69–74
- Alpsoy E, Akman-Karakas A, Uzun S (2015) Geographic variations in epidemiology of two autoimmune bullous diseases: pemphigus and bullous pemphigoid. *Arch Dermatol Res* 307:291–298
- Arzi B, Murphy B, Cox DP et al (2010) Presence and quantification of mast cells in the gingival of cats with tooth resorption, periodontitis and chronic stomatitis. *Arch Oral Biol* 55:148–154
- Arzi B, Kol A, Murphy B, Walker NJ, Wood JA, Clark K, Verstraete FJM, Borjesson DL (2015) Feline foamy virus adversely affects feline mesenchymal stem cell culture and expansion: implications for animal model development. *Stem Cells Dev* 24(7):814–823
- Arzi B, Mills-Ko E, Verstraete FJM, Kol A, Walker NJ, Badgley MR, Fazel N, Murphy WJ, Vapniarsky N, Borjesson DL (2016) Therapeutic efficacy of fresh, autologous mesenchymal stem cells for severe refractory gingivostomatitis in cats. *Stem Cells Transl Med* 5:75–86

- Cardoso MT, Pinheiro AO, Vidane AS, Casals JB, de Oliveira VC, Gonçalves NJN, Martins DS, Ambrósio CE (2016) Characterization of teratogenic potential and gene expression in canine and feline amniotic membrane-derived stem cells. *Reprod Domest Anim* 51(Suppl. 3):1–7
- Cocayne CG, Reinero CR, DeClue AE (2011) Subclinical airway inflammation despite high-dose oral corticosteroid therapy in cats with lower airway disease. *J Feline Med Surg* 13:558–563
- Dean E, Meunier V (2013) Feline eosinophilic keratoconjunctivitis: a retrospective study of 45 cases (56 eyes). *J Feline Med Surg* 15:661–666
- Durrani SR, Viswanathan RK, Busse WW (2011) What effect does asthma treatment have on airway remodeling? Current perspectives. *J Allergy Clin Immunol* 128:439–448
- Freishtat RJ, Nagaraju K, Jusko W, Hoffman EP (2010) Glucocorticoid efficacy in asthma: is improved tissue remodeling upstream of anti-inflammation. *J Investig Med* 58:19–22
- Gómez MC, Qin Q, Biancardi MN, Galiguis J, Dumas C, MacLean RA, Wang G, Pope E (2015) Characterization and multilineage differentiation of domestic and black-footed cat mesenchymal stromal/stem cells from abdominal and subcutaneous adipose tissue. *Cell Reprogram* 17(5):376. <https://doi.org/10.1089/cell.2015.0040>
- Glage S, Klinge PM, Miller MC, Wallrapp C, Geigle P, Hedrich HJ, Brinker T (2011) Therapeutic concentrations of glucagon-like peptide-1 in cerebrospinal fluid following cell-based delivery into the cerebral ventricles of cats. *Fluids Barriers CNS* 8:18
- Iacono E, Cunto M, Zambelli D, Ricci F, Tazzari PL, Merlo B (2012) Could fetal fluid and membranes be an alternative source for mesenchymal stem cells (MSCs) in the feline species? A preliminary study. *Vet Res Commun* 36:107–118
- Janecko S, Atwater D, Bogel E et al (2008) The relationship of mucosal bacteria to duodenal histopathology, cytokine mRNA, and clinical disease activity in cats with inflammatory bowel disease. *Vet Microbiol* 128:178–193
- Jennings MW, Lewis JR, Soltero-Rivera MM et al (2015) Effect of tooth extraction on stomatitis in cats: 95 cases (2000–2013). *J Am Vet Med Assoc* 246:654–660
- Jin G-Z, Yin X-J, Yu X-F, Cho S-J, Lee H-S, Lee H-J, Kong I-K (2007) Enhanced tyrosine hydroxylase expression in PC12 cells co-cultured with feline mesenchymal stem cells. *J Vet Sci* 8(4):377–382
- Jin G-Z, Yin X-J, Yu X-F, Cho S-J, Choi E-G, Lee Y-S, Jeon J-T, Yee S-T, Kong I-K (2008) Generation of neuronal-like cells from umbilical cord blood-derived mesenchymal stem cells of a RFP-transgenic cloned cat. *J Vet Med Sci* 70(7):723–726
- Kim H-R, Lee J, Byeon JS, Gu N-Y, Lee J, Cho I-S, Cha S-H (2017) Extensive characterization of feline intra-abdominal adipose-derived mesenchymal stem cells. *J Vet Sci* 18(3):299–306
- Kono S, Kazama T, Kano K, Harada K, Uechi M, Matsumoto T (2014) Phenotypic and functional properties of feline dedifferentiated fat cells and adipose-derived stem cells. *Vet J* 199:88–96
- Lawson J, Elliott J, Wheeler-Jones C, Syme H, Jepson R (2015) Renal fibrosis in feline chronic kidney disease: known mediators and mechanisms of injury. *Vet J* 203:18–26
- Lee B-Y, Li Q, Song W-J, Chae H-K, Kweon K, Ahn J-O, Youn H-Y (2018) Altered properties of feline adipose-derived mesenchymal stem cells during continuous *in vitro* cultivation. *J Vet Med Sci* 80(6):930–938
- Li XM, Qi J, Juorio AV, Boulton AA (1997) Reciprocal regulation of the content of aromatic L-amino acid decarboxylase and tyrosine hydroxylase mRNA by NGF in PC12 cells. *J Neurosci Res* 47:449–454
- Maggs DJ (2008) Cornea and sclera. In: Maggs DJ, Miller PE, Ofri R, Slatter DH (eds) *Slatter's fundamentals of veterinary ophthalmology*, 4th edn. Elsevier Saunders, St. Louis, pp 175–202
- Martin DR, Cox NR, Hathcock TL et al (2002) Isolation and characterization of multipotential mesenchymal stem cells from feline bone marrow. *Exp Hematol* 30:879–886
- Mumaw JL, Schmiedt CW, Breidling S et al (2015) Feline mesenchymal stem cells and super-natant inhibit reactive oxygen species production in cultured feline neutrophils. *Res Vet Sci* 103:60–69
- Munoz JL, Greco SG, Patel SA, Sherman LS, Bhatt S, Bhatt RS, Shrensel JA, Guan Y-Z, Xie G, Ye J-H, Rameshwar P, Siegel A (2012) Feline bone marrow-derived mesenchymal stromal

- cells (MSCs) show similar phenotype and functions with regards to neuronal differentiation as human MSCs. *Differentiation* 84(2):214–222
- Murdoch JR, Lloyd CM (2010) Chronic inflammation and asthma. *Mutat Res* 690:24–39
- Norris Reinero CR, Decile KC, Berghaus RD et al (2004) An experimental model of allergic asthma in cats sensitized to house dust mite or Bermuda grass allergen. *Int Arch Allergy Immunol* 135:117–131
- Parys M, Nelson N, Koehl K, Miller R, Kaneene JB, Kruger JM, Yuzbasiyan-Gurkan V (2016) Safety of intraperitoneal injection of adipose tissue-derived autologous mesenchymal stem cells in cats. *J Vet Intern Med* 30:157–163
- Penha EM, Aguiar PHP, Barrouin-Melo SM, de Lima RS, da Silveira ACC, Otelto ARS, Pinheiro CMB, Ribeiro-dos-Santos R, Soares MBP (2012) Clinical neurofunctional rehabilitation of a cat with spinal cord injury after hemilaminectomy and autologous stem cell transplantation. *Int J Stem Cells* 5(2):146–150
- Quimby JM, Borjesson DL (2018) Mesenchymal stem cell therapy in cats current knowledge and future potential. *J Feline Med Surg* 20:208–216
- Quimby JM, Webb TL, Gibbons DS, Dow SW (2011) Evaluation of intrarenal mesenchymal stem cell injection for treatment of chronic kidney disease in cats: a pilot study. *J Feline Med Surg* 13:418–426
- Quimby JM, Webb TL, Habenicht LM, Dow SW (2013) Safety and efficacy of intravenous infusion of allogeneic cryopreserved mesenchymal stem cells for treatment of chronic kidney disease in cats: results of three sequential pilot studies. *Stem Cell Res Ther* 4:48
- Quimby JM, Webb TL, Randall E, Marolf A, Valdes-Martinez A, Dow SW (2016) Assessment of intravenous adipose derived allogeneic mesenchymal stem cells for the treatment of feline chronic kidney disease: a randomized, placebo-controlled clinical trial in eight cats. *J Feline Med Surg* 18(2):165–171
- Rosselli DD, Mumaw JL, Dickerson V, Brown CA, Brown SA, Schmiedt CW (2016) Efficacy of allogeneic mesenchymal stem cell administration in a model of acute ischemic kidney injury in cats. *Res Vet Sci* 108:18–24
- Sato K, Yamawaki-Ogata A, Kanemoto I, Usui A, Narita Y (2016) Isolation and characterisation of peripheral blood-derived feline mesenchymal stem cells. *Vet J* 216:183–188
- Simonaro CM, Sachot S, Ge Y, He X, DeAngelis VA, Eliyahu E, Leong D, Sun HB, Mason JB, Haskins ME, Richardson DW, Schuchman EH (2013) Acid ceramidase maintains the chondrogenic phenotype of expanded primary chondrocytes and improves the chondrogenic differentiation of bone marrow-derived mesenchymal stem cells. *PLoS One* 8(4):e62715
- Taghavi S, Sharp TE, Duran JM, Makarewich CA, Berretta RM, Starosta T, Kubo H, Barbe M (2015) Autologous c-kit+ mesenchymal stem cell injections provide superior therapeutic benefit as compared to c-kit+ cardiac-derived stem cells in a feline model of isoproterenol-induced cardiomyopathy. *Clin Transl Sci* 8(5):425–431
- Trepanier L (2009) Idiopathic inflammatory bowel disease in cats. Rational treatment selection. *J Feline Med Surg* 11:32–38
- Trzil JE, Masseau I, Webb TL, Chang C-H, Dodam JR, Cohn LA, Liu H, Quimby JM, Dow SW, Reinero CR (2014) Long term evaluation of mesenchymal stem cell therapy in a feline model of chronic allergic asthma. *Clin Exp Allergy* 44(12):1546–1557
- Trzil JE, Masseau I, Webb TL, Chang C-H, Dodam JR, Liu H, Quimby JM, Dow SW, Reinero CR (2016) Intravenous adipose-derived mesenchymal stem cell therapy for the treatment of feline asthma: a pilot study. *J Feline Med Surg* 18:981. <https://doi.org/10.1177/1098612X15604351>
- Vidane AS, Souza AF, Sampaio RV, Bressan FF, Pieri NC, Martins DS, Meirelles FV, Miglino MA, Ambrósio CE (2014) Cat amniotic membrane multipotent cells are nontumorigenic and are safe for use in cell transplantation. *Stem Cells Cloning: Adv Appl* 7:71–78
- Vidane AS, Pinheiro AO, Casals JB, Passarelli D, Hage MCFNS, Bueno RS, Martins DS, Ambrósio CE (2016) Transplantation of amniotic membrane-derived multipotent cells ameliorates and delays the progression of chronic kidney disease in cats. *Reprod Domest Anim* 51(Suppl. 3):1–11

- Villatoro AJ, Fernández V, Claros S, Rico-Llanos GA, Becerra J, Andrades JA (2015) Use of adipose-derived mesenchymal stem cells in keratoconjunctivitis sicca in a canine model. *Biomed Res Int* 2015:1
- Villatoro AJ, Claros S, Fernández V, Alcoholad C, Fariñas F, Moreno A, Becerra J, Andrades JA (2018) Safety and efficacy of the mesenchymal stem cell in feline eosinophilic keratitis treatment. *BMC Vet Res* 14:116
- Webb TL, Webb CB (2015) Stem cell therapy in cats with chronic enteropathy: a proof-of-concept study. *J Feline Med Surg* 17:901. <https://doi.org/10.1177/1098612X14561105>
- Webb TL, Quimby JM, Dow SW (2011) In vitro comparison of feline bone marrow derived and adipose tissue-derived mesenchymal stem cells. *J Feline Med Surg* 14(2):165–168
- Zhou J, Bradford HF, Stern GM (1994) The stimulatory effect of brain-derived neurotrophic factor on dopaminergic phenotype expression of embryonic rat cortical neurons *in vitro*. *Brain Res Dev Brain Res* 81:318–324
- Zhou J, Pliego-Rivero B, Bradford HF, Stern GM, Jauniaux ER (1998) Induction of tyrosine hydroxylase gene expression in human foetal cerebral cortex. *Neurosci Lett* 252:215–217



Dog Mesenchymal Stem Cell Basic Research and Potential Applications

11

M. B. Gugjoo, Amar Pal, and G. T. Sharma

Abstract

Mesenchymal stem cells (MSCs) being available in numerous adult tissues and foetal membranes have been harvested from almost all these sources in dog. Due to the presence of miniscule number of MSCs in these tissues, culture expansion followed by their characterization becomes imperative. These characterized cells have been made subject in preclinical experimental and clinical settings in dogs. The studies have variably but undeniably supported their therapeutic effects, although currently inconclusive. Number of dog clinical ailments compeer to that of human. Therapeutic benefits of dMSCs in these ailments may provide proof-of-therapeutic principle for human medicine. The current chapter details dMSCs culture characteristics and potential applications.

11.1 Introduction

The population of pets like dog is increasing in the world. These pets are considered as family members and owners spent many dollars to keep them healthy. Dog, *Canis familiaris*, suffers from numerous ailments that lack standard treatment. Many of

M. B. Gugjoo (✉)

Division of Veterinary Clinical Complex, FVSc & AH, SKUAST-Kashmir,
Jammu and Kashmir, India

A. Pal

Division of Surgery, Indian Veterinary Research Institute, Izatnagar, UP, India

G. T. Sharma

Division of Physiology and Climatology, Indian Veterinary Research Institute,
Izatnagar, UP, India

© The Editor(s) (if applicable) and The Author(s), under exclusive license to
Springer Nature Singapore Pte Ltd. 2020

M. B. Gugjoo, A. Pal (eds.), *Mesenchymal Stem Cell in Veterinary Sciences*,
https://doi.org/10.1007/978-981-15-6037-8_11

213

their ailments have patho-physiological resemblance to that of human ailments, involving genetic basis as well (Starkey et al. 2005). This makes dog a suitable model animal for human. Dog offers many advantages including similarity in lifespan. This allows long-term studies on the safety and efficacy of therapeutic agents (Baird et al. 2015). To address issues of these enigmatic ailments and/or to promote early healing, regenerative medicine involving stem cell therapy is currently being studied. Among various types of stem cells, MSCs mainly contribute to the therapeutics in dog.

11.2 In Vitro Studies

11.2.1 Sources of Dog MSCs

A diverse set of tissues have been evaluated for stem cell reserve in dogs. This includes adult tissues and the foetal membranes (Table 11.1) (Gugjoo et al. 2019a). These cells have also been harvested from induced pluripotent stem cells (Chow et al. 2017). The current tissue harvesting procedures are invasive; efforts are being made to develop advanced harvesting procedures that reduce animal sufferings. For muscle tissue harvesting, minimally invasive technique of microbiopsy (leads to insignificant muscle damage) had been demonstrated as reasonably safe way to harvest MSCs (Ceusters et al. 2017). Foetal membrane tissues are limited in dogs as pets undergo elective castration in juvenile age (Sultana et al. 2018). dMSCs in compensation, may be harvested from ovaries and/or uterus of spayed animals (Sultana et al. 2018).

11.2.2 Cellular Variability with Respect to Sources

11.2.2.1 Cell Concentration

The tissues harbour dMSCs in limited and variable concentrations. The cell yield per gram of tissue varies. At day 16 of culture, the highest cell yield had been obtained from periosteum (19.4×10^6) followed in descending order by muscle (3.37×10^6), adipose tissue (2.33×10^6) and bone marrow (1.45×10^6) (Kisiel et al. 2012). dMSCs tend to lose their self-renewal potential after certain passages. With extended passaging, these cells had shown reduced proliferation potential and appeared senescent. dMSCs had been demonstrated to maintain their consistent population doubling time (PDT) up to 20 passages, though other factors like cell source and culture environment may also affect such properties (Spencer et al. 2012; Kresic et al. 2017). One of the studies had evaluated MSCs from various sources and it was demonstrated that highest cell proliferation in all these sources was at passage 3. Among these tissue sources, average PDT was lowest for adipose tissue MSCs (15 h) followed in ascending order by placenta-MSCs (21.2 h), bone marrow MSCs (26.2 h) and umbilical cord MSCs (41.9 h) (Zhan et al. 2019). Early passage dMSCs may therefore be clinically more useful than those obtained after extended passaging.

Table 11.1 Dog Mesenchymal stem cell (MSC) sources and their characterization

Positive markers	Negative markers	Differentiation	References
<i>Adipose MSCs (AD-MSCs)</i>			
CD44, CD90, MHC-I, CD34 (partially)	CD14 and CD45	Osteogenic	Kang et al. (2008)
OCT4, NANOG, SOX2	Not available	Osteogenic, Chondrogenic and limited Adipogenic	Neupane et al. (2008)
CD44, CD90, CD105	CD14, CD34, CD45	Osteogenic, Adipogenic, neurogenic	Ryu et al. (2009)
CD44, CD29, CD90	CD14, CD34, CD45, CD117, CD13, CD105, CD73	Osteogenic, Adipogenic, Chondrogenic, myogenic	Vieira et al. (2010)
CD90, CD44, CD140a, CD117	CD34, CD45	Osteogenic, Adipogenic, myogenic	Martinello et al. (2011)
CD29, CD44, thy 1.1	CD31, CD34, CD73, CD105	Osteogenic, Adipogenic, Chondrogenic, myogenic, neurogenic	Oh et al. (2011)
OCT4, NANOG, SOX2	–	Osteogenic, Adipogenic, chondrogenic	Guercio et al. (2012)
CD44, CD90 SOX2, OCT4, NANOG	CD34, CD45, CD146	Osteogenic, Adipogenic	Kisiel et al. (2012)
CD29, CD44, CD 90, SSEA-1 (low), OCT3/4, SOX2, NANOG	CD34, CD45, SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81	Osteogenic, Adipogenic	Takemitsu et al. (2012)
CD44, CD73, CD90, CD105	CD14, CD34, CD45	Neurogenic	Ryu et al. (2012)
CD29, CD90 and STRO-1	CD34, CD45 and MHC-II	Osteogenic, Adipogenic, Chondrogenic	Villatoro et al. (2015)
CD90, CD44, CD166, Oct4, Sox2	CD34, CD45	Primordial germ cell-like cells and male germ cells	Wei et al. (2016)
CD 90, CD 105	CD 14, CD 45	Osteogenic, Adipogenic, Chondrogenic, neurogenic	Blecker et al. (2017)
CD9, CD44, CD90, and CD105, NANOG, OCT4, and SOX2	CD34, CD45 and STRO-1	Osteogenic, Adipogenic, Chondrogenic	Bearden et al. (2017)

(continued)

Table 11.1 (continued)

Positive markers	Negative markers	Differentiation	References
CD44, CD90	CD34, CD45	Osteogenic, Adipogenic, neurogenic	Kriston-Pál et al. (2017)
CD90, CD44, NANOG, TERT, SOX2, OCT4	CD34, CD45	Osteogenic, Chondrogenic, Adipogenic, neurogenic, endoderm, primordial germ cells	Trindade et al. (2017)
CD90, CD44, Sox2 and Nanog	CD45	Osteogenic, Chondrogenic	Ayala-Cuellar et al. (2019)
CD73, CD90, CD44	CD45, CD14, HLA-DR and CD34.	Osteogenic, Chondrogenic, Adipogenic	Bçrziød et al. (2018)
CD90, OCT4, SOX9, RUNX2, PPARG	CD34, CD45, MCH-II	–	Enciso et al. (2018)
CD90, CD44	CD45, CD11b	Osteogenic, Chondrogenic, Adipogenic	Sasaki et al. (2018)
CD44, CD90	CD 34 and CD45	Osteogenic, Chondrogenic	Shah et al. (2018)
CD29, CD44, CD73, CD90 and STRO-1	CD11/18, CD34, CD45, MHC-II	Osteogenic, Adipogenic, Chondrogenic	Villatoro et al. (2018)
<i>Amniotic fluid MSCs</i>			
Oct-4, CD44, DLA-DRA1 and DLA-79	CD34, CD45	Osteogenic, Chondrogenic, Adipogenic, neurogenic	Uranio et al. (2011)
<i>Amniotic membrane MSCs</i>			
CD90, CD105	CD3, CD11c, CD28, CD38, CD62L, CD34, CD45, CD41a	Osteogenic, Chondrogenic, Adipogenic, neurogenic	Park et al. (2012a)
Oct-4, CD44, CD184 and CD29	CD34, CD45	Osteogenic, Chondrogenic, Adipogenic, neurogenic	Uranio et al. (2011)
<i>Bone marrow MSCs</i>			
CD90, MHC-I	CD34, CD45, MHC-II	Neurogenic	Kamishina et al. (2006)
CD105, CD90	CD45, CD34	Osteogenic, Chondrogenic, Adipogenic	Csaki et al. (2007)

(continued)

Table 11.1 (continued)

Positive markers	Negative markers	Differentiation	References
CD10, CD13, CD29, CD44, CD73/SH-3, CD90/Thy-1 and CD106/VCAM-1	CD3, CD14, CD34, CD45	–	Lee et al. (2011b)
CD 44 and CD 90	CD34	Osteogenic Chondrogenic, Adipogenic, neurogenic	Tharasanit et al. (2011)
CD44, STRO-1	CD34, CD45	Chondrogenic	Hodgkiss-Geere et al. (2012)
CD44, CD90, SOX2, OCT4, NANOG	CD34, CD45, CD146	Osteogenic, Adipogenic	Kisiel et al. (2012)
CD44, CD73, CD90, CD105	CD14, CD34, CD45	Neurogenic	Ryu et al. (2012)
CD29, CD44, CD 90, OCT3/4, SOX2, NANOG	CD34, CD45, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81	Osteogenic, Adipogenic	Takemitsu et al. (2012)
CD44, CD105	CD34, CD45	–	Kazemi et al. (2017)
CD29, CD44, CD90	CD45 and CD11b	Osteogenic, Adipogenic	Matsuda et al. (2017)
CD90, OCT4, SOX9, RUNX2, PPARγ	CD34, CD45, MCH-II	–	Enciso et al. (2018)
CD 166, CD 29, CD 90, CD 105 and CD 44	CD45 and CD 34	Osteogenic, Chondrogenic	Li et al. (2018)
CD90, CD44	CD45, CD11b	Osteogenic, Chondrogenic, Adipogenic	Sasaki et al. (2018)
CD9, CD44, CD90, and CD105, NANOG, OCT4, and SOX2	CD34, CD45 and STRO-1	Osteogenic, Adipogenic, Chondrogenic	Bearden et al. (2017)
<i>Corneal MSCs</i>			
CD90, CD73, CD105, N-cadherin, Pax6	CD34	Osteogenic, Chondrogenic, Adipogenic	Kafarnik et al. (2020)
<i>Dental pulp MSCs</i>			
CD90, CD44, CD146, SSEA-4 (low)	–	Osteogenic, Chondrogenic, Adipogenic	Khorsand et al. (2013)

(continued)

Table 11.1 (continued)

Positive markers	Negative markers	Differentiation	References
<i>Endometrium MSCs</i>			
CD44, CD29, CD90, CD13, CD133, CD73, CD105, Oct4	CD45, CD34, CD31 (low to nil)	Osteogenic, Chondrogenic, Adipogenic	De Cesaris et al. (2017)
<i>Limbal epithelium MSCs</i>			
CD90, CD44, CD49	CD45, CD11b/c	Osteogenic, Chondrogenic, Adipogenic, keratinocyte	Sancak et al. (2014)
<i>Muscle MSCs</i>			
CD44, CD90, SOX2, OCT4, NANOG	CD34, CD45	Osteogenic, Adipogenic	Kisiel et al. (2012)
<i>Olfactory epithelium MSCs</i>			
CD73, CD90, CD105	CD34, CD45, CD79	Osteogenic, Chondrogenic, Adipogenic, neurogenic	Pinheiro et al. (2016)
<i>Omental MSCs</i>			
CD90, CD44	CD45, CD11b	Osteogenic, Chondrogenic, Adipogenic	Bahamondes et al. (2017)
<i>Ovarian MSCs</i>			
CD90, CD44, NANOG, TERT, SOX2, OCT4	CD34, CD45	Osteogenic, Chondrogenic, Adipogenic, neurogenic, endoderm, primordial germ cells	Trindade et al. (2017)
<i>Periodontal ligament MSCs</i>			
–	–	Adipogenic, Osteogenic	Sedigh et al. (2010)
<i>Periosteal MSCs</i>			
CD44, CD90, SOX2, OCT4, NANOG	CD34, CD45	Osteogenic, Adipogenic	Kisiel et al. (2012)
<i>Synovial membrane MSCs</i>			
CD9, CD44, CD90, and CD105, NANOG, OCT4, and SOX2.	CD34, CD45 and STRO-1	Osteogenic, Adipogenic, Chondrogenic	Bearden et al. (2017)
CD90, CD44	CD45, CD11b	Osteogenic, Chondrogenic, Adipogenic	Sasaki et al. (2018)
<i>Peripheral blood MSCs</i>			
CD34(low)	CD34	Osteocalcin	Huss et al. (2000)

(continued)

Table 11.1 (continued)

Positive markers	Negative markers	Differentiation	References
<i>Placental MSCs</i>			
CD90, CD29, CD44, CD105, CD73, SOX2 (low)	CD45, CD34 and MHC-II, NANOG, OCT4	Osteogenic, Chondrogenic, Adipogenic	Saulnier et al. (2016)
CD44, CD29, CD90	MHC-II, CD45, CD34	Osteogenic, Adipogenic, Chondrogenic	Cabon et al. (2019)
<i>Umbilical cord MSCs</i>			
CD29, CD33, CD44, CD105, CD184, Oct4	CD4, CD8a, CD10, CD14, CD20, CD24, CD31, CD34, CD38, CD41a, CD45, CD49b, CD41/61, CD62p, CD73, CD90, CD133, HLA-DR	Osteogenic, Chondrogenic, neurogenic	Seo et al. (2009)
<i>Umbilical cord tissue MSCs</i>			
CD44, CD54, CD61, CD80, CD90, CD105, Flk1, HMGA2, Sox2, Nanog, Oct3/4	CD4, CD8A, CD25, CD33, CD34 and CD117	Chondrogenic	Lee et al. (2013)
Oct-4, CD44, CD184 and CD29	CD34, CD45	Osteogenic, Chondrogenic, Adipogenic, neurogenic	Uranio et al. (2011)
<i>Umbilical cord vein MSCs</i>			
CD44, CD29, CD90	CD14, CD34, CD45, CD117	Osteogenic, Adipogenic, Chondrogenic	Zuconni et al. (2010)
CD44, CD73, CD90, CD105	CD14, CD34, CD45	Neurogenic	Ryu et al. (2012)
<i>Wharton's jelly MSCs</i>			
CD90, CD105	CD3, CD11c, CD28, CD38, CD62L, CD34, CD45, CD41a	Osteogenic, Chondrogenic, neurogenic	Seo et al. (2012)
CD44, CD73, CD90, CD105	CD14, CD34, CD45	Neurogenic	Ryu et al. (2012)

AD Adipose tissue, AF Amniotic fluid, AM Amniotic membrane, BM Bone marrow, DP Dental pulp, End Endometrium, LE Limbal epithelium, Li liver, MSC Mesenchymal stem cell, Mu Muscle, OE Olfactory epithelium, Om Omentum, Ov Ovary, PL Periodontal ligament, Pe Periosteum, PB Peripheral blood, Pl Placenta, Sy synovium, UCB Umbilical cord blood, UCT Umbilical cord tissue, UCV Umbilical cord vein, WJ Wharton's jelly

11.2.3 Characterization of dMSCs

The characterization criteria followed in dMSCs is similar to that of human including plastic adherence, surface marker expression and differentiation potential (Dominici et al. 2006). dMSCs show plastic adherence and appear morphologically as spindle-shaped fibroblasts, although morphologies resembling slender and/

elongated to cuboidal shapes too have been demonstrated (Huss et al. 2000; Csaki et al. 2007; de Bakker et al. 2013).

11.2.3.1 Expression Profile of dMSCs

dMSCs in general express various surface markers as per the laid down criteria except for few surface markers as detailed in Table 11.1. dMSCs show plasticity and variably express pluripotency markers depending upon their tissue source as enlisted in Table 11.1. dBM-MSCs and dAD-MSCs had comparable expression of Oct2/4 and Sox2 although Nanog expression was 2.5 times higher in the latter as compared to the former cells (Takemitsu et al. 2012). Such an expression remains comparable in foetal adnexa MSCs at various stages of gestation (Uranio et al. 2014). One study on placental MSCs, however, had failed to demonstrate expression profile of some of the pluripotency markers like Oct3/4 and Nanog (Saulnier et al. 2016). The basis of these differences needs to be understood as it may have bearing on in vivo therapeutic results.

11.2.3.2 dMSCs Differentiation Potential

dMSCs undergo recommended tri-lineage differentiation involving osteogenic, adipogenic and chondrogenic. In two studies MSCs adipogenic differentiation had failed to occur (Neupane et al. 2008; Seo et al. 2009) but was effectuated in presence of rabbit serum (Neupane et al. 2008). Since dMSCs express pluripotency markers, extended differentiation potential into neural cell-, myocyte-, keratinocyte-, endothelial cell- and primordial germ cell-like cells too has been established (Table 11.1).

11.2.4 Comparative Source Based dMSC Characteristics

dMSCs from various sources show variable characteristics. Dog endometrium-(dEnd-) MSCs had been demonstrated to have lower replicative potential as compared to dAD-MSCs (De Cesaris et al. 2017). Surface marker expression may not be similar in all the cases. Among various surface markers consistent expression of CD13 and CD105 has been demonstrated from different tissue sources. Other important surface marker (CD73) has also been consistently expressed barring a single study (Vieira et al. 2010). A study on dog peripheral blood MSCs (dPB-MSCs) had reported partial expression of haematopoietic markers (CD34) and could be attributed to their dynamic shift in blood (Huss et al. 2000). Overall expression of dMSCs derived from bone marrow, synovium and adipose tissue had been comparable except for CD105 expression that was more in adipose tissue (AD-) MSCs and synovial (Sy-) MSCs.

Osteogenesis had been significantly higher in Sy-MSCs and BM-MSCs while pellet size in chondrogenic medium was significantly more in AD-MSCs and Sy-MSCs (Bearden et al. 2017). Other study had shown higher chondrogenic

potential of dSy-MSCs than dBM-MSCs and MSCs derived from inguinal and infrapatellar fat pad (Sasaki et al. 2018). Between dBM-MSCs and dLi-MSCs, chondrogenic potential had been higher in former while liver specific genes were better expressed in the latter (Malagola et al. 2016). Among various sources like adipose tissue, bone marrow, umbilical cord and Wharton's Jelly, dAD-MSCs had higher potential to proliferate. Vascular endothelial growth factor expression (VEGF) had been higher in dBM-MSCs. At early phase dAD-MSCs and dog umbilical cord blood- (dUCB-) MSCs had greater ability of osteogenesis, however, comparable osteogenesis was demonstrated in all these cell lines (Kang et al. 2012). From all these studies it can be inferred that tissue source plays an important role in MSCs proliferation and differentiation properties. Musculoskeletal tissue (synovium and bone marrow) derived MSCs may be preferable for musculoskeletal tissue regeneration.

Location of the particular tissue may also affect MSCs properties. Omentum and subcutaneous adipose tissue derived AD-MSCs had comparable trophic, vasculogenic and immuno-modulatory properties. Omentum, however, had significantly higher cell yield as compared to others (Bahamondes et al. 2017). dMSCs from subcutaneous fat had shown multipotent differentiation up to passage 8 while such a potential of MSCs from visceral (periovaric) fat had been only up to passage 4. The former cell type additionally had higher osteogenic expression (Yaneselli et al. 2018). Among various BM-MSCs sources ilium derived cells had been most proliferative (Volk et al. 2012). Mandible MSCs may carry higher cell proliferation in comparison to the femur (Bugueño et al. 2017). However, considering factors like collection technique and cell characteristics, humerus had been considered to be better source (Laura et al. 2008).

11.2.5 Effect of Donor Age on dMSCs

Ageing may reduce dMSCs proliferation and differentiation potential. MSCs from aged dog bone marrow had a significant (40%) reduction in proliferation potential, in addition to the osteogenic potential as compared to young ones (Volk et al. 2012). Other similar study had demonstrated young donor AD-MSCs carried higher population doubling potential as compared to those of aged dog MSCs. These cells had also expressed higher surface and pluripotency markers (Lee et al. 2017). Other study though had reported comparable osteogenic gene and fibroblast growth factor-10 expression in young and aged dMSCs but tumour necrosis factor and interferon gamma expression had increased in aged dAD-MSCs (Taguchi et al. 2019). Thus, available environment may affect dMSCs expression potential.

11.2.6 Effect of Drugs

dMSCs may be affected by steroids (Marycz et al. 2014; Guo et al. 2015; Jo et al. 2017) and vitamin C (Guo et al. 2015). Low dose steroids may improve dMSCs

activity while higher doses might be cytotoxic (Marycz et al. 2014). Vitamin C may also improve dMSCs proliferation (Guo et al. 2015).

11.2.7 Breed Variability and MSCs

Dog breeds may vary in their genetics and are expected to show some cellular differences including of MSCs. Most of the breeds including German shepherd, Labrador, Golden retriever, Border collie and Malinois had comparable initial BM-MSCs population except one (Hovawart) that had much lower cell population. MSCs from these breeds (German shepherd, Labrador, Golden retriever and Border collie), however, had undergone early senescence. The cellular differentiation potential in these breeds may also vary (Bertolo et al. 2015).

11.3 In Vivo Potential Therapeutic Applications

MSCs are increasingly being utilized in dog clinical practice. dMSCs have been studied in various types of musculoskeletal and non-musculoskeletal tissues (Tables 11.2, 11.3, 11.4, 11.5, 11.6, 11.7, 11.8, 11.9, 11.10, 11.12, and 11.13) (Gugjoo et al. 2019a). The in vivo studies have largely supported dMSCs therapeutic potential (Hall et al. 2010; Sousa et al. 2011; Kim et al. 2011; Cuervo et al. 2014; Muir et al., 2016). Many of these studies have failed to report any major adverse effect (Cuervo et al. 2014; Bęrzioń et al. 2018; Villatoro et al. 2018) while some have reported adverse reactions like self-controllable localized inflammation and pulmonary parenchymal oedema and haemorrhage (Mokbel et al. 2011; Park et al. 2012a, b; Kang and Park 2014).

In vivo MSCs viability remains important for their effectiveness. MSCs in vivo implantation exposes them to noxious environment. As such the scaffold seeded cells are being implanted. dAD-MSCs encapsulated in alginate beads had improved retention and the cells were viable up to 7 days (Koh et al. 2017). The studies described in this chapter range from preclinical experimental models to the clinical trials. The clinical studies described are mostly uncontrolled and have been conducted as case report, case series and/or comparative studies.

11.3.1 Musculoskeletal Tissues

11.3.1.1 Osteoarthritis

Osteoarthritis (OA) affects larger dog population (20%) (Johnston, 1997). Currently, no successful clinical treatment is available (Gugjoo et al. 2016; Gugjoo et al. 2017; Li et al. 2018; Gugjoo et al. 2019b). As such stem cell applications in dog are being evaluated under experimental settings (Table 11.2) and in also clinical trials (Table 11.3).

Table 11.2 In vivo chondrogenic preclinical experimental mesenchymal stem cell studies in dogs

Model type	Number of animals	Model defect size/Study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Partial thickness chondral defects of lateral femoral condyle	32 (8 control; 12 group II; 12 group III)	3 mm diameter and 1 mm depth/8 weeks	Autologous BM-MSCs	1.4–1.6 × 10 ⁶	Morphological, histological and fluorescence analysis	Recovery was significant both clinically and histologically in the two cell treated groups (group II and III) as compared to the control (group I). In the meantime, group II showed better results at 8 weeks than group III. Homing was confirmed by the incorporation of injected GFP-labelled MSCs within the newly formed cartilage	Mokbel et al. (2011)
Osteochondral defects of medial femoral condyles	Group I: Cell + scaffold; group II: Control	4.2 mm diameter and 6 mm depth/3 months and 6 months	Chondrogenically-induced BMSCs and scaffold	–	Gross morphology and by histological, biochemical, biomechanical and micro-CT analyses	Statistically significant improvement in gross and histology and cartilage stiffness in cell/scaffold treated animals as compared to control. Comparable micro-CT analysis of the subchondral bone in two groups. Better results in later period than at early period	Yang et al. (2011)

(continued)

Table 11.2 (continued)

Model type	Number of animals	Model defect size/Study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Osteonecrosis of the femoral head	24 (54 hip joints) group I: Transgenic BM-MSCs; group II: BM-MSCs group III: Control	2 mm diameter and 2 mm width/12 weeks	VEGF 165 transgenic bone marrow mesenchymal stem cells or simple BM-MSCs	2×10^7	Radiography, single-photon emission, computed tomography, histopathology, histomorphometric analysis and immunofluorescent staining for von Willebrand factor	Better results in group I as compared to group II and group III. A regular arrangement of trabeculae and obvious bone regeneration with significantly increased capillaries in group I animals	Hang et al. (2012)
Osteochondral defects	12 (24 (defects) group I: Cell + PRF treated; group II: Control	6 mm diameter and depth of 5 mm/4–24 weeks	Autologous BM-MSCs + platelet rich fibrin (PRF)	1×10^6	Macroscopic and histopathology	Group I had statistically significant improvement in gross and histological parameters as compared to control at 16 weeks. Compared to cell treated group gross parameters were deteriorated with time in control group animals	Kazemi et al. (2017)

Chondral defects of stifle joint	24 (48 defects) group I: Cell + HA treated; group II hyaluronic acid; group III: Control	4 mm/28 weeks	BM-MSCs + hyaluronic acid (HA)	1×10^7	Macroscopy, magnetic resonance imaging (MRI), histopathology, immunohistochemistry for type II collagen	Group I had regenerated more cartilage-like tissue than did HA alone or saline. Macroscopic evaluation and histological assessment score were significantly improved in cartilage defects of group I as compared to those in the other groups. MRI demonstrated cartilage-like signal at defect sites, and the surface of cartilage was relatively smooth. No obvious defect was found and cartilage-like tissue with the same thickness of the surrounding normal tissue was formed in group I. For group II, cartilage-like signal was also observed, but the thicknesses of tissue at the defect sites were thinner than those at normal. For group III, no cartilage-like signal was observed	Li et al. (2018)
----------------------------------	--	---------------	--------------------------------	-----------------	---	---	------------------

Table 11.3 In vivo chondrogenic clinical mesenchymal stem cell studies in dogs

Clinical condition/ ailment	Number of animals included	Study period	Study type	Cell source	Cell dose	Evaluation criteria	Overall result	References
Chronic osteoarthritis of Coxo-femoral joint	21	90 days	Case series (randomized, double blinded, placebo- controlled trial)	Autologous AD-MSCs	5×10^6 (20 dogs) and 4.5×10^6 (1 dog)	Orthopaedic examination scores, lameness and composite scores and size effect	Statistically significant improvement in scores for lameness and the compiled scores for lameness, pain and range of motion as compared to control dogs	Black et al. (2007)
Chronic osteoarthritis of humero-radial joint	14	180 days	Case series (randomized, double blinded, non-placebo- controlled trial)	Autologous AD-MSCs	$3-5 \times 10^6$	Orthopaedic examination score and size effect	Statistically significant improvement in lameness, pain on manipulation, range of motion and functional disability in cell treated animals	Black et al. (2008)
Chronic osteoarthritis of humero-radial joint	4	30 days	Case series (uncontrolled study)	Autologous AD-MSCs	$3-5 \times 10^6$ (laden in platelet rich plasma or hyaluronic acid)	Clinical tests like trot pain on palpation and functional improvement in disability	Improvement with time as per owner although without any statistical testing	Guercio et al. (2012)
Chronic arthritis of the hip joint	8	180		Autologous AD-MSCs	15×10^6	Force platform analysis	Significant improvement in clinical parameters in cell treated cases	Vilar et al. (2013)

Hip osteoarthritis	39 (autologous AD-MSCs vs PRGF)	180 days	Randomized comparative clinical trial	Autologous AD-MSCs and PRGF	30×10^6	Visual analog scale (VAS), bioearth scale assessment, range of motion (ROM), radiography	MSCs and PRGF implantation was safe and effective in the functional analysis at 1, 3 and 6 months; provide a significant improvement, reducing dog's pain, and improving physical function. With respect to basal levels for every parameter in patients with hip OA, MSCs showed better results at 6 months	Cuervo et al. (2014)
Hip dysplasia	9 (group I: SVF, $n = 4$; group II: AD-MSCs; $n = 5$)	30 days	Comparative study	Allogeneic AD-MSCs at acupoints or stromal vascular fraction (SVF)	$0.2-0.8 \times 10^6$	Physical and orthopaedic examinations	Positive results were more clearly seen in the SVF-treated group. All dogs had shown improvement in range of motion, lameness at trot and pain on manipulation of the joints, except for one ASC-treated patient. These results had shown that autologous SVF or allogeneic ASCs can be safely used in acupoint injection for treating hip dysplasia in dogs and represent an important therapeutic alternative for this type of pathology	Marx et al. (2014)

(continued)

Table 11.3 (continued)

Clinical condition/ ailment	Number of animals included	Study period	Study type	Cell source	Cell dose	Evaluation criteria	Overall result	References
Chronic arthritis of the hip joint	15 (group I: MSCs treated, $n = 10$; sound dogs, $n = 5$)	180 days	Case control (blinded control study)	Autologous AD-MSCs	15×10^6	X-ray and platform gait analysis: Mean values of peak vertical force (PVF) and vertical impulse (VI)	Mean values of PVF and VI were significantly improved within the first 3 months cell post- treatment in the OA group, increasing 9% and 2.5% body weight, respectively, at day 30. After this, the effect seems to decrease reaching initial values	Vilar et al. (2014)
Osteoarthritis of hip, elbow, stifle, and or shoulder joints	82 (bilateral joints: Treated, $n = 35$; placebo control, $n = 32$) and (single joint: Treated: $n = 8$; placebo control, $n = 7$)	60 days	Case series study (prospective, randomized, masked and placebo- controlled)	Allogeneic AD-MSCs	12×10^6 (cryopreserved with 85.1 % viability)	Owner client-specific outcome measurement (CSOM) and secondary measures included veterinary pain on manipulation, veterinary global score, and owner global score	Success in the primary outcome was variable. CSOM was statistically improved in the treated dogs compared to the placebo dogs. The veterinary pain on manipulation score and the veterinary global score were both statistically improved in treated dogs as compared to placebo	Harman et al. (2016)

Elbow dysplasia and elbow osteoarthritis	30 dogs (39 elbow joints)	1 year	Case series (uncontrolled study)	Allogeneic AD-MSCs + hyaluronic acid (0.5%)	$12 \times 10^6 \pm 3.2 \times 10^6$ cells	Owner and veterinarian examination, arthroscopy and histopathology	A highly significant improvement was achieved after cell implantation without any medication as demonstrated by the degree of lameness during the follow-up period. Control arthroscopy of 1 transplanted dog indicated that the cartilage had regenerated. Histological analysis of the cartilage biopsy confirmed that the regenerated cartilage was of hyaline type	Kriston-Pál et al. (2017)
Hip, knee, radiocarpal, intercarpal, elbow and ulnar osteoarthritis	10 patients ($n = 5$ evaluated for 90 days and $n = 5$ for 1–4 years)	30–90 days/4 years (5 animals)	Case reports	Autologous AD-MSCs	3×10^7	Physical examination and assessment for lameness, pain on manipulation, range of motion of the joint and functional disability	Functional outcomes for all analysed characteristics improved significantly at the end of this evaluation compared with the baseline. Long-lasting positive effects on two out of five analysed characteristics	Dražilov et al. (2018)

(continued)

Table 11.3 (continued)

Clinical condition/ ailment	Number of animals included	Study period	Study type	Cell source	Cell dose	Evaluation criteria	Overall result	References
Degenerative arthritis	203 (divided into groups as per age, weight)	10 weeks	Controlled clinical study	Allogeneic AD-MSCs	128 received a single dose of intra-articular injection in the affected joint. 65 dogs received a single intravenous injection, and 10 dogs received both IA and IV injections	Quality of life score (QoL), lameness and pain score	Ninety percent of young dogs (<9 years) had shown excellent improvement in pain and mobility and were able to run and resume normal activity. Sixty percent of older dogs showed good improvement. However, 12% of dogs did not exhibit any change in symptoms; one dog showed worsening of the symptoms	Shah et al. (2018)
Osteoarthritis (hip, knee, elbow and tarsal joints)	22	6 months and 2 years	Non- randomized, open and monocentric study	Allogeneic MSCs	10×10^6 (repeated injection after 6 months in 8 dogs or 11 joints)	Clinical evaluation, flow Cytometric Crossmatch analysis of Humoral response against cellular product	Veterinary clinical evaluation showed a significant and durable clinical improvement (up to 6 months) following MSC administration. Eight dogs (11 joints) were re-injected 6 months apart, sustaining clinical benefits up to 1 year. Owner's global satisfaction reached 75% at 2 years post-treatment	Cabon et al. (2019)

Elbow osteoarthritis	6	6 weeks	Case control	Xenogenic chondrogenic induced PB-MSCs (equine source)	1×10^6 cells	Orthopaedic examination, synovial fluid examination, radiography	There was no significant difference in the orthopaedic examination parameters, the radiographic examination, synovial fluid sampling and pressure plate analysis between placebo and MSC treatment. A single intra-articular administration of MSCs proved to be a well-tolerated treatment, which reduced lameness and pain according to the owner's evaluations as compared to a placebo treatment	Daems et al. (2019)
----------------------	---	---------	--------------	--	-----------------------	--	--	---------------------

In these studies a single dose of dMSCs has been injected locally into joints except for a study that had implanted cells more than once (Cabon et al. 2019). One study had utilized acupoint as site of implantation (Marx et al. 2014). Either only cells have been utilized (Marx et al. 2014; Vilar et al. 2014) or along with platelet rich plasma/fibrin (Vilar et al. 2013; Kazemi et al. 2017), biphasic scaffold (Yang et al. 2011) or hyaluronic acid (Guercio et al. 2012; Kriston-Pál et al. 2017; Li et al. 2018). Even chondrogenically differentiated (Yang et al. 2011) or vascular endothelial growth factor transduced dMSCs have also been evaluated (Hang et al. 2012). In general, these studies have variably supported therapeutic applications of dMSCs.

Variable evaluation parameters have been undertaken in these studies. Variable follow-up period ranging from as low as 1 month (Guercio et al. 2012; Marx et al. 2014) to a maximum period of 5 years (Yoon et al. 2012) had been conducted. Therapeutic evaluation of dMSCs has been made on the basis of clinical evaluation parameters like Pain scales, and MRI, CT scan and histological examination. Quantitative Force Platform Gait Analysis tends to have greater accuracy and concordance for assessment of AO lameness as compared to pain assessment scales (Vilar et al. 2014). Improvement on the basis of clinical parameters, arthroscopic evaluation and histology has been demonstrated (Kriston-Pál et al. 2017). The histological scores of healed tissues in MSCs-treated groups were higher as compared to control but complete hyaline tissue was lacking. The healed tissues had been mixed fibrocartilage and/hyaline lacking complete integration to the native cartilage (Yang et al. 2011; Kazemi et al. 2017). Some of the studies, however, had failed to demonstrate significant improvement (Daems et al. 2019) and even some failed to demonstrate long-lasting improvement with MSCs applications (Vilar et al. 2014).

Several comparative studies have also been conducted. dAD-MSCs had been compared to platelet rich growth factors (PRGF) (Cuervo et al. 2014), stromal vascular fraction (SVF) (Marx et al. 2014), in addition to the VEGF transgenic dBM-MSCs against simple BM-MSCs. MSCs though had provided better outcome as compared to PRGF but not in comparison to SVF. SVF also contain growth factors and as such might have improved osteochondral defect healing (Kazemi et al. 2017). VEGF transgenic dBM-MSCs had better osteochondral healing potential as compared to simple MSCs (Hang et al. 2012). There is need for studies that compares dMSCs to the currently available surgical techniques.

11.3.1.2 Osteogenic Defects

Bone usually heal fast but may sometimes be hampered, resulting in delayed union and/non-union. Varied conditions like infection, extensive damage, old age, tumours and/or osteoporosis impair bone healing. Dog fracture non-union rate remains at 3.4% of the total bone fractures (Millis and Jackson, 2003).

MSCs have been studied in various dog preclinical experimental models (Table 11.4) and in clinical cases (Table 11.5). MSCs have been implanted along with various scaffolds like β -TCP (Arinzech et al. 2003), β -TCP/poly

Table 11.4 In vivo Preclinical experimental osteogenic mesenchymal stem cell studies

Model type	Number of animals	Model defect size/study period	Study type	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Critical size defect of femora	15 (n = 6 in cell treated and n = 6 ceramic treated and 3 in control)	21 mm length/4–16 weeks	Comparative experimental study (treatment group Vs porous ceramic cylinder treated group Vs control)	Autologous BM-MSCs + porous ceramic cylinder containing hydroxyapatite (65%) and beta-tricalcium phosphate ceramic (35%)	–	Histology and morphometry, radiography	Atrophic non-union occurred in untreated defects. Only a small amount of trabecular bone formed at the cut ends of the cortex of the host bone in this group. In contrast, radiographic union was established rapidly at the interface between the host bone and the implants in cell loaded. Histological and morphometric analyses demonstrated that both woven and lamellar bone had filled the pores of the implants that had been loaded with mesenchymal stem cells. The amount of bone was significantly greater than the pores of the implants that had not been loaded with cells. The collar of bone remodelled during the sixteen-week period of study, resulting in a size and shape that were comparable with those of the segment of bone that had been resected. Callus did not develop around the cortex of the host bone or around the defect in any of the specimens in the other two groups.	Bruder et al. (1998)

(continued)

Table 11.4 (continued)

Model type	Number of animals	Model defect size/study period	Study type	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Critical size defect of femoral diaphysis	12	21 mm length/16 weeks	Comparative experimental study (allogeneic MSCs + beta-tricalcium phosphate vs autologous MSCs vs control)	Allogeneic MSCs + beta-tricalcium phosphate	–	Histology and radiography	Defects treated with allogeneic mesenchymal stem cell implants showed no adverse host response. Histologically, no lymphocytic infiltration occurred and no antibodies against allogeneic cells were detected. Histologically, by eight weeks, a callus spanned the length of the defect, and lamellar bone filled the pores of the implant at the host bone-implant interface. Fluorescently labelled allogeneic cells were also detected. At sixteen weeks, new bone had formed throughout the implant. These results were consistent with those seen in implants loaded with autologous cells. Implants loaded with allogeneic or autologous stem cells had significantly greater amounts of bone within the available pore space than did cell-free implants at 16 weeks	Arinze et al. (2003)

Radial critical size defect	6 ($n = 3$ in treatment group and $n = 3$ in control)	1.5 cm/12 weeks	Experimental study (allogeneic UCB-MSCs + beta-tricalcium phosphate Vs control)	Allogeneic UCB-MSCs + beta-tricalcium phosphate covered with β -TCP/poly L-lactide-co- ϵ -caprolactone composite supported with compression plate	1×10^6	Radiography, histology	Radiographically, continuity between implant and host bone interfaces in control by 12 weeks, but in three of six interfaces in cell treated group. Radiolucency was found only near the bone end in control group at 12 weeks after implantation, but in the entire graft in cell treated group. Histologically, bone formation was observed around beta-TCP in longitudinal sections of implant in both groups. Histomorphometric analysis revealed significantly increased new bone formation in cell treated group at 12 weeks after implantation.	Jang et al. (2008)
Critical size ulnar bone defect	9 (18 forelimb defects)	2.5 cm/12 weeks	Comparative study (groups of different BM-MSC concentration + rBMP concentration)	BM-MSCs + rBMP (E. coli)	560 $\mu\text{g}/10^7$ cells, 560 $\mu\text{g}/10^5$ cells, 560 $\mu\text{g}/0$ cells, 140 $\mu\text{g}/10^7$, 140 $\mu\text{g}/10^5$, 140 $\mu\text{g}/0$	Radiography, micro-CT, histological	Enhanced bone formation, with improved bone mineral density and reduced non-uniformity of the regenerated bone in cell and BMP treated group. Higher cell and growth factor concentration in general improved healing. Micro-CT confirmed bone mineral density was improved in the groups that received cells (10^7 or 10^5) along with the 560 μg of rBMP. The non-uniformity of the bone mineral density was improved in animals that received 560 μg rBMP with 10^7 and 10^5 cells and 140 $\mu\text{g}/10^7$ cells.	Itoi et al. (2016)

Table 11.5 In vivo clinical osteogenic mesenchymal stem cell studies

Clinical case	Number of animals included	Study period	Study type	Cell source	Cell dose	Route	Evaluation criteria	Overall result	References
Radial fracture with sclerotic ends	1	90 days	Case report	Autologous AD-MSCs + hydroxyapatite and chitosan	3.2×10^7	Local implantation + compression plate	Radiography and lameness	The composition scaffold of HA containing ADSCs had induced new bone formation	Lee et al. (2009a)
Radial non-union fracture	1	8 weeks	Case report	Umbilical cord MSCs + cortical allograft	1×10^7 repeated after 1 month	Local implantation	Radiography	Fracture healing was identified by 6 weeks after cell injection. Increased opacity between radius and allograft bone, and a decreased radiolucent gap was checked. With time, the radiolucent site gradually decreased and the radiolucent fracture line disappeared	Jang et al. (2008)
Oligotrophic non-union left radial fracture	1 dog	9 weeks	Case report	Bone morphogenetic protein-7 AD-MSC sheet	1×10^4 cells/cm ²	Local implantation + locking plate	Lameness and radiography	Completely healed fracture	Song et al. (2017)
Tibial tuberosity osteotomy	9 (control, $n = 5$; cell treated, $n = 4$)	15, 30, 60 and 120 days	Double-blind experimental study	MSCs	1.5×10^6	Local application	Radiography, image analysis software	Cell treated group had significant ossification mean 36.45% greater than the control at 30 days	dos Santos et al. (2018)

L-lactide-co- ϵ -caprolactone (Jang et al. 2008), coral scaffold (Cui et al. 2017) and porous ceramic cylinder (Bruder et al. 1998). All these studies have demonstrated improved bone density with uniform growth of regenerated tissue upon dMSCs implantation. Concentration of the cells and of the growth factor (rBMP) used too may have effect on bone healing (Itoi et al. 2016). Contrary to *ex vivo* reports, dMSCs from varied sources had provided comparable *in vivo* osteogenesis (Kang et al. 2012).

Apart from experimental studies, clinical application of MSCs too has supported their role in enhancement of healing. BMP-7 transgenic dAD-MSC sheets implanted in a clinical oligotrophic non-union radial fracture and autologous AD-MSCs in combination with hydroxyapatite and chitosan implanted in a radial fracture with sclerotic ends had complete healing in about 9 weeks (Song et al. 2017) and 12 weeks (Lee et al. 2009a), respectively. All these studies have utilized MSCs along with variable scaffolds and/growth factors. There is need to perform comparative uniform studies incorporating various scaffolds and growth factors. Besides, these studies have to be compared against the current surgical treatment options for their feasibility and effectiveness.

11.3.1.3 Periodontal Defects

Periodontitis has limited outcome with currently available regenerative therapies (guided tissue regeneration and enamel matrix derivatives). These techniques are associated with poor clinical predictability (Esposito et al. 2009). To address the issue, stem cell application for periodontal regeneration is currently being evaluated (Table 11.6).

In experimental studies, dMSCs have improved periodontal defect repair, although variably. The *in vivo* implanted cells had migrated to these structures and showed differentiation into osteoblast- and fibroblast-like cells (Akizuki et al. 2005; Wei et al. 2010), and odontoblast-like cells (Shi and Gronthos, 2003). MSCs from various sources have been utilized (Table 11.6). dMSCs from alveolar periosteum, bone marrow and periodontal ligament (PDL) had variably improved healing. PDL-MSCs had led to the significant periodontal regeneration in comparison to the other cell types. The healed tissue with PDL-MSC implantation included well-oriented PDL fibres, regenerated nerve, cementum and alveolar bone (Tsumanuma et al. 2011). To improve cell survival and effectivity, MSCs along with scaffold matrices and osteo-inductive material had successfully regenerated the tissue in furcation defects (Takewaki et al. 2017). Further, cryopreserved MSCs had comparable periodontal healing potential to that of fresh MSCs (Li et al. 2009).

These model studies evaluated MSCs in variable classes of furcation defects. MSCs concentration, passage number, concentrations and sources has been variable in these studies. Addition of scaffolds and growth factors too makes these studies non-uniform.

Table 11.6 In vivo preclinical experimental mesenchymal studies on periodontal regeneration

Model type	Number of animals	Model defect size/ study period	Study type	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Periodontal fenestration defects	Five dogs (26 teeth)	5 mm × 5 mm (apical to the cemento-enamel junction)/8 weeks	Comparative experimental study (fresh BM-MSCs Vs cryopreserved BM-MSCs)	Freshly isolated and cryopreserved BM-MSCs seeded on collagen scaffold	5×10^6	Histological and histomorphometric parameters	Both freshly isolated and cryopreserved BMSC transplants induced significantly better periodontal regeneration with newly formed cementum, alveolar bone and periodontal ligament as compared to collagen scaffold applied cases.	Li et al. (2009)
Class II furcation defects	7 (control group: Coronally positioned flap, bioabsorbable membranes: GTR, sponge group: Carrier + GTR, cell group: Carrier + PDL cells + GTR	6 months	Experimental study (cell treated Vs control)	Autologous periodontal ligament derived MSCs		Histometric parameters, scanning electron microscopy (SEM)	PDL cells had significantly promoted periodontal regeneration. Cell group presented a superior length of new cementum, a greater extension of periodontal regeneration and a larger area of new bone as compared to sponge, GTR and control group, respectively	Suaid et al. (2011)

One-wall intrabony defect	4	5 mm in depth, 5 mm mesio-distal width/8 weeks	Comparative study (PDL-MSCs Vs alveolar periosteum MSCs (AP) Vs BM-MSCs)	Autologous cells in concerned groups	3–5 × 10 ⁴ cell sheets	Histological and histometric parameters	Significant periodontal regeneration (Cementum and well-oriented PDL fibres) in the PDL-MSCs group than others. Nerve regeneration and alveolar bone formation are also better in PDL-MSC group.	Tsumanuma et al. (2011)
Class II furcation defects	3	5 mm (apico-occlusal direction) and 2 mm (buccolingual direction)/8 weeks	Comparative experimental study (BM-MSCs + PRP Vs PRP Vs control)	Autologous BM-MSCs + PRP	1 × 10 ⁷	Histomorphometric analysis and histological analysis	Periodontal regeneration with complete filling of class II furcation defects with cementum, alveolar bone and periodontal ligament all cases healed equally.	Simsek et al. (2012)
Class III furcation defects	7	5.0 mm in the apico-occlusal direction/3 months	Comparative study (PDL-MSCs + sponge carrier vs sponge treated vs control)	Autologous periodontal ligament derived MSCs + carrier + sponge	3 × 10 ⁵	Histomorphometrically	PDL cells with sponge had significantly promoted periodontal regeneration	Suaid et al. (2012)

(continued)

Table 11.6 (continued)

Model type	Number of animals	Model defect size/ study period	Study type	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
3-walled periodontal defects in first lower premolar teeth	10 (one side treated and other side untreated)	3 mm buccolingually, 5 mm apico-coronally, and 8 mm mesiodistally created defect/8 weeks	Experimental study (cell treated Vs control)	Autologous dental pulp MSCs and bio-Oss granules after 4 weeks	2×10^7	Histologically and histomorphometrically	Cementum regeneration and periodontal ligament regeneration significantly higher in treated group than control	Khorsand et al. (2013)
Class III periodontal tissue defects	8	5 mm (height from the top of the dental root bifurcation to the reduced alveolar crest in the class III defect) /2 months	Comparative experimental study (cell + PRP vs PRP Vs non treated groups)	Autologous AD-MSCs and PRP	1.5×10^7	Histologic, immuno-histologic and radiography	Periodontal tissue with the correct architecture including alveolar bone, cementum-like structures and periodontal ligament-like structures regenerated in cell and PRP treated cases	Tobita et al. (2013)
Premolar class III furcation defect	7 (24 right and left premolar defects)	Height from the cement-enamel junction to the reduced alveolar crest was 4 mm/12 weeks	Experimental study (cell treated Vs control)	Autologous BM-MSCs and osteogenic induced BM-MSCs	7×10^4 cell sheet	Micro-computed tomography, histological analysis and morphometric analysis	Successful periodontal tissue regeneration with BM-MSCs as compared to control. Rapid alveolar bone formation with osteogenic differentiated BM-MSCs	Takewaki et al. (2017)

11.3.1.4 Ligaments/Tendon/Muscle Injuries

Rupture of cranial cruciate ligament (CCL) causes hind limb lameness in dog (Johnson et al. 1994). Currently, no specific and effective treatment is available and as such MSCs are being evaluated (Table 11.7). Intra-articularly implanted dBM-MSCs tend to engraft CCL (Linon et al. 2014). An overall improvement, although variably has been demonstrated with MSCs (Canapp Jr. et al. 2016; Muir et al. 2016). A retrospective study had reported an improvement with AD-MSCs and BM-MSCs along with platelet rich plasma on partial cranial cruciate ligament rupture. Available arthroscopic findings had represented fully healed CCL (marked neo-vascularization and a normal fibre pattern) in the disrupted areas in most of the cases (Canapp Jr. et al. 2016). Further, MSCs implanted in a tibial plateau levelling osteotomy (TPLO) treated CCL had significantly improved bone healing at 1 month period in comparison to non-steroidal anti-inflammatory drugs (NSAIDs) treated ones. However, the improvement could not be maintained for long as non-significant differences in clinical parameters at later periods (up to 3 months) had been demonstrated (Taroni et al. 2017). Further extensive studies of longer follow-up periods are therefore desired.

MSCs may impart clinical healing either by fibroblastic differentiation or by the release of trophic factors (Yoon et al. 2012). MSCs like AD-MSCs may modulate tendon inflammation by increasing M2 macrophages, inhibiting inflammation (prostaglandin reductase 1). The cells may introduce progenitor cell, reduce mononuclear cell infiltration to the damaged site and increase pro-tenogenic proteins (periostin). Incorporation of recombinant bone morphogenetic protein-12 (rBMP-12) may further potentiate these effects possibly through induction of more activated M2 macrophages (Gelberman et al. 2017).

Athletic dogs undergo frequent muscle injuries (Lee et al. 2012). Muscle lack innate ability to regenerate. As MSCs tend to differentiate into myocyte like cells under ex vivo studies they have prospect to repair muscles under in vivo system. MSCs implanted into the incised infraspinatus tendon tend to align between its collagen fibres. These aligned cells may express higher tenomodulin, MMP13 and collagen type I in the composite (Omae et al. 2009). In dog clinical cases, these cells have promoted muscle repair and prevented fibrosis. The treated animals were able to work being characterized by improved functional gait and lack of lameness (Brown et al. 2012; Case et al. 2013; Gibson et al. 2017). Ultrasonographic examination, however, had demonstrated lack of proper arrangement of gastrocnemius muscle fibres (Case et al., 2013). Muscle derived MSCs may also improve muscular dystrophy. The implanted cells in immune-suppressed dogs had led to the gradual improvement of dystrophied muscles (Lorant et al. 2018).

11.3.1.5 Myocardial Diseases

Dilated cardiomyopathy is one of the commonly seen cardiac ailments in dogs. AD-MSCs application in occult-dilated cardiomyopathy cases had been safe but without any observable clinical benefits (Pogue et al. 2013). Further studies are

Table 11.7 In vivo clinical mesenchymal stem cell studies on tendon/ligament/muscle injuries

Clinical case	Number of animals included	Study period	Study type	Cell source	Cell dose	Route	Evaluation criteria	Overall result	References
Chronic Chagas cardiomyopathy	5	6 months	Case series	Autologous BM-MSC	100×10^6	Right and left coronary artery implantation	Electrocardiography and echocardiography	Significant improvement in cardiac function (peak velocity of aortic flow) after cell implantation	Sousa et al. (2011)
Semitendinosus myopathy	2	19 weeks (case 1) and 22 weeks (case 2)	Case report	Autologous AD-MSCs	3.7×10^6	Local implantation	Ultrasonography, muscle biopsy, visual assessment of dog's improvement in affected tasks and surgeon assessments	AD-MSCs therapy enhanced muscle healing and prevented fibrosis in these clinical cases, similar to the reported literature in laboratory animals. The dogs returned to their previous training and occupations with a functional gait and no lameness.	Brown et al. (2012)
Grade IV patellar luxation, arthritis	1	5 years	Case report	Autologous AD-MSC	1×10^6 + hyaluronic acid	Local implantation	Lameness and radiography	Normal walking and reduced osteophytes and osteochondral lesions had been reported after cell implantation.	Yoon et al. (2012)

Gastrocnemius tendon injury	1	631 days	Case report	Autologous BM-MSC	>20 × 10 ⁶ (cryopreservation)	Local implantation	Serial orthopaedic examinations, ultrasonography and long-term force-plate gait analysis	Lameness subjectively resolved and peak vertical force increased from 43% to 92% of the contralateral pelvic limb. Serial ultrasonographic examinations had revealed improved but incomplete restoration of normal linear fibre pattern of the gastrocnemius tendon.	Case et al. (2013)
-----------------------------	---	----------	-------------	-------------------	--	--------------------	--	--	--------------------

(continued)

Table 11.7 (continued)

Clinical case	Number of animals included	Study period	Study type	Cell source	Cell dose	Route	Evaluation criteria	Overall result	References
Partial cranial cruciate ligament tear treated with TPLO	36 (<i>n</i> = 19 for BMC + PRP) and (<i>n</i> = 17 for AD-PCs + PRP)	90 days	Retrospective comparative study	Bone marrow concentrate + PRP vs AD-MSCs + PRP	2–4 mL (BMC + PRP) and 1–2 mL (AD-PCs + PRP)	Local joint implantation	Objective gait analysis, diagnostic arthroscopy, and validated functional questionnaire	Promising healing potential of these treatments was demonstrated. Arthroscopic findings of 13 cases out of 36 cases at 90 days interval were available. Nine dogs had fully healed CCL represented by marked neo-vascularization and a normal fibre pattern in the disrupted areas. Out of other four cases, one had improved healing and was subjected to another injection while rest three underwent tibial plateau levelling osteotomy	Canapp Jr. et al. (2016)

Cranial cruciate ligament rupture	12	8 weeks	Case series (case controlled clinical study)	Autologous BM-MSCs	2×10^6 and 5×10^6	Intravenous and local intra-articular	Radiography, arthroscopy, serum and synovial cytokines and C-reactive protein	No adverse events. With CD8+ T lymphocytes after cell treatment were detected. Serum C-reactive protein (CRP) was decreased at 4 weeks and serum CXCL8 was increased at 8 weeks. Synovial CRP in the complete CR stifle was decreased at 8 weeks. Synovial IFN γ was also lower in both stifles after cell injection. Systemic and intra-articular injection of autologous BM-MSCs in dogs with partial CR suppresses systemic and stifle joint inflammation, including CRP concentrations.	Muir et al. (2016)
-----------------------------------	----	---------	--	--------------------	-------------------------------------	---------------------------------------	---	--	--------------------

(continued)

Table 11.7 (continued)

Clinical case	Number of animals included	Study period	Study type	Cell source	Cell dose	Route	Evaluation criteria	Overall result	References
Semitendinosus myopathy	9 dogs (11 cases)	Short-term (6 months) and long-term (1 year) follow-up	Uncontrolled retrospective comparative clinical trial	AD-MSCs	–	Local implantation	Ultrasonography, visual assessment score (VAS),	At short-term follow-up ultrasound and gait analysis showed a mean reduction in the overall intramuscular lesion size and reduction in the visual assessment score (VAS). At long-term follow-up, in 8 cases had a normal gait and in 3 cases the dogs had an improved gait as compared with initial examination. All 8 dogs had returned to active police work.	Gibson et al. (2017)
Cranial cruciate ligament rupture	14 (9 MSCs and 5 NSAIDs)	180 days	Comparative study (blinded)	Allogeneic foetal adnexa MSCs	10 × 10 ⁶	Local implantation	Clinical score and gait and bone healing	Better healing in MSC treated animal at 1 month with comparable gait and clinical score at 6 months	Taroni et al. (2017)

Muscular dystrophy	16 (group received cells with transient immunosuppression (GRMDMU/tr-IS), <i>n</i> = 4; group received cells without immunosuppression (GRMDMU/no-IS), <i>n</i> = 4; group received no cells without immunosuppression, <i>n</i> = 3; group received no cells with transient immunosuppression, <i>n</i> = 5)	5 and 9 months	Case series	Allogeneic muscle derived MSCs + immunosuppression	6.3×10^7 to 1.2×10^8 per kg	Intrafemoral injections	Immunohistochemistry, histomorphometry, mononuclear cell proliferation, western blotting	Persistent clinical improvement of the GRMDMU/tr-IS dogs was observed. GRMDMU/no-IS dogs exhibited no benefit. Histologically, only 9-month-old GRMDMU/tr-IS dogs showed an increased muscle regenerative activity. A mixed cell reaction with the host peripheral blood mononuclear cells (PBMCs) and corresponding donor cells revealed undetectable to weak lymphocyte proliferation in GRMDMU/tr-IS dogs compared with a significant proliferation in GRMDMU/no-IS dogs	Lorant et al. (2018)
--------------------	---	----------------	-------------	--	---	-------------------------	--	---	----------------------

desired involving genetically modified cells to confirm their role in cardiac ailments, if any (Lu et al. 2013).

Dog has been used as myocardial ischemic model animal for human though the condition has little relevance in dog clinical practice. An increased vascularity and cardiac functional improvement (Memon et al. 2005; Silva et al. 2005; Bartunek et al. 2007; Perin et al. 2008) has been reported with MSCs implantation. As stem cells have self-renewal and multipotent differentiation properties, infarcted myocardium may have been repaired (Linke et al. 2005).

11.3.2 Non-Musculoskeletal Tissues

11.3.2.1 Wounds/Ulcers

Numerous MSCs studies are being conducted to evaluate the healing potential of MSCs in wound and ulcer. In dog MSCs have been employed in management of wounds (Tables 11.8 and 11.9), and ulcers (Table 11.8). Even dAD-MSCs may suffice as canine dermal papillae substitutes (Bae et al. 2015). Local implantation of the MSCs like BM-MSCs appears more effective in induced oral ulcers as compared to the systemic implantation (Aly et al. 2014).

An extensive study on full thickness wound model had shown that implantation of allogeneic BM-MSCs of various concentrations (1×10^4 , 1×10^5 , 1×10^6 and 1×10^7) had led to wound epithelial regeneration by day 7. Although, collagen was higher in healed wounds implanted with highest cell concentration but was statistically insignificant. Further, pro-healing factor like bovine fibroblast growth factor (bFGF) was highly expressed while inflammatory mediators like IL-2 and INF- γ were decreased at day 7 in comparison to control confirming their anti-inflammatory and anti-fibrotic role in skin wounds (Kim et al. 2013). MSCs extracellular vesicles (microvesicles) may also improve healing of wounds in dogs (El-Tookhy et al. 2017). In chronic clinical cases previously unresponsive to conventional therapy, MSCs had promoted healing (Madhu et al. 2014; Zubin et al. 2015). In our own experience allogeneic BM-MSCs had improved and led to faster healing of an extensive wound in a dog (Madhu et al. 2014).

MSCs tend to decrease inflammation (Yang et al. 2018), suppress collagenous matrix degradation (Jeon et al. 2010), favour angiogenesis (Kim et al. 2012) and accelerate epithelialization of wound (Rodriguez-Menocal et al. 2015).

11.3.2.2 Vocal Fold Injury

Vocal fold injury demands scar free healing for effective functioning (Rousseau et al. 2003). dMSCs and/conditioned medium had improved healing of vocal fold and laryngotracheal stenosis (Kanemaru et al. 2003; Lee et al. 2006; Ohno et al. 2011; Hu et al. 2014; Iravani et al. 2017) (Table 11.8). MSCs anti-inflammatory activity might prevent pseudomembrane formation and may also promote rational arrangement of the extracellular matrix proteins in the lamina propria (Kumai et al. 2010).

Table 11.8 In vivo preclinical experimental mesenchymal stem cell studies on wound/oral ulcer healing

Model type	Number of animals	Model defect size/study period	Study type	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Oral ulcers (formosol induced)	18 (3 groups with $n = 6$) group I; high dose; group II low dose and group III: Control	0 day to 15 days	Experimental study (cell treated Vs control)	BM-MSCs	1×10^7 (low dose) and 2×10^7 (high dose)	Clinical evaluation (wound assessment parameter scoring tool) and polymerase chain reaction for collagen gene	Groups I and II had shown clinical improvement in their wounds within 7 days following administration of MSCs, and the wounds showed a steady overall decrease in wound size. After 15 days, there was no statistically significant difference among the three groups. Groups II and III had shown better healing by histopathologic examination of oral tissue biopsies at 2 weeks as compared to the control group. An increased expression of both collagen and VEGF genes in MSCs-treated ulcers as compared to controls was demonstrated. Expression of VEGF was significantly higher in case of the group I as compared to group II	El-Menoufy et al. (2010)

(continued)

Table 11.8 (continued)

Model type	Number of animals	Model defect size/study period	Study type	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Full thickness skin wound	10 (24 wounds)	7 days	Experimental study	Allogeneic BM-MSCs	1×10^4 , 1×10^5 , 1×10^6 and 1×10^7 cells /300 μ L of PBS, 200 μ L (wound bed and 100 μ L in wound edges)	Histopathology, immunohisto-chemistry and PCR	Wound epithelial regeneration was seen with epithelial gap significantly smaller in cell treated groups as compared to untreated. Compared to control significantly higher proliferating cell nuclear antigen (PCNA), angiogenesis and collagen synthesis was seen in cell treated groups. Although, collagen was higher in 1×10^7 cell treated groups but was statistically non-significant. Bovine fibroblast growth factor (bFGF) was higher and IL-2 and INF- γ were lower at day 7	Kim et al. (2013)
Induced Oral ulcers	18 (6 in each group)	15 days	Comparative experimental study (intravenous Vs local implantation Vs control)	BM-MSCs	2×10^7	Clinical and histopathological	The treatment resulted in dramatic wound edge activation and resurfacing of oral mucosa wound. Local application hastens such healing compared to systemic application	Aly et al. (2014)
Laryngotracheal stenosis	7	6 weeks	Experimental study (cell/ conditioned media treated Vs control)	BM-MSCs	2×10^6 and conditioned media	Histology	Prominent healing effect of the cells or conditioned media in wounds though statistically insignificant was reported	Iravani et al. (2017)

Table 11.9 In vivo clinical mesenchymal stem cell studies on wound healing

Clinical case	Number of animals included	Study period	Study type	Cell source	Cell dose	Route	Evaluation criteria	Overall result	References
Large skin wound	1	480 day	Case report	AD-MSCs + PRP	2×10^6	Local spraying	Clinical evaluation	Successful healing of complicated wound	Zubin et al. (2015)
Large skin wound	1	50 days	Case report	Allogeneic BM-MSCs post caudal superficial epigastric axial pattern flap application	5×10^6	Local implantation on wound margins	Clinical evaluation	Uneventful wound healing	Madhu et al. (2014)

11.3.2.3 Immune-Mediated and/Anti-Inflammatory Effects

MSCs immuno-modulatory and anti-inflammatory properties makes them a suitable candidate to address immune-mediated disorders like inflammatory bowel disease (IBD), anal furunculosis or keratoconjunctivitis (Table 11.10) (Kang et al. 2008; Stevenson et al. 2012; Pérez-Merino et al. 2015a).

11.3.2.4 Anal Furunculosis/Fistula

Anal furunculosis, an immune-mediated disorder tends to progress to perianal fistulas, commonly seen in middle aged German shepherd dogs (Day, 1993). Clinically, condition relapses frequently and thus, demands lifelong medication. The condition is akin to human anal condition known as Crohn's disease (Ferrer et al. 2016).

An open label trial had demonstrated significant improvement in the condition with local injection of single dose of human embryonic stem cell derived MSCs (hESC-MSCs) after 3 months. Animals had been free of fistulae by 3 months and required less than half of the dose of the cyclosporine. However, the condition had recurred in few cases after 6 months (Ferrer et al. 2016). Further studies are thus, desired that can incorporate multiple doses and be performed for longer periods to confirm utility of MSCs in anal fistula.

11.3.2.5 Inflammatory Bowel Disease (IBS)

Allogeneic AD-MSCs had reduced the clinical symptoms in IBS (Pérez-Merino et al. 2015b). The intestinal tissues had better histology (Pérez-Merino et al. 2015a) (Table 11.10). Thus, further extensive studies are desired.

11.3.2.6 Keratoconjunctivitis Sicca (KCS) and Corneal Ulcer

It is an immune-mediated disease of dog resembling to that of human (Barabino and Dana 2004; Abelson et al. 2009). Currently, KCS requires lifelong treatment (Lemp, 2008). Few clinical studies conducted on dog KCS had shown significantly improved results with the application of allogeneic AD-MSCs. Even immune-suppressants were not required in the treated cases (Villatoro et al. 2015; Bittencourt et al. 2016). These studies had shown improved results with variable dose of cells and had been conducted for a maximum of 12 months. Thus, uniform studies need to be conducted for a longer duration.

A single case of recurrent corneal ulcer in a diabetic poodle dog treated by topical application of two doses of allogeneic AD-MSCs had improved clinical parameters. The fluorescein test had been negative by day 69 (Arantes-Tsuzuki et al. 2019). The patho-physiological basis of the recurrent corneal ulcer, however, requires to be understood.

Table 11.10 In vivo clinical mesenchymal stem cell studies on allergic/ auto-immune diseases

Clinical case	Number of animals included	Study period	Study type	Cell source	Cell dose	Route	Evaluation criteria	Overall Result	References
Atopic dermatitis	5	90 days	Case series (pilot study)	Autologous AD-MSCs	1.3 × 10 ⁶ /kg b. wt.	Intravenous	Canine atopic dermatitis extent and severity score and visual pruritis score	Non-significant reduction in clinical signs	Hall et al. (2010)
Atopic dermatitis (for at least 12 months)	26 (22 animals completed study)	6 months	Controlled clinical case series	Allogeneic AD-MSCs	1.5 × 10 ⁶ /kg b. wt	Intravenous	Clinical signs, haematological and biochemistry profiles, and AD severity (canine atopic dermatitis extent and severity index, version 4 (CADESI-04)), visual analogue scale	Pruritus and CADESI-04 scores decreased significantly after 1 week or month of treatment, respectively, and remained stable for 6 months. Owner's global assessment score was 2.15 ± 1.15 for all the animals in the study	Villatoro et al. (2018)

(continued)

Table 11.10 (continued)

Clinical case	Number of animals included	Study period	Study type	Cell source	Cell dose	Route	Evaluation criteria	Overall Result	References
Inflammatory bowel disease	11	42	Case series (Unblinded, non-comparator study)	Allogeneic AD-MSCs	2×10^6 /kg b. wt	Intravascular	Clinical inflammatory bowel disease activity index (CIBDAI) and canine chronic Enteropathy clinical activity index (CCECAI), and C-reactive protein (CRP)	Significantly decreased CIBDAI and CCECAI but no effect on CRP	Pérez-Merino et al. (2015a, b)
Keratoconjunctivitis sicca	15 (24 eyes)	12 months	Case series	Allogeneic AD-MSCs	1×10^6	Lacrimal glands (dorsal and third eyelid)	Schirmer tear test, ocular surface evaluation	Statistically significant improvement in Schirmer tear test from 28 days	Bittencourt et al. (2016)
Keratoconjunctivitis sicca	12 (24 eyes)	9 months	Case series	Allogeneic AD-MSCs	5×10^6 & 3×10^6	Around lacrimal glands and third eyelid	Schirmer tear test, ocular surface evaluation	Statistically significant improvement in Schirmer tear test and ocular surface	Bittencourt et al. (2016)

Recurrent corneal ulcer	1	69 days after first treatment	Case report	Allogeneic AD-MSCs	3×10^6 (two doses at 48 days later)	Topical application	Clinical testing	Improved clinical parameters like blepharospasm, conjunctival hyperaemia, mucopurulent ocular discharge, photophobia, corneal opacity, chemosis, pigmentation, neo-vascularization and pain. The fluorescein test was negative by day 69	Arantes-Tsuzuki et al. (2019)
Perianal fistula	6	6 months	Case series	Human embryonic stem cell derived MSCs + cyclosporine	Commercial preparation	Local implantation	Clinical evaluation	Completely healed fistula at 3 months while 2 cases had relapse at 6 months	Ferrer et al. (2016)

11.3.2.7 Atopic Dermatitis

One of the initial MSCs application study on dog allergic atopic dermatitis had failed to provide satisfactory results (Hall et al. 2010). However, a recent study had demonstrated improvement in clinical symptoms with MSCs as early as 1 week after treatment (Villatoro et al. 2018). Thus, further studies may be conducted to standardize the process.

11.3.2.8 Nervous System

Nervous tissue regeneration is currently not possible and as such MSCs are being evaluated for the purpose. To achieve therapeutic concentration of these cells in a brain, it is important to locate the suitable route of implantation. In case of meningo-encephalomyelitis of unknown origin (MUO), the stem cells implanted either by intrathecal and intra-arterial route had shorter time of reaction to therapy as compared to those receiving through intrathecal and intravenous route (Zeira et al. 2015). Another brain study had shown that intra-cerebro-ventricular implantation of xenogeneic UC-MSCs (human) in dogs had distributed to various brain structures. These cells had even enhanced sub-ventricular zone neural stem cell (Park et al. 2016).

In dogs spinal cord injury (SCI) is common and may occur due to spinal fractures and or disc degeneration (Fluehmann et al. 2006). The disease resembles to that of the human condition (Sarmento et al. 2014). As no successful therapy is available stem cells are being evaluated (Granger et al. 2012; Roszek et al. 2016).

Stem cells characteristic properties make them potential candidates to heal nervous tissues (Roszek et al. 2016). MSCs in various dog preclinical SCI models created mostly by balloon catheter have been evaluated (Table 11.11). In addition, MSCs regenerative potential has also been evaluated in peripheral nerve injury models (Ding et al. 2010) and disc disease models (Hiyama et al. 2008; Lee et al. 2009b). These cells have mostly been implanted after a week's period (Lee et al. 2011a; Park et al. 2012a, b). Apart from experimental studies, dMSCs have also been evaluated in clinical spinal cord injuries and meningo-encephalomyelitis of unknown origin (MUO) enlisted in Table 11.12.

MSCs from various sources like umbilical cord (UC), UCB (Lim et al. 2007; Lee et al. 2011b; Park et al. 2011; Besalti et al. 2015), WJ (Ryu et al. 2012), BM (Penha et al. 2014; Bhat et al. 2019) or AD (Park et al. 2012b) have been evaluated in SCI models. MSCs from autologous, allogeneic or xenogeneic sources have been implanted. Evaluation has been made on the basis of clinical scores, histology and/or magnetic resonance imaging (MRI). In general, MSCs have improved repair of injured spinal cord. In our own clinical study spinal cord injured dogs (n = 44), two doses of allogeneic BM-MSCs implanted in dogs at 2 weeks interval had significantly improved hind limb reflexes and Olby scores in comparison to those managed by the conventional treatment (Bhat et al. 2019).

dMSCs harvested from different tissues tend to show variable spinal cord repair potential. Umbilical cord blood MSCs (UCB-MSCs) had proven better results as

Table 11.11 In vivo preclinical experimental mesenchymal stem cell studies on spinal cord and associated tissues

Model type	Number of animals	Model defect size/study period	Study type	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Spinal cord injury	25 (5 per group)	Durotomy at L4/8 weeks	Comparative experimental study (recombinant methionyl human granulocyte colony-stimulating factor + UCB-MSCs Vs UCB-MSCs Vs saline treatment Vs control)	Allogeneic umbilical cord blood (UCB)-MSCs	1×10^6	The Olby score, magnetic resonance imaging, somatosensory-evoked potentials and histopathological examinations	Significant improvement in the nerve conduction velocity. Distinct structural consistency of the nerve cell bodies noted in the lesion of the spinal cord of the UCB-MSC and UCBG groups.	Lim et al. (2007)
Spinal cord injury	10 (7 cell transplanted)	5 studied till end (between L2 and L3)/3 months to 1 year	Experimental study (cell treated vs no treatment)	Human umbilical cord derived MSCs	1×10^6 or 2×10^6	Behavioural assessment, MRI, Histology and immunohistochemistry	Remyelination of peripheral-type myelin sheaths	Lee et al. (2011a)
Spinal cord injury	12 (4 per group)	Injury created at L1/8 weeks	Comparative experimental study (neural differentiated AD-MSC + Matrigel Vs Matrigel Vs control)	Neural differentiated AD-MSCs and Matrigel	1×10^7	Behavioural assessment, Histopathologic and immunofluorescence assessment, western blot	The combination of Matrigel NMSC produced beneficial effects in dogs with regard to functional recovery following SCI through enhancement of anti-inflammation, anti-astrogliosis, neuronal extension and neuronal regeneration effects	Park et al. (2012b)

(continued)

Table 11.11 (continued)

Model type	Number of animals	Model defect size/study period	Study type	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Spinal cord injury (hemilaminectomy, L4) (treated 7 days after injury)	20 (control, Matrigel loaded, $n = 4$; Matrigel + BM-MSCs, $n = 4$; Matrigel + AD-MSCs, $n = 4$; Matrigel + WJ-MSCs, $n = 4$; Matrigel + UC-MSCs, $n = 4$)	8 weeks	Experimental study	BM-MSCs, AD-MSCs, WJ-MSCs, UC-MSCs, Matrigel	1×10^5 cells/ μ L (total 60 μ L at cranial, epicentre and caudal lesions after 1 week of injury)	Histopathological, immunofluorescence, quantitative analysis of inflammatory cytokines and surviving nerves	MSC groups showed significant improvements in locomotion. This recovery was accompanied by increased numbers of surviving neuron and neurofilament-positive fibres in the lesion site. Compared to the control, the lesion sizes were smaller, and fewer microglia and reactive astrocytes were found in the spinal cord epicentre of all MSC groups. Although there were no significant differences in functional recovery among the MSCs groups, UCB derived MSCs (UCSCs) induced more nerve regeneration and anti-inflammation activity ($P < 0.05$). Transplanted MSCs survived for 8 weeks and reduced IL-6 and COX-2 levels, which may have promoted neuronal regeneration in the spinal cord.	Ryu et al. (2012)

Acute spinal cord injury	16	Hemilaminectomy (L1)	Comparative study (control, MPSS, AD-MSCs, and AD-MSCs + MPSS.)	Methylprednisolone sodium succinate (MPSS), AD-MSCs	1×10^7 (3 doses intravenous)	Revised Tarlov scale, markers for oxidative metabolism (3-nitrotyrosine, 4-hydroxynonenal, and protein carbonyl) and inflammation (cyclooxygenase-2, interleukin-6, and tumour necrosis factor- α)	At 7 days post-treatment, hindlimb movement had improved in the AD-MSCs and AD-MSCs + MPSS groups. Hematoxylin and eosin staining revealed fewer haemorrhages and lesser microglial infiltration in the AD-MSCs group. Levels of 3-nitrotyrosine were decreased in the MPSS and AD-MSCs + MPSS groups; 4-hydroxynonenal and cyclooxygenase-2 levels were decreased in all treatment groups; and interleukin-6, tumour necrosis factor- α , and phosphorylated-signal transducer and activator transcription 3 levels were decreased in the AD-MSCs and AD-MSCs + MPSS groups	Kim et al. (2015)
--------------------------	----	----------------------	---	---	---------------------------------------	--	---	-------------------

(continued)

Table 11.11 (continued)

Model type	Number of animals	Model defect size/study period	Study type	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Acute spinal cord injury	8	8 weeks	Comparative experimental study (AD-MSCs-Haemoxigenase-1 vs GFPAD-MSCs)	AD-MSCs (haemoxigenase-1 overexpression) or GFP AD-MSCs	1×10^7	Histopathology, immune-fluorescence, and western blot analyses	In AD- MSC-HO-1 group basso, Beattie, Bresnahan (BBB) score significantly improved at 7 weeks significantly higher motor recovery in the AD-MSC-HO-1 group and reduction of fibrotic changes as compared to GFPAD-MSC group	Lee et al. (2017c)
Intervertebral disc disease	18	Nucleotomy (L3/4, L4/5, and L5/6)/12 weeks	Experimental study (cell treated group Vs control)	Autologous BM-MSCs	1×10^6	Radiological, MRI, macroscopy, histology, immunohisto-chemistry, RT-PCR (FasL)	Regeneration of degenerated discs with expression of FasL gene in cell treated group	Hiyama et al. (2008)
Spontaneous degenerative lumbosacral IVD (L7-S1; decompressive surgery treated)	20 (MSCs + microcarrier, n = 11; MSCs + TGF- β 1 + microcarrier, n = 6; microcarrier only, n = 3)	10 months	Prospective clinical study	Autologous BM-MSCs, collagen microcarrier	3×10^6 cells/250 mg on collagen	MRI	In vivo injection was successful in all dogs, without any visible leakage, and clinical functioning was restored back to normality. Pfirrmann grade remained identical in all dogs, and formation of Schmorl's nodes was detected in 45% of dogs. This side effect was reduced by halving the injection volume, which was then observed only in 11% of dogs	Steffen et al. (2018)

Sciatic nerve resection (a 35 mm long)	20 (negative control, LOCC, hUC-MSc in combination with LOCC (LOCC/LOCC-MSc), and autograft (positive control) groups	9 months	Human umbilical cord MSCs	1 × 10 ⁶ , longitudinally oriented collagen conduit (LOCC)	Local implantation	Electrophysiological, electron microscopy, histological analysis	The LOCC and hUC-MSCs synergistically promoted regeneration and improved the functional recovery in a dog model of sciatic nerve injury	Cui et al. (2017)
--	---	----------	---------------------------	---	--------------------	--	---	-------------------

Table 11.12 In vivo clinical mesenchymal stem cell studies on various neurological affections

Clinical case	Number of animals included	Study period	Study type	Cell source	Cell dose	Route	Evaluation criteria	Overall result	References
Chronic spinal cord injury	23 (10 BM-MSCs and 13 control)	6 months to 35 months	Case series (uncontrolled, non-blinded clinical study)	Autologous BM-MSCs	1.4×10^6 – 5.6×10^6	Subarachnoid space implantation between L5 and L6	TCSIS system (involves evaluation of each pelvic limb separately and has 3 components: Gait, proprioceptive positioning and nociception)	Improvement in locomotor function in 6 cell treated dogs	Nishida et al. (2011)
Spinal cord injury	1	180 days	Case report	Autologous bone marrow mononuclear cells (BMMNCs)	20×10^6 & 4.16×10^6 BMMNCs + thermoreversible gelatin polymer	Local post at T12 left hemilaminectomy and intravenous implantation	Neurological examination, Olby scores	Functional recovery demonstrated with cell implantation	William et al. (2011)
Acute spinal cord injury	85 (34 randomized subjects out of which 23 cell treated and 11 no cell treatment)	6 months	Case series (controlled, randomized double-blinded trial)	Autologous olfactory mucosal stem cells	5×10^6 cells	Intralesional	Gait analysis, somatosensory-evoked potential, Transcranial magnetic motor-evoked potentials, Urodynamics	Recipients of olfactory mucosal stem cell transplants gained significantly better forehand coordination than those dogs that did not received cells. Intraspinal olfactory mucosal cell transplantation improves communication across the damaged region of the injured spinal cord	Granger et al. (2012)

Spinal cord compression due to herniated intervertebral disks (T12 to L5 vertebrae)	4	18 months	Case reports (uncontrolled clinical study)	Autologous BM-MSCs	1×10^6 + collagen gelfoam scaffold	Local lesion implantation post-surgical decompression	Clinically, radiographically, and by nuclear magnetic resonance imaging (MRI)	Post MSCs implantation, a progressive recovery of the panniculus reflex and diminished superficial and deep pain response, although there were still low proprioceptive reflexes in addition to a hyperreflexia in the ataxic hind limb movement responses was demonstrated at day 10. Each dog demonstrated an improvement in these gains over time. Conscious reflex recovery occurred simultaneously with moderate improvement in intestine and urinary bladder functions in two of the four dogs. By the 18th month of clinical monitoring, a remarkable clinical amelioration accompanied by improved movement, in three of the four dogs was reported. However, no clinical gain was associated with alterations in magnetic resonance imaging.
---	---	-----------	--	--------------------	---	---	---	---

(continued)

Table 11.12 (continued)

Clinical case	Number of animals included	Study period	Study type	Cell source	Cell dose	Route	Evaluation criteria	Overall result	References
Chronic spinal cord injury	7	90 days	Case series (uncontrolled pilot study)	Allogeneic BM-MSCs	1×10^6	Intralesional and caudal to lesion implantation	MRI and Olby scale	All dogs improved locomotor and sensory function, tail tone was observed in seven dogs, pain reflexes and defecation return were observed in five dogs.	Sarmiento et al. (2014)
Paraplegia	13	12 months	Case series (uncontrolled clinical study)	Autologous neurogenically-induced BM-MSCs	5.0×10^6 (2 doses after 21 days of interval post hemilaminectomy/laminectomy)	Percutaneous transplantation	Texas spinal cord injury scale (TSCIS), somatosensory-evoked potentials (SEP) and motor-evoked potentials (MEP)	Improvement in gait, nociception, proprioception, SEP and MEP results (2 cases), only gait score improvement (6 cases) and no improvement was recorded in (5 cases)	Besalti et al. (2015)
Chronic spinal injury/IVDD	6	16 weeks	Case series (pilot clinical test)	Allogeneic AD-MSCs	1×10^7	Intralesional implantation	Functional evaluation of Locomotor activity	Only single dog regained ability to walk	Escalhão et al. (2017)
Meningo-encephalomyelitis of unknown origin (MUO)	8	2 years	Clinical series	Autologous BM-MSC	2×10^6 , 4×10^6 (3 dogs) and 2×10^6 , 0.5×10^6 (4 dogs)	Intrathecal (IT)/right Intracarotid (IC) & intrathecal and intravenous	MRI, neurological examination and histopathology	All the dogs had presented early improvement in their general and neurological conditions, with particular effect on cervical pain. The group of dogs treated by IT+IA administration showed a shorter time of reaction to therapy compared to the group treated by IT+IC administration	Zeira et al. (2015)

Canine distemper	8 (<i>n</i> = each group; group I: Cell + standard medicine; group II: Standard medicine)	15 days	Case series (double-blind randomized trial)	Foetal olfactory epithelium stem cells	1.0×10^6 /kg b. wt.	Intravenous	RT-PCR and laboratory investigations	MSC therapy resulted in no significant improvement when administered during the acute phase of canine distemper disease, and a prevalence of animals with high mortality rate was found in both groups due to the severity of symptoms	Pinheiro et al. (2016)
Canine distemper	4	1 year	Case series	AD-MSCs	1.0×10^7 (3 doses at monthly interval)	Intravenous	Neurological examination	During the first year after cellular therapy, the animals did not demonstrate significant changes in their locomotive abilities. However, the intense (grade V) myoclonus in three animals was reduced to a moderate (grade IV) level. At 1 year after the mesenchymal stem cell infusions, three animals regained functional ambulation (grade I), and all four dogs started to move independently (grades I and II). In two animals, the myoclonic severity had become mild (grade III)	Pinheiro et al. (2019)

compared to BM-/AD-/WJ-MSCs. Among these cells BM-MSCs had lowest survival number (Ryu et al. 2012). Allogeneic or autologous BM-MSCs had improved the pelvic limb function scores in dogs. Olby scores, however, were higher in autologous cell implanted cases as compared to the allogeneic implanted ones (Jung et al. 2009). MSCs have mostly been implanted through local intrathecal route. Besides, MSCs percutaneous transplantation has also been made. Human UCB-MSCs applied through that route also had improved healing of the injured spinal cord (Lee et al. 2009b). dMSCs had been implanted at early (12 h), delayed (1 week) or late (2 weeks). The better results had been shown to occur by implanting the cells at a delay of 1 week. Peak inflammation at early injury period may hamper MSCs activity while more delayed cell implantation (2 weeks) may encounter already developed glial cell reaction (Park et al. 2011).

To further improve their effectiveness, various unorthodox options like MSCs genetic engineering, in vitro differentiated MSCs or their conditioned medium are being evaluated. In SCI, haemoxygenase-1 transfected dAD-MSCs had led to superior functional recovery as compared to the simple dAD-MSCs (Lee et al. 2017a). The haemoxygenase-1 has anti-oxidative and anti-inflammatory action, immune-modulation and might improve local microcirculation (Zhang et al. 2014). Combined local implantation and intravenous injection of brain derived nerve factor (BDNF) transfected MSCs had led to the significant functional recovery with improved histological features in SCI cases as compared to ones that received transfected dAD-MSCs locally (Lee et al. 2016). Contrarily, one of the studies had failed to demonstrate additive effects of BMP-2 and TGF- β 1 on dBM-MSCs healing of nucleus pulposus (Bach et al. 2017). MSCs conditioned medium may also improve SCI repair (Al Delfi et al. 2016).

MSCs have also been demonstrated to improve peripheral nerve injury. MSCs loaded on chitosan/poly(lactic-co-glycolic acid) (PLGA)-based neural scaffold had led to the significant sciatic nerve regeneration and functional recovery in 50 mm sciatic nerve defect model. The healing had been comparable to that with nerve auto-grafting (Ding et al. 2010).

Disc regeneration is one of the important challenges. Degenerated disc repair may be improved with MSCs, although concentration has a role to play. One million cells had given better repair as compared to 0.1 million and ten million cells (Serigano et al. 2010). MSCs neural differentiation is currently questionable (Wu et al. 2003) but due to their trophic action nerve protection and repair may be enhanced (Ohta et al. 2004; Hokari et al. 2008; Saito et al. 2008; Park et al. 2011; Park et al. 2012a, b; Chung et al. 2013; Lim et al. 2017; Long et al. 2018).

11.3.3 Miscellaneous Studies

Miniscule number of MSCs studies related to various other ailments like diabetes (Zhu et al. 2011; Gautam et al. 2016), liver fibrosis (Matsuda et al. 2017) and acute kidney injury (Lee et al. 2017b) have also been undertaken as enlisted in Table 11.13. In a dog hepatocutaneous syndrome, multiple intrahepatic implantations of

Table 11.13 Miscellaneous in vivo mesenchymal stem cell studies in dog

Model type	Number of animals	Model defect size/study period	Study type	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Diabetic model (alloxan and streptozotocin induced)	30 dogs (6 in each group)	16 weeks	Comparative experimental study (group 1: MSCs transfected with retrovirus carrying insulin and EGFP gene) Vs group 2: MSCs transfected with retrovirus carrying EGFP gene Vs group 3: MSCs without virus infection) Vs group 4: Cell-free group 5: Normal dogs)	Autologous BM-MSCs carrying insulin and EGFP gene	2.53106 / mL (total volume 20 mL injected	Analysis of C peptide, immunohistochemistry and immunocytochemistry, glucose tolerance test, measurement of blood glucose, insulin detection	Group 1 had insulin secretion through implanted cells; animals also responded to glucose challenge however β -cells were undetectable as no C peptide secretion was recorded. Rest of the groups had no control on glucose challenge. Glucose level was statistically controlled in group 1 compared to group 4	Zhu et al. (2011)

(continued)

Table 11.13 (continued)

Model type	Number of animals	Model defect size/study period	Study type	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Dilated cardiomyopathy	15	2 months	A pilot study	Stromal-derived factor-1 transduced AD-MSCs	Retrograde coronary venous delivery	Electrocardiograms (ECG), echocardiograms and Holter monitoring	Echocardiographic measurements of left ventricular size and function had shown continued progression of disease. Stem cell therapy in dogs with occult-dilated cardiomyopathy does not appear to offer advantage as compared to recently published survival data in similarly affected Doberman dogs	Pogue et al. (2013)
Acute kidney disease	14 (3 in MSCs group, 8 in phosphate buffer saline treated group and 3 normal dogs	Bilateral renal corticomedullary/7 weeks junction induction of AKI	Experimental study (cell treated) Vs phosphate buffer saline Vs normal dogs	UCB-MSCs	1 × 10 ⁶	Renal function and histology	Improved renal tubular function and moderate tubular necrosis in cell treated group compared to PBS group	Lee et al. (2017c)

Canine liver fibrosis (CCl ₄)	16 (BM-MSCs injected, <i>n</i> = 8; control, <i>n</i> = 8)	4 weeks	Experimental study (cell treated) Vs control	<i>Autologous BM-MSCs</i>	4 x 10 ⁵ /kg	CE-CT IMAGING, liver biopsy, histology, histomorphometry, DNA microarray analysis, PCR	BM-MSCs improved liver fibrosis without adverse effects. COL1A2, COL3, TIMP-1, EDNRA and EDNRB expression levels were significantly lower in the BMSC group compared with the control group. Alanine aminotransferase, albumin, and antithrombin 3 levels significantly improved in the BMSC group	Matsuda et al. (2017)
---	--	---------	--	---------------------------	-------------------------	--	--	-----------------------

allogeneic AD-MSCs had been made along with other managemental practices. An improvement in the clinical signs like hair regrowth and resolution of skin lesions was demonstrated. The dog, however, had died 32 months after diagnosis. The necropsy had revealed liver fibrosis and superficial necrolytic dermatitis (Nam et al. 2017). MSCs may also act drug (paclitaxel) carriers and can be used to target tumours like glioma and glioblastoma (Bonomi et al. 2017). Combined application of MSCs, recombinant human BMP-2 and cisplatin may prove useful in canine osteosarcoma (Rici et al. 2018). However, MSCs soluble factors may promote tumour cell proliferation and invasion (Teshima et al. 2018) and thus, studies are desired to confirm their safety, feasibility and actual utility.

11.4 Conclusion(S)

Numerous dog tissue sources harbour MSCs although concentration and properties may be variable. The cellular features also vary with age and health status of the donor. dMSCs have been evaluated for their therapeutic benefits on musculoskeletal and non-musculoskeletal tissue ailments. MSCs studies in dog tend to provide proof-of-therapeutic potential in human medicine. Due to the lack of understanding in their basic cellular properties, an initial step should be deliberated upon these properties. Further, case controlled uniform clinical trials need to be conducted to provide evidence based medicine for clinical conditions. Several issues like MSCs source, cell concentration, mode of delivery, cell survival on transplantation and frequency of stem cells need further deliberation and standardization.

References

- Abelson MB, Ousler GW, Maffei C (2009) Dry eye in 2008. *Curr Opin Ophthalmol* 20(4):282–286
- Akizuki T, Odam S, Komaki M, Tsuchioka H, Kawakatsu N, Kikuchi A, Yamato M, Okano T, Ishikawa I (2005) Application of periodontal ligament cell sheet for periodontal regeneration: a pilot study in beagle dogs. *J Periodontal Res* 40:245–251
- Al Delfi IR, Sheard JJ, Wood CR, Vernallis A, Innes JF, Myint P, Johnson WEB (2016) Canine mesenchymal stem cells are neurotrophic and angiogenic: an in vitro assessment of their paracrine activity. *Vet J* 217:10–17
- Aly LA, El-Menoufy H, Sadeq HS, Ragae A, Sabry D (2014) Efficiency of systemic versus intralesional bone marrow derived stem cells in regeneration of oral mucosa after induction of formocresol induced ulcers in dogs. *Dent Res J (Isfahan)* 11(2):212–221
- Arantes-Tsuzuki PDM, Mazzonetto PC, Lo Turco EG (2019) Treatment for canine corneal ulcer using adipose Tissue-derived Mesenchymal stem cell therapy - Case report. *Preprints* 2019:120185
- Arinzech TL, Peter SJ, Archambault MP, van den Bos C, Gordon S, Kraus K, Smith A, Kadiyala S (2003) Allogeneic mesenchymal stem cells regenerate bone in a critical-sized canine segmental defect. *J Bone Joint Surg Am* 85-A:1927–1935
- Ayala-Cuellar AP, Kim CW, Hwang KA, Kang JH, Lee G, Cho J, Choi KC (2019) Characterization of canine adipose tissue-derived mesenchymal stem cells immortalized by SV40-T retrovirus for therapeutic use. *J Cell Physiol* 234(9):16630–16642

- Bach FC, Miranda-Bedate A, van Heel FW, Riemers FM, Müller MC, Creemers LB, Ito K, Benz K, Meij BP, Tryfonidou MA (2017) Bone morphogenetic Protein-2, but not Mesenchymal stromal cells, exert regenerative effects on canine and human nucleus Pulposus cells. *Tissue Eng A* 23(5–6):233–242
- Bae S, Kim J, Li L, Lee A, Lim H, Jeong J, Lee SH, Kweon O-K, Kim WH (2015) Canine adipose-derived stem cell aggregates as a viable substitute to actual canine dermal papillae. *Macedonian Vet Rev* 38(2):167–173
- Bahamondes F, Flores E, Cattaneo G, Bruna F, Conget P (2017) Omental adipose tissue is a more suitable source of canine Mesenchymal stem cells. *BMC Vet Res* 13:166
- Baird AEG, Barsby T, Guest DJ (2015) Derivation of canine induced pluripotent stem cells. *Reprod Domest Anim* 50(4):669–676
- Barabino S, Dana MR (2004) Animal models of dry eye: a critical assessment of opportunities and limitations. *Invest Ophthalmol Vis Sci* 45(6):1641–1646
- Bartunek J, Croissant JD, Wijns W, Gofflot S, de Lavareille A, Vanderheyden M, Kaluzhny Y, Mazouz N, Willemsen P, Penicka M et al (2007) Pre-treatment of adult bone marrow mesenchymal stem cells with cardiomyogenic growth factors and repair of the chronically infarcted myocardium. *Am J Physiol* 292:H1095–H1104
- Bçrziðð U, Matise-VanHoutana I, Pçtersone I, Dûrtis I, Òikuïðins S, Bogdanova-Jâtiece A, Kâlis M, Svirskis Ð, Skrastiða D, Ezerta A et al (2018) Characterisation and *In Vivo* safety of canine adipose-derived stem cells. *Proc Latvian Acad Sci* 72(3):160–171
- Bearden RN, Huggins SS, Cummings KJ, Smith R, Gregory CA, Saunders WB (2017) In-vitro characterization of canine multipotent stromal cells isolated from synovium, bone marrow, and adipose tissue: a donor-matched comparative study. *Stem Cell Res Ther* 8:218
- Bertolo A, Steffen F, Malonzo-Marty C, Stoyanov J (2015) Canine Mesenchymal stem cell potential and the importance of dog breed: implication for cell-based therapies. *Cell Transplant* 24:1969–1980
- Besalti O, Can P, Akpinar E, Aktas Z, Elcin AE, Elcin YM (2015) Intraspinal transplantation of autologous neurogenically-induced bone marrow-derived mesenchymal stem cells in the treatment of paraplegic dogs without deep pain perception secondary to intervertebral disk disease. *Turk Neurosurg* 25:625–632
- Bhat IA, Sivanarayanan TB, Somal A, Pandey S, Bharti MK, Panda BSK, Indu B, Verma M, Anand J, Sonwane A et al (2019) An allogenic therapeutic strategy for canine spinal cord injury using mesenchymal stem cells. *J Cell Physiol* 234(3):2705–2718
- Bittencourt MKW, Barros MA, Martins JFP, Vasconcellos JPC, Morais BP, Pompeia C, Bittencourt MD, Evangelho KS, Kerkis I, Wenceslau CV (2016) Allogeneic Mesenchymal stem cell transplantation in dogs with Keratoconjunctivitis Sicca. *Cell Med* 8:63–77
- Black LL, Gaynor J, Gahring D, Adams C, Aron D, Harman S, Gingerich DA, Harman R (2007) Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints: a randomized, double-blinded, multicenter, controlled trial. *Vet Ther* 8:272–284
- Black LL, Gaynor J, Adams C, Dhupa S, Sams AE, Taylor R, Harman S, Gingerich DA, Harman R (2008) Effect of intraarticular injection of autologous adipose-derived mesenchymal stem and regenerative cells on clinical signs of chronic osteoarthritis of the elbow joint in dogs. *Vet Ther* 9:192–200
- Blecker D, Elashry MI, Heimann M, Wenisch S, Arnhold S (2017) New insights into the neural differentiation potential of canine adipose tissue-derived Mesenchymal stem cells. *Anatomia Histologia Embryologia* 46(3):304–315
- Bonomi A, Ghezzi E, Pascucci L, Aralla M, Ceserani V, Pettinari L, Coccè V, Guercio A, Alessandri G, Parati E et al (2017) Effect of canine mesenchymal stromal cells loaded with paclitaxel on growth of canine glioma and human glioblastoma cell lines. *Vet J* 223:41–47
- Brown SG, Harman RJ, Black LL (2012) Adipose-derived stem cell therapy for severe muscle tears in working German shepherds: two case reports. *Stem Cell Discov* 2(2):41–44

- Bruder SP, Kraus KH, Goldberg VM, Kadiyala S (1998) The effect of implants loaded with autologous mesenchymal stem cells on the healing of canine segmental bone defects. *J Bone Joint Surg Am* 80:985–996
- Bugueño J, Li W, Salat P, Qin L, Akintoye SO (2017) Canine mesenchymal stem cell bone regenerative capacity is regulated by sitespecific multi-lineage differentiation. *Oral Surg Oral Med Oral Pathol Oral Radiol* 123(2):163–172
- Cabon Q, Febre M, Gomez N, Cachon T, Pillard P, Carozzo C, Saulnier N, Robert C, Livet V, Rakic R, Plantier N, Saas P, Maddens S, Viguier E (2019) Long-term safety and efficacy of single or repeated intra-articular injection of allogeneic neonatal Mesenchymal stromal cells for managing pain and lameness in moderate to severe canine osteoarthritis without anti-inflammatory pharmacological support: pilot clinical study. *Front Vet Sci* 6:10
- Canapp SO Jr, Leasure CS, Cox C, Ibrahim V, Carr BJ (2016) Partial cranial cruciate ligament tears treated with stem cell and platelet-rich plasma combination therapy in 36 dogs: A retrospective study. *Front Vet Sci* 3:112
- Case JB, Palmer R, Valdes-Martinez A, Egger EL, Haussler KK (2013) Gastrocnemius tendon strain in a dog treated with autologous mesenchymal stem cells and a custom orthosis. *Vet Surg* 42:355–360
- Ceusters J, Lejeune J-P, Sandersen C, Niesten A, Lagneaux L, Serteyn D (2017) From skeletal muscle to stem cells: an innovative and minimally invasive process for multiple species. *Sci Rep* 7:696
- Chow L, Johnson V, Regan D, Wheat W, Webb S, Koch P, Dow S (2017) Safety and immune regulatory properties of canine induced pluripotent stem cell derived mesenchymal stem cells. *Stem Cell Res* 25:221–232
- Chung C-S, Fujita N, Kawahara N, Yui S, Nam E, Nishimura R (2013) A comparison of neurosphere differentiation potential of canine bone marrow-derived mesenchymal stem cells and adipose-derived mesenchymal stem cells. *J Vet Med Sci* 75:879–886
- Csaki C, Matis U, Mobasheri A, Ye H, Shakibaei M (2007) Chondrogenesis, osteogenesis and adipogenesis of canine mesenchymal stem cells: a biochemical, morphological and ultrastructural study. *Histochem Cell Biol* 128(6):507–520
- Cuervo B, Rubio M, Sopena J, Dominguez JM, Vilar J, Morales M, Cugat R, Carrillo JM (2014) Hip osteoarthritis in dogs: A randomized study using Mesenchymal stem cells from adipose tissue and plasma rich in growth factors. *Int J Mol Sci* 15:13437–13460
- Cui L, Liu B, Liu G, Zhang W, Cen L, Sun J, Yin S, Liu W, Cao Y (2017) Repair of cranial bone defects with adipose derived stem cells and coral scaffold in a canine model. *Biomaterials* 28(36):5477–5486
- Daems R, Van Hecke L, Schwarzkopf I, Depuydt E, Broeckx SY, David M, Beerts C, Vandekerckhove P, Spaas JH (2019) A feasibility study on the use of equine Chondrogenic induced Mesenchymal stem cells as a treatment for natural occurring osteoarthritis in dogs. *Stem Cells Int* 2019:4587594
- Day M (1993) Immunopathology of anal furunculosis in the dog. *J Small Anim Pract* 34(8):381–389
- de Bakker E, Van Ryssen B, De Schauwer C, Meyer E (2013) Canine mesenchymal stem cells: state of the art, perspectives as therapy for dogs and as a model for man. *Vet Q* 33(4):225–233
- De Cesaris V, Grolli S, Bresciani C, Conti V, Basini G, Parmigiani E, Bigliardi E (2017) Isolation, proliferation and characterization of endometrial canine stem cells. *Reprod Domest Anim* 52(2):235–242
- Ding F, Wu J, Yang Y, Hu W, Zhu Q, Tang X, Liu J, Gu X (2010) Use of tissue-engineered nerve grafts consisting of a chitosan/poly(lactic-co-glycolic acid)- based scaffold included with bone marrow mesenchymal cells for bridging 50-mm dog sciatic nerve gaps. *Tissue Eng A* 16:3779–3790
- Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytherapy* 8(4):315–317

- dos Santos CR, da Rocha Filgueiras R, Malard PF, da Cunha Barreto-Vianna AR, Nogueira K, da Silva Leite C, Mendes de Lima EM (2018) Mesenchymal stem cells in osteotomy repair after tibial tuberosity advancement in dogs with cranial cruciate ligament injury. *J Exp Orthopaed* 5:17
- Dražilov SS, Mrkovački J, Spasovski V, Fazlagić A, Pavlović S, Nikčević G (2018) The use of canine Mesenchymal stem cells for the autologous treatment of osteoarthritis. *Acta Vet Hung* 66(3):376–389
- El-Menoufy H, Aly LA, Aziz MT, Atta HM, Roshdy NK, Rashed LA, Sabry D (2010) The role of bone marrow-derived mesenchymal stem cells in treating formocresol induced oral ulcers in dogs. *J Oral Pathol Med* 39:281–289
- El-Tookhy OS, Shamaa AA, Shehab GG, Abdallah AN, Azzam OM (2017) Histological evaluation of experimentally induced critical size defect skin wounds using Exosomal solution of Mesenchymal stem cells derived microvesicles. *Inter J Stem cells* 10(2):144–153
- Enciso N, Ostronoff LLK, Mejías G, León LG, Fermín ML, Merino E, Fragio C, Avedillo L, Tejero C (2018) Stem cell factor supports migration in canine mesenchymal stem cells. *Vet Res Commun* 42(1):29–38
- Escalhão CCM, Ramos IP, Hochman-Mendez C, Brunswick THK, Souza SAL, Gutflen B, Goldenberg RCS, Coelho-Sampaio T (2017) Safety of allogeneic canine adipose tissue-derived Mesenchymal stem cell intraspinal transplantation in dogs with chronic spinal cord injury. *Stem Cells Int* 2017:3053759
- Espósito M, Grusovin MG, Papanikolaou N, Coulthard P, Worthington HV (2009) Enamel matrix derivative (Emdogain(R)) for periodontal tissue regeneration in intrabony defects. *Cochrane database. Syst Rev* 2009:CD003875
- Ferrer L, Kimbrel EA, Lam A, Falk EB, Zewe C, Juopperi T, Lanza R, Hoffman A (2016) Treatment of perianal fistulas with human embryonic stem cell-derived mesenchymal stem cells: a canine model of human fistulizing Crohn's disease. *Regen Med* 11(1):33–43
- Fluehmann G, Doherr MG, Jaggy A (2006) Canine neurological diseases in a referral hospital population between 1989 and 2000 in Switzerland. *J Small Anim Pract* 47:582–587
- Gautam P, Foale RD, Recino A, Zhao J, Gan SU, Wallberg M, Calne R, Lever AML (2016) Promoter optimisation of Lentiviral vectors for efficient insulin gene expression in canine Mesenchymal stromal cells: potential surrogate Beta cells. *J Gene Med* 18(10):312–321
- Gelberman RH, Linderman SW, Jayaram R, Dikina AD, Sakiyama-Elbert H, Alsborg E, Thomopoulos S, Shen H (2017) Combined administration of ASCs and BMP-12 promotes an M2 macrophage phenotype and enhances tendon healing. *Clin Orthopaed Relat Res* 475(9):2318–2331
- Gibson MA, Brown SG, Brown NO (2017) Semitendinosus myopathy and treatment with adipose-derived stem cells in working German shepherd police dogs. *Can Vet J* 58:241–246
- Granger N, Blamires H, Franklin RJM, Jeffery ND (2012) Autologous olfactory mucosal cell transplants in clinical spinal cord injury: a randomized double-blinded trial in a canine translational model. *Brain* 135:3227–3237
- Guercio A, Di Marco P, Casella S, Cannella V, Russotto L, Purpari G, Di Bella S, Piccione G (2012) Production of canine mesenchymal stem cells from adipose tissue and their application in dogs with chronic osteoarthritis of the humeroradial joints. *Cell Biol Int* 36:189–194
- Gugjoo MB, Amarpal, Sharma GT, Kinjavdekar P, Aithal HP, Pawde AM (2016) Cartilage tissue engineering: role of mesenchymal stem cells along with growth factors and scaffolds. *Indian J Med Res* 144(3):339–347
- Gugjoo MB, Amarpal, Abdelbaset-Ismail A, Aithal HP, Kinjavdekar P, Pawde AM, Kumar GS, Sharma GT (2017) Mesenchymal stem cells with IGF-1 and TGF- β 1 in laminin gel for osteochondral defects in rabbits. *Biomed Pharmacother* 93:1165–1174
- Gugjoo MB, Amarpal A, Sharma GT (2019a) Mesenchymal stem cell basic research and applications in dog medicine. *J Cell Physiol* 234(10):16779–16811
- Gugjoo MB, Amarpal, Makhdoomi DM, Sharma GT (2019b) Equine mesenchymal stem cells: properties, sources, characterization and potential therapeutic applications. *J Equine Vet Sci* 72:16–27

- Guo P, Zeng J-J, Zhou N (2015) A novel experimental study on the fabrication and biological characteristics of canine bone marrow Mesenchymal stem cells sheet using vitamin C. *Scanning* 9999:1–7
- Hall MN, Rosenkrantz WS, Hong JH, Griffin CE, Mendelsohn CM (2010) Evaluation of the potential use of adipose-derived mesenchymal stromal cells in the treatment of canine atopic dermatitis: a pilot study. *Vet Ther* 11:E1–E14
- Hang D, Wang Q, Guo C, Chen Z, Yan Z (2012) Treatment of osteonecrosis of the femoral head with VEGF165 transgenic bone marrow mesenchymal stem cells in mongrel dogs. *Cells Tissues Organs* 195:495–506
- Harman R, Carlson K, Gaynor J, Gustafson S, Dhupa S, Clement K, Hoelzler M, McCarthy T, Schwartz P, Adams C (2016) A prospective, randomized, masked, and placebo-controlled efficacy study of Intraarticular allogeneic adipose stem cells for the treatment of osteoarthritis in dogs. *Front Vet Sci* 3:81
- Hiyama A, Mochida J, Iwashina T, Omi H, Watanabe T, Serigano K, Tamaru F, Sakai D (2008) Transplantation of mesenchymal stem cells in a canine disc degeneration model. *J Orthop Res* 26:589–600
- Hodgkiss-Geere HM, Argyle DJ, Corcoran BM, Whitelaw B, Milne E, Bennett D, Argyle SA (2012) Characterisation and differentiation potential of bone marrow derived canine mesenchymal stem cells. *Vet J* 194:361–368
- Hokari M, Kuroda S, Shichinohe H, Yano S, Hida K, Iwasaki Y (2008) Bone marrow stromal cells protect and repair damaged neurons through multiple mechanisms. *J Neurosci Res* 86:1024–1035
- Hu R, Ling W, Xu W, Han D (2014) Fibroblast-like cells differentiated from adipose-derived mesenchymal stem cells for vocal fold wound healing. *PLoS One* 9(3):e92676
- Huss R, Claudia L, Weissinger EM, Kolb HJ, Thalmeier K (2000) Evidence of peripheral blood-derived, plastic-adherent CD34^{low} hematopoietic stem cell clones with mesenchymal stem cell characteristics. *Stem Cells* 18:252–260
- Iravani K, Sobhanmanesh A, Ashraf MJ, Hashemi SB, Mehrabani D, Zare S (2017) The healing effect of conditioned media and bone marrow-derived stem cells in laryngotracheal stenosis: a comparison in experimental dog model. *World J Plast Surg* 6(2):190–197
- Itoi T, Harada Y, Irie H, Sakamoto M, Tamura K, Yogo T, Soeta S, Amasaki H, Hara Y, Tagawa M (2016) Escherichia coli-derived recombinant human bone morphogenetic protein-2 combined with bone marrow-derived mesenchymal stromal cells improves bone regeneration in canine segmental ulnar defects. *BMC Vet Res* 12:201
- Jang BJ, Byeon YE, Lim JH, Ryu HH, Kim WH, Koyama Y, Kikuchi M, Kang KS, Kweon OK (2008) Implantation of canine umbilical cord blood-derived mesenchymal stem cells mixed with beta-tricalcium phosphate enhances osteogenesis in bone defect model dogs. *J Vet Sci* 9:387–393
- Jeon YK, Jang YH, Yoo DR, Kim SN, Lee SK, Nam MJ (2010) Mesenchymal stem cells' interaction with skin: wound-healing effect on fibroblast cells and skin tissue. *Wound Repair Regen* 18:655–661
- Jo K, Kim Y, Lee SH, Yoon YS, Kim WH, Kweon O-K (2017) Effect of canine cortical bone demineralization on osteogenic differentiation of adipose- derived mesenchymal stromal cells. *Heliyon* 3(8):e00383
- Johnson JA, Austin C, Breur GJ (1994) Incidence of canine appendicular musculo-skeletal disorders in 16 veterinary teaching hospitals from 1980 through 1989. *Vet Comp Orthop Traumatol* 7:56–69
- Johnston SA (1997) Osteoarthritis. Joint anatomy, physiology, and pathobiology. *Vet Clin North Am Small Anim Pract* 27:699–723
- Jung DI, Ha J, Kang BT, Kim JW, Quan FS, Lee JH, Woo EJ, Park HM (2009) A comparison of autologous and allogenic bone marrow-derived mesenchymal stem cell transplantation in canine spinal cord injury. *J Neurol Sci* 285:67–77

- Kafarnik C, McClellan A, Dziasko M, Daniels JT, Guest DJ (2020) Canine corneal stromal cells have multipotent mesenchymal stromal cell properties in vitro. *Stem Cells Dev.* <https://doi.org/10.1089/scd.2019.0163>
- Kamishina H, Deng J, Oji T, Cheeseman JA, Clemmons RM (2006) Expression of neural markers on bone-marrow-derived canine mesenchymal stem cells. *Am J Vet Res* 67:1921–1928
- Kanemaru S, Nakamura T, Omori K, Kojima H, Magrúfov A, Hiratsuka Y, Hirano S, Ito J, Shimizu Y (2003) Regeneration of the vocal fold using autologous mesenchymal stem cells. *Ann Oto Rhinol Laryngol* 112:915–920
- Kang MH, Park HM (2014) Evaluation of adverse reactions in dogs following intravenous mesenchymal stem cell transplantation. *Acta Vet Scand* 56:16
- Kang JW, Kang K-S, Koo HC, Park JR, Choi EW, Park YH (2008) Soluble factors-mediated immunomodulatory effects of canine adipose tissue-derived mesenchymal stem cells. *Stem Cells Develop* 17(4):681–693
- Kang BJ, Ryu HH, Park SS, Koyama Y, Kikuchi M, Woo HM, Kim WH, Kweon OK (2012) Comparing the osteogenic potential of canine mesenchymal stem cells derived from adipose tissues, bone marrow, umbilical cord blood, and Wharton's jelly for treating bone defects. *J Vet Sci* 13:299–310
- Kazemi D, Asenjan S, Dehdilani K, Parsa H (2017) Canine articular cartilage regeneration using mesenchymal stem cells seeded on platelet rich fibrin. *Bone Joint Res* 6:98–107
- Khorsand A, Eslaminejad MB, Arabsolghar M, Paknejad M, Ghaedi B, Rokn AR, Moslemi N, Nazarian H, Jahangir S (2013) Autologous dental pulp stem cells in regeneration of defect created in canine periodontal tissue. *J of Oral Implantol* 39(4):433–443
- Kim YK, Lee SY, Park SJ, Lee SS, Kim JH, Lee HC (2011) Clinical application of mesenchymal stem cells in a dog with intervertebral disc disease. *J Vet Clin* 28:122–127
- Kim S, Zhang H, Guo L, Kim J, Kim MH (2012) Amniotic mesenchymal stem cells enhance wound healing in diabetic NOD/SCID mice through high angiogenic and engraftment capabilities. *PLoS One* 7:1–11
- Kim JW, Lee JH, Lyoo YS, Jung DI, Park HM (2013) The effects of topical mesenchymal stem cell transplantation in canine experimental cutaneous wounds. *Vet Dermatol* 24:242–e53
- Kim Y, Jo S-H, Kim WH, Kweon O-K (2015) Antioxidant and anti-inflammatory effects of intravenously injected adipose derived mesenchymal stem cells in dogs with acute spinal cord injury. *Stem Cell Res Ther* 6:229
- Kisiel AH, McDuffee LA, Masaoud E, Bailey TR, Esparza Gonzalez BP, Nino-Fong R (2012) Isolation, characterization, and in vitro proliferation of canine mesenchymal stem cells derived from bone marrow, adipose tissue, muscle, and periosteum. *Am J Vet Res* 73(8):1305–1317
- Koh E, Jung YC, Woo H-M, Kang B-J (2017) Injectable alginate-microencapsulated canine adipose tissue-derived mesenchymal stem cells for enhanced viable cell retention. *J Vet Med Sci* 79(3):492–501
- Kresic N, Simic I, Lojkić I, Bedeković T (2017) Canine adipose derived mesenchymal stem cells transcriptome composition alterations: A step towards standardizing therapeutic. *Stem Cells Int* 2017:12. Art ID:4176292
- Kriston-Pál É, Czibula Á, Gyuris Z, Balka G, Seregi A, Sükösd F, Süth M, Kiss-Tóth E, Harácska L, Uher F, Monostori É (2017) Characterization and therapeutic application of canine adipose mesenchymal stem cells to treat elbow osteoarthritis. *Can J Vet Res* 81:73–78
- Kumai Y, Kobler JB, Park H, Galindo M, Herrera VL, Zeitels SM (2010) Modulation of vocal fold scar fibroblasts by adipose-derived stem/stromal cells. *Laryngoscope* 120:330–337
- Laura C, Groza I, Oana L, Pall E, Pestean C, Cătană R, Cenariu M (2008) Canine Mesenchymal stem cells isolation from bone marrow aspirates. *Bull UASVM Vet Med* 65(2):96–101
- Lee BJ, Wang SG, Lee JC, Jung JS, Bae YC, Jeong HJ, Kim HW, Lorenz RR (2006) The prevention of vocal fold scarring using autologous adipose tissue-derived stromal cells. *Cells Tissue Organs* 184:184–204
- Lee HB, Chung YS, Heo SY, Kim NS (2009a) Augmentation of bone healing of nonunion fracture using stem cell based tissue engineering in a dog: a case report. *Vet Med* 54(4):198–203

- Lee JH, Chang HS, Kang EH, Chung DJ, Choi CB, Lee JH, Hwang SH, Han H, Kim HY (2009b) Percutaneous transplantation of human umbilical cord blood-derived multipotent stem cells in a canine model of spinal cord injury. *J Neurosurg Spine* 11(6):749–757
- Lee WS, Suzuki Y, Graves SS, Iwata M, Venkataraman GM, Mielcarek M, Peterson LJ, Ikehara S, Torok-Storb B, Storb R (2011a) Canine bone-marrow-derived mesenchymal stromal cells suppress alloreactive lymphocyte proliferation in vitro but fail to enhance engraftment in canine bone marrow transplantation. *Biol Blood Marrow Transplant* 17:465–475
- Lee JH, Chung WH, Kang EH, Chung DJ, Choi CB, Chang HS, Lee JH, Hwang SH, Han H, Choe BY et al (2011b) Schwann cell-like remyelination following transplantation of human umbilical cord blood (hUCB)-derived mesenchymal stem cells in dogs with acute spinal cord injury. *J Neurol Sci* 300:86–96
- Lee JC, Mitchell AWM, Healy JC (2012) Imaging of muscle injuries. *Br J Radiol* 85(1016):1173–1185
- Lee KS, Cha SH, Kang HW, Song JY, Lee KW, Ko KB, Lee HT (2013) Effects of serial passage on the characteristics and chondrogenic differentiation of canine umbilical cord matrix derived mesenchymal stem cells. *Asian Australas J Anim Sci* 26(4):588595
- Lee SH, Kim Y, Rhew D, Kim A, Jo KR, Yoon Y, Choi KU, Jung T, Kim WH, Kweon OK (2016) Impact of local injection of brain-derived neurotrophic factor-expressing mesenchymal stromal cells (MSCs) combined with intravenous MSC delivery in a canine model of chronic spinal cord injury. *Cytherapy* S1465-3249(16):30540
- Lee SH, Kim Y, Rhew D, Kim A, Jo KR, Yoon Y, Choi KU, Jung T, Kim WH, Kweon O-K (2017a) Effect of canine mesenchymal stromal cells overexpressing heme oxygenase-1 in spinal cord injury. *J Vet Sci* 18(3):377–386
- Lee S-J, Ryu M-O, Seo M-S, Park S-B, Ahn J-O, Han S-M, Kang K-S, Bhang D-H, Youn H-Y (2017b) Mesenchymal stem cells contribute to improvement of renal function in a canine kidney injury model. *In Vivo* 31(6):1115–1124
- Lee J, Lee KS, Kim C-L, Byeon JS, Gu N-Y, Cho I-S, Cha S-H (2017c) Effect of donor age on the proliferation and multipotency of canine adipose-derived mesenchymal stem cells. *J Vet Sci* 18(2):141–148
- Lemp MA (2008) Management of dry eye disease. *Am J Manag Care* 14(3):S88–S101
- Li H, Yan F, Lei L, Li Y, Xiao Y (2009) Application of autologous cryopreserved bone marrow mesenchymal stem cells for periodontal regeneration in dogs. *Cells Tissues Organs* 190:94–101
- Li L, Duan X, Fan Z, Chen L, Xing F, Xu Z, Chen Q, Xiang Z (2018) Mesenchymal stem cells in combination with hyaluronic acid for articular cartilage defects. *Sci Rep* 8:9900
- Lim JH, Byeon YE, Ryu HH, Jeong YH, Lee YW, Kim WH, Kang K-S, Kweon O-K (2007) Transplantation of canine umbilical cord blood-derived mesenchymal stem cells in experimentally induced spinal cord injured dogs. *J Vet Sci* 8:275–282
- Lim J-H, Koh S, Thomas R, Breen M, Olby NJ (2017) Evaluation of gene expression and DNA copy number profiles of adipose tissue-derived stromal cells and consecutive neurosphere-like cells generated from dogs with naturally occurring spinal cord injury. *Am J Vet Res* 78:371–380
- Linke A, Ller PM, Nurzynska D, Casarsa C, Torella D, Nascimbene A, Castaldo C, Cascapera S, Böhm M, Quaini F et al (2005) Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function. *PNAS* 102(25):8966–8971
- Linon E, Spreng D, Rytz U, Forterre S (2014) Engraftment of autologous bone marrow cells into the injured cranial cruciate ligament in dogs. *Vet J* 202(3):448–454
- Long C, Lankford L, Kumar P, Grahn R, Borjesson DL, Farmer D, Wang A (2018) Isolation and characterization of canine placenta-derived Mesenchymal stromal cells for the treatment of neurological disorders in dogs. *Cytometry A* 93(1):82–92
- Lorant J, Larcher T, Jaulin N, Hedan B, Lardenois A et al (2018) Vascular delivery of allogeneic MuStem cells in dystrophic dogs requires only short-term immunosuppression to avoid host immunity and generate clinical/tissue benefits. *Cell Transplant Cognizant Commun Corporat* 27(7):1096–1110

- Lu W, Yaoming N, Boli R, Jun C, Changhai Z, Yang Z, Zhiyuan S (2013) mHCN4 genetically modified canine Mesenchymal stem cells provide biological Pacemaking function in complete dogs with Atrioventricular block. *PACE* 36:1138–1149
- Madhu DN, Ahmad RA, Amarpal, Gugjoo MB, Pawde AM, Kinjavedkar P, Aithal HP, Ansari M, Chandra V, Sharma GT (2014) Caudal superficial epigastric axial pattern flap and stem cell therapy for the management of large wound on medial aspect of thigh in a dog. *Adv Anim Vet Sci* 2(3):188–191
- Malagola E, Teunissen M, van der Laan LJ, Verstegen MM, Schotanus BA, van Steenbeek FG, Penning LC, van Wolferen ME, Tryfonidou MA, Spee B (2016) Stem characterization and comparison of canine multipotent stromal cells derived from liver and bone marrow. *Stem Cells Develop* 25(2):139–150
- Martinello T, Bronzini I, Maccatrozzo L, Mollo A, Sampaolesi M, Mascarello F, Decaminada M, Patruno M (2011) Canine adipose-derived-mesenchymal stem cells do not lose stem features after a long-term cryopreservation. *Res Vet Sci* 91(1):18–24
- Marx C, Silveira MD, Selbach I, da Silva AS, Gomes de Macedo Braga LM, Camassola M, Nardi NB (2014) Acupoint injection of autologous stromal vascular fraction and allogeneic adipose-derived stem cells to treat hip dysplasia in dogs. *Stem Cells Int* 2014:391274
- Marycz K, Smieszek A, Grzesiak J, Nicpoń JE (2014) Effects of steroids on the morphology and proliferation of canine and equine mesenchymal stem cells of adipose origin - in vitro research. *Acta Vet Hung* 62(3):317–333
- Matsuda T, Takami T, Sasaki R, Nishimura T, Aibe Diaz Paredes B, Quintanilha LF, Matsumoto T, Ishikawa T, Yamamoto N, Tani K et al (2017) A canine liver fibrosis model to develop a therapy for liver cirrhosis using cultured bone marrow-derived cells. *Hepatol Commun* 1(7):691–703
- Memon IA, Sawa Y, Miyagawa S, Taketani S, Matsuda H (2005) Combined autologous cellular cardiomyoplasty with skeletal myoblasts and bone marrow cells in canine hearts for ischemic cardiomyopathy. *Journal of Thoracic & Cardiovascular Surgery* 130:646–653
- Millis DL, Jackson AM (2003) Delayed union, nonunion, and malunions. 1849–1861. In: Slatter D (ed) *Textbook of small animal surgery*, 3rd edn. W.R. Saunders, Philadelphia, p 2714
- Mokbel A, El-Tookhy O, Shamaa AA, Sabry D, Rashed L, Mostafa A (2011) Homing and efficacy of intra-articular injection of autologous mesenchymal stem cells in experimental chondral defects in dogs. *Clin Exp Rheumatol* 29:275–284
- Muir P, Hans EC, Racette M, Volstad N, Sample SJ, Heaton C, Holzman G, Schaefer SL, Bloom DD, Bleedorn JA et al (2016) Autologous bone marrow-derived Mesenchymal stem cells modulate molecular markers of inflammation in dogs with cruciate ligament rupture. *PLoS One* 11(8):e0159095
- Nam A, Han S-M, Go D-M, Kim D-Y, Seo K-W, Youn H-Y (2017) Long-term management with adipose tissue-derived mesenchymal stem cells and conventional treatment in a dog with Hepatocutaneous syndrome. *J Vet Intern Med* 31:1514–1519
- Neupane M, Chang C-C, Kiupel M, Yuzbasiyan-Gurkan V (2008) Isolation and characterization of canine adipose-derived mesenchymal stem cells. *Tissue Eng A* 14(6):1007–1015
- Nishida H, Nakayama M, Tanaka H, Kitamura M, Hatoya S, Sugiura K, Suzuki Y, Ide C, Inaba T (2011) Evaluation of transplantation of autologous bone marrow stromal cells into the cerebrospinal fluid for treatment of chronic spinal cord injury in dogs. *Am J Vet Res* 72:1118–1123
- Oh HJ, Park JE, Kim MJ, Hong SG, Ra JC, Jo JY, Kang SK, Jang G, Lee BC (2011) Recloned dogs derived from adipose stem cells of a transgenic cloned beagle. *Theriogenology* 75:1221–1231
- Ohno S, Hirano S, Kanemaru S, Kitani Y, Kojima T, Tateya I, Nakamura T, Ito J (2011) Implantation of an atelocollagen sponge with autologous bone marrow-derived Mesenchymal stromal cells for treatment of vocal fold scarring in a canine model. *Ann OtoRhinol Laryngol* 120:401–408
- Ohta M, Suzuki Y, Noda T, Ejiri Y, Dezawa M, Kataoka K, Chou H, Ishikawa N, Matsumoto N, Iwashita Y et al (2004) Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation. *Exp Neurol* 187:266–278
- Omae H, Zhao C, Sun YL (2009) Multilayer tendon slices seeded with bone marrow stromal cells: a novel composite for tendon engineering. *J Orthop Res* 27:937–942

- Park SS, Byeon YE, Ryu HH, Kang BJ, Kim YS, Kim W-H, Kang K-S, Han H-J, Kweon O-K (2011) Comparison of canine umbilical cord blood derived mesenchymal stem cell transplantation times: involvement of astrogliosis, inflammation, intracellular actin cytoskeletal pathways, and neurotrophin-3. *Cell Transplant* 20:1867–1880
- Park SS, Lee YJ, Lee SH, Lee D, Choi K, Kim WH, Kweon OK, Han HJ (2012a) Functional recovery after spinal cord injury in dogs treated with a combination of matrigel and neural-induced adipose-derived mesenchymal stem cells. *Cytherapy* 14:584–597
- Park S-B, Seo M-S, Kim H-S, Kang K-S (2012b) Isolation and characterization of canine amniotic membrane-derived multipotent stem cells. *PLoS One* 7(9):e44693
- Park SE, Jung NY, Lee NK, Lee J, Hyung B, Myeong SH, Kim HS, Suh YL, Lee JI, Cho KR et al (2016) Distribution of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) in canines after intracerebroventricular injection. *Neurobiol Aging* 47:192–200
- Penha EM, Meira CS, Guimarães ET, Mendonça MVP, Gravelly FA, Pinheiro CMB, Pinheiro TMB, Barrouin-Melo SM, Ribeiro-dos-Santos R, Soares MBP (2014) Use of autologous Mesenchymal stem cells derived from bone marrow for the treatment of naturally injured spinal cord in dogs. *Stem Cells Int* 2014:437521
- Pérez-Merino EM, Uson-Casaus JM, Duque-Carrasco J, Zaragoza-Bayle C, Marinas-Pardo L, Hermida-Prieto M, Vilafnca-Compte M, Barrera-Chacon R, Gualtieri M (2015a) Safety and efficacy of allogeneic adipose tissue-derived mesenchymal stem cells for treatment of dogs with inflammatory bowel disease: endoscopic and histological outcomes. *Vet J* 206:391–397
- Pérez-Merino EM, Uson-Casaus JM, Zaragoza-Bayle C, Duque-Carrasco J, Marinas-Pardo L, Hermida-Prieto M, Barrera-Chacon R, Gualtieri M (2015b) Safety and efficacy of allogeneic adipose tissue-derived mesenchymal stem cells for treatment of dogs with inflammatory bowel disease: clinical and laboratory outcomes. *Vet J* 206:385–390
- Perin EC, Silva GV, Assad JA, Vela D, Buja LM, Sousa AL, Litovsky S, Lin J, Vaughn WK, Coulter S, Fernandes MR, Willerson JT (2008) Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. *J Mol Cel Cardiol* 44:486–495
- Pinheiro AO, Cardoso MT, Vidane AS, Casals JB, Passarelli D, Alencar ALF, Sousa RLM, Fantinato-Neto P, Oliveira VC, Lara VM et al (2016) Controversial results of therapy with mesenchymal stem cells in the acute phase of canine distemper disease. *Genet Mol Res* 15(2). <https://doi.org/10.4238/gmr.15028310>
- Pinheiro LL, de Lima AR, Martins DM, de Oliveira EHC, Souza MPC, de Carvalho Miranda CMF, Beltrão-Braga PCB, Russo FB, Conceição Pignatari G, da Silva Filho E, Branco E (2019) Mesenchymal stem cells in dogs with demyelinating leukoencephalitis as an experimental model of multiple sclerosis. *Heliyon* 5(6):e01857
- Pogue B, Estrada AH, Sosa-Samper I, Maisenbacher HW, Lamb KE, Mincey BD, Erger KE, Conlon TJ (2013) Stem-cell therapy for dilated cardiomyopathy: A pilot study evaluating retrograde coronary venous delivery. *J Small Anim Pract* 54:361–366
- Rici REG, Will S, Luna ACL, Melo LF, Santos AC, Rodrigues RF, Leandro RM, Maria DA (2018) Combination therapy of canine osteosarcoma with canine bone marrow stem cells, bone morphogenetic protein and carboplatin in an in vivo model. *Vet Comp Oncol* 16(4):478–488
- Rodriguez-Menocal L, Shareef S, Salgado M, Shabbir A, Van Badiavas E (2015) Role of whole bone marrow, whole bone marrow cultured cells, and mesenchymal stem cells in chronic wound healing. *Stem Cell Res Ther* 6:1–11
- Roszek K, Makowska N, Czarnecka J, Porowska D, Dabrowski M, Danielewska J, Nowak W (2016) Canine adipose-derived stem cells: purinergic characterization and neurogenic potential for therapeutic applications. *J Cell Biochem* 9999:1–8
- Rousseau B, Hirano S, Scheidt TD, Welham NV, Thibeault SL, Ford CN, Bless DM (2003) Characterization of vocal fold scarring in a canine model. *Laryngoscope* 113:620–627
- Ryu HH, Lim JH, Byeon YE, Park JR, Seo MS, Lee YW, Kim WH, Kang KS, Kweon OK (2009) Functional recovery and neural differentiation after transplantation of allogenic adipose-derived stem cells in a canine model of acute spinal cord injury. *J Vet Sci* 10:273–284

- Ryu H-H, Kang B-J, Park S-S, Kim Y, Sung G-J, Woo H-M, Kim WH, Kweon O-K (2012) Comparison of Mesenchymal stem cells derived from fat, bone marrow, Wharton's jelly, and umbilical cord blood for treating spinal cord injuries in dogs. *J Vet Med Sci* 74(12):1617–1630
- Saito F, Nakatani T, Iwase M, Maeda Y, Hirakawa A, Murao Y, Suzuki Y, Onodera R, Fukushima M, Ide C (2008) Spinal cord injury treatment with intrathecal autologous bone marrow stromal cell transplantation: the first clinical trial case report. *J Trauma* 64:53–59
- Sancak IG, Özen A, Pinarli FA, Tiryaki M, Ceylan A, Acar U, Delibaşı T (2014) Limbal stem cells in dogs and cats their identification culture and differentiation into keratinocytes. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 20(6):909–914
- Sarmiento CAP, Rodrigues MN, Bocabello RZ, Mess AM, Miglino MA (2014) Pilot study: bone marrow stem cells as a treatment for dogs with chronic spinal cord injury. *Regenerat Med Res* 2:9
- Sasaki A, Mizuno M, Ozeki N, Katano H, Otabe K, Tsuji K, Koga H, Mochizuki M, Sekiya I (2018) Canine mesenchymal stem cells from synovium have a higher chondrogenic potential than those from infrapatellar fat pad, adipose tissue, and bone marrow. *PLoS One* 13(8):e0202922
- Saulnier N, Loriau J, Febre M, Robert C, Rakic R, Bonte T, Buff S, Maddens S (2016) Canine placenta: A promising potential source of highly proliferative and immunomodulatory mesenchymal stromal cells? *Vet Immunol Immunopathol* 171:47–55
- Sedigh HS, Saffarpour A, Jamshidi S, Ashouri M, Nassiri SM, Sharifi D, Torkzaban P, Kajbafzadeh AM (2010) Canine periodontal stem cells: isolation, differentiation potential and electronic microscopic characterization. *Iranian J Vet Surg* 5(1,2):19–28
- Seo MS, Jeong YH, Park JR, Park SB, Rho KH, Kim HS, Yu KR, Lee SH, Jung JW, Lee YS, Kang KS (2009) Isolation and characterization of canine umbilical cord blood-derived mesenchymal stem cells. *J Vet Sci* 10:181–187
- Seo MS, Park SB, Kang KS (2012) Isolation and characterization of canine Wharton's jelly-derived Mesenchymal stem cells. *Cell Transplant* 21:1493–1502
- Serigano K, Sakai D, Hiyama A, Tamura F, Tanaka M, Mochida J (2010) Effect of cell number on mesenchymal stem cell transplantation in a canine disc degeneration model. *J Orthop Res* 28:1267–1275
- Shah K, Drury T, Roic I, Hansen P, Malin M, Boyd R, Sumer H, Ferguson R (2018) Outcome of allogeneic adult stem cell therapy in dogs suffering from osteoarthritis and other joint defects. *Stem Cells Int* 2018:7309201
- Shi S, Gronthos S (2003) Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J Bone Miner Res* 18:696–704
- Silva GV, Litovsky S, Assad JA, Sousa AL, Martin BJ, Vela D, Coulter SC, Lin J, Ober J, Vaughn WK et al (2005) Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model. *Circulation* 111:150–156
- Simsek SB, Keles GC, Baris S, Cetinkaya BO (2012) Comparison of mesenchymal stem cells and autogenous cortical bone graft in the treatment of class II furcation defects in dogs. *Clin Oral Investig* 16:251–258
- Song J, Kim Y, Kweon O-K, Kang B-J (2017) Use of stem-cell sheets expressing bone morphogenetic protein-7 in the management of a nonunion radial fracture in a toy poodle. *J Vet Sci* 18(4):555–558
- Sousa MG, Paulino-Junior D, Pascon JPE, Pereira-Neto GB, Carareto R, Champion T, Camacho AA (2011) Cardiac function in dogs with chronic Chagas cardiomyopathy undergoing autologous stem cell transplantation into the coronary arteries. *Can Vet J* 52(8):869–874
- Spencer ND, Chun R, Vidal MA, Gimble JM, Lopez MJ (2012) In vitro expansion and differentiation of fresh and revitalized adult canine bone marrow-derived and adipose tissue-derived stromal cells. *Vet J* 191(2):231–239
- Starkey MP, Scase TJ, Mellersh CS, Murphy S (2005) Dogs really are man's best friend—canine genomics has applications in veterinary and human medicine! *Brief Funct Genomic Proteomic* 4:112–128

- Steffen F, Bertolo A, Affentranger R, Ferguson SJ, Stoyanov J (2018) Treatment of naturally degenerated canine lumbosacral intervertebral discs with autologous Mesenchymal stromal cells and collagen microcarriers: A prospective clinical study. *Cell Transplant*. <https://doi.org/10.1177/0963689718815459>
- Stevenson W, Chauhan SK, Dana R (2012) Dry eye disease: an immune-mediated ocular surface disorder. *Arch Ophthalmol* 130(1):90–100
- Suaid FF, Ribeiro FV, Gomes TRLES, Silvério KG, Carvalho MD, Nociti FH Jr, Casati MZ, Sallum EA (2011) Autologous periodontal ligament cells in the treatment of class III furcation defects: a study in dogs. *J Clin Periodontol* 39:377–384
- Suaid FF, Ribeiro FV, Silva Gomes TRLE, Gonzales Silvério K, Carvalho MD, Nociti FH Jr, Casati MZ, Antônio SE (2012) Autologous periodontal ligament cells in the treatment of class III furcation defects: a study in dogs. *J Clin Periodontol* 39(4):377–384
- Sultana T, Lee S, Yoon H-Y, Lee JI (2018) Current status of canine umbilical cord blood-derived Mesenchymal stem cells in veterinary medicine. *Stem Cells Int* 2018:8329174
- Taguchi T, Borjesson DL, Osmond C, Griffon DJ (2019) Influence of Donor's age on Immunomodulatory properties of canine adipose tissue-derived Mesenchymal stem cells. *Stem Cells Dev* 28(23):1562–1571
- Takemitsu H, Zhao D, Yamamoto I, Harada Y, Michishita M, Arai T (2012) Comparison of bone marrow and adipose tissue-derived canine mesenchymal stem cells. *BMC Vet Res* 8:150
- Takewaki M, Kajiya M, Takeda K, Sasaki S, Motoike S, Komatsu N, Matsuda S, Ouhara K, Mizuno N, Fujita T et al (2017) MSC/ECM cellular complexes induce periodontal tissue regeneration. *J Dent Res* 96(9):984–991
- Taroni M, Cabon Q, Fèbre M, Cachon T, Saulnier N, Carozzo C, Maddens S, Labadie F, Robert C, Viguier E (2017) Evaluation of the effect of a single intra-articular injection of allogeneic neonatal Mesenchymal stromal cells compared to Oral non-steroidal anti-inflammatory treatment on the postoperative musculoskeletal status and gait of dogs over a 6-month period after Tibial plateau leveling osteotomy: A pilot study. *Front Vet Sci* 4:83
- Teshima T, Matsumoto H, Koyama H (2018) Soluble factors from adipose tissue derived mesenchymal stem cells promote canine hepatocellular carcinoma cell proliferation and invasion. *PLoS One* 13(1):e0191539
- Tharasanit T, Phutikanit N, Wangdee C, Soontornvipart K, Tantrajak S, Kaewamatawong T, Suwimonterabutr J, Supaphol J, Techakumphu M (2011) Differentiation potentials of canine bone marrow Mesenchymal stem cells. *Thailand J Vet Med* 41(1):79–86
- Tobita M, Uysal CA, Guo X, Hyakusoku H, Mizuno H (2013) Periodontal tissue regeneration by combined implantation of adipose tissue-derived stem cells and platelet-rich plasma in a canine model. *Cytotherapy* 15(12):1517–1526
- Trindade AB, Therrien J, Garcia JM, Smith LC (2017) Mesenchymal-like stem cells in canine ovary show high differentiation potential. *Cell Prolif* 50(6):e12391
- Tsumanuma Y, Iwata T, Washio K, Yoshida T, Yamada A, Takagi R, Ohno T, Lin K, Yamato M, Ishikawa I et al (2011) Comparison of different tissue-derived stem cell sheets for periodontal regeneration in a canine 1-wall defect model. *Biomaterials* 32:5819–5825
- Uranio MF, Valentini L, Lange-Consiglio A, Caira M, Guaricci AC, L'abbate A, Catacchio CR, Ventura M, Cremonesi F, Dell'aquila ME (2011) Isolation, proliferation, cytogenetic, and molecular characterization and in vitro differentiation potency of canine stem cells from Foetal adnexa: A comparative study of amniotic fluid, amnion, and umbilical cord matrix. *Mol Reprod Develop* 78:361–373
- Uranio MF, Dell'aquila ME, Caira M, Guaricci AC, Ventura M, Catacchio C, Martino NA, Valentini L (2014) Characterization and in vitro differentiation potency of early-passage canine amnion- and umbilical cord-derived mesenchymal stem cells as related to gestational age. *Mol Reprod Develop* 81:539–551
- Vieira NM, Brandalise V, Zucconi E, Secco M, Strauss BE, Zatz M (2010) Isolation, characterization, and differentiation potential of canine adipose-derived stem cells. *Cell Transplant* 19:279–289

- Vilar JM, Morales M, Santana A, Spinella G, Rubio M, Cuervo B, Cugat R, Carrillo JM (2013) Controlled, blinded force platform analysis of the effect of intraarticular injection of autologous adipose-derived Mesenchymal stem cells associated to PRGF-Endoret in osteoarthritic dogs. *BMC Vet Res* 9:131
- Vilar JM, Batista M, Morales M, Santana A, Cuervo B, Rubio M, Cugat R, Sopena J, Carrillo JM (2014) Assessment of the effect of intraarticular injection of autologous adipose-derived mesenchymal stem cells in osteoarthritic dogs using a double blinded force platform analysis. *BMC Vet Res* 10:143
- Villatoro AJ, Fernández V, Claros S, Rico-Llanos GA, Becerra J, Andrades JA (2015) Use of adipose-derived Mesenchymal stem cells in Keratoconjunctivitis Sicca in a canine model. *Biomed Res Int* 2015:527926
- Villatoro AJ, Hermida-Prieto M, Fernández V, Fariñas F, Alcoholado C, Rodríguez-García MI, Mariñas-Pardo L, Becerra J (2018) Allogeneic adipose-derived mesenchymal stem cell therapy in dogs with refractory atopic dermatitis: clinical efficacy and safety. *Vet Record* 183(21):654. <https://doi.org/10.1136/vr.104867>
- Volk SW, Wang Y, Hankenson KD (2012) Effects of donor characteristics and ex vivo expansion on canine Mesenchymal stem cell properties: implications for MSC-based therapies. *Cell Transplant* 21:2189–2200
- Wei N, Gong P, Liao D, Yang X, Li X, Liu Y, Yuan Q, Tan Z (2010) Auto transplanted mesenchymal stromal cell fate in periodontal tissue of beagle dogs. *Cytotherapy* 12:514–521
- Wei Y, Fang J, Cai S, Lv C, Zhang S, Hua J (2016) Primordial germ cell-like cells derived from canine adipose mesenchymal stem cells. *Cell Prolif* 49(4):503–511
- William JB, Prabakaran R, Ayyappan S, Puskhinraj H, Rao D, Manjunath SR, Thamaraikannan P, Dedeepiya VD, Kuroda S, Yoshioka H et al (2011) Functional recovery of spinal cord injury following application of Intralesional bone marrow mononuclear cells embedded in polymer scaffold—two year follow-up in a canine. *J Stem Cell Res Ther* 1:110
- Wu S, Suzuki Y, Ejiri Y, Noda T, Bai H, Kitada M, Kataoka K, Ohta M, Chou H, Ide C (2003) Bone marrow stromal cells enhance differentiation of cocultured neurosphere cells and promote regeneration of injured spinal cord. *J Neurosci Res* 72:343–351
- Yaneselli KM, Kuhl CP, Terraciano PB, de Oliveira FS, Pizzato SB, Pazza K, Magrisso AB, Torman V, Rial A, Moreno M, Llambí S, Cirne-Lima E, Maisonnave J (2018) Comparison of the characteristics of canine adipose tissue-derived mesenchymal stem cells extracted from different sites and at different passage numbers. *J Vet Sci* 19(1):13–20
- Yang Q, Peng J, Lu SB, Guo QY, Zhao B, Zhang L, Wang AY, Xu WJ, Xia Q, Ma XL, Hu YC, Xu BS (2011) Evaluation of an extracellular matrix-derived acellular biphasic scaffold/cell construct in the repair of a large articular high-load-bearing osteochondral defect in a canine model. *Chin Med J (Engl)* 124:3930–3938
- Yang H-M, Song W-J, Qiang L, Kim S-Y, Kim H-J, Ryu M-O, Ahn J-O, Youn H-Y (2018) Canine mesenchymal stem cells treated with TNF- α and IFN- γ enhance anti-inflammatory effects through the COX-2/PGE2 pathway. *Res Vet Sci* 119:19–26
- Yoon HY, Lee JH, Jeong SW (2012) Long-term follow-up after implantation of autologous adipose tissue derived mesenchymal stem cells to treat a dog with stifle joint osteoarthritis. *J Vet Clin* 29:82–86
- Zeira O, Asiag N, Aralla M, Ghezzi E, Pettinari L, Martinelli L, Zahirpour D, Dumas MP, Lupi D, Scaccia S et al (2015) Adult autologous mesenchymal stem cells for the treatment of suspected non-infectious inflammatory diseases of the canine central nervous system: safety, feasibility and preliminary clinical findings. *J Neuroinflammation* 12:181
- Zhan X-S, El-Ashram S, Luo D-Z, Luo H-N, Wang B-Y, Chen S-F, Bai Y-S, Chen Z-S, Liu C-Y, Ji H-Q (2019) A comparative study of biological characteristics and transcriptome profiles of mesenchymal stem cells from different canine tissues. *Int J Mol Sci* 20:1485
- Zhang LF, Qi J, Zuo G, Jia P, Shen X, Shao J, Kang H, Yang H, Deng L (2014) Osteoblast-secreted factors promote proliferation and osteogenic differentiation of bone marrow stromal cells via VEGF/heme-oxygenase-1 pathway. *PLoS One* 9(6):e99946

- Zhu S, Lu Y, Zhu J, Xu J, Huang H, Zhu M, Chen Y, Zhou Y, Fan X, Wang Z (2011) Effects of intrahepatic bone-derived mesenchymal stem cells autotransplantation on the diabetic beagle dogs. *J Surg Res* 168:213–223
- Zubin E, Conti V, Leonardi F, Zanichelli S, Ramoni R, Grolli S (2015) Regenerative therapy for the management of a large skin wound in a dog. *Clin Case Rep* 3(7):598–603
- Zucconi E, Vieira NM, Bueno DF, Secco M, Jazedje T, Ambrosio CE, Passos-Bueno MR, Miglino MA, Zatz M (2010) Mesenchymal stem cells derived from canine umbilical cord vein—a novel source for cell therapy studies. *Stem Cells Develop* 19:395–402



Equine Mesenchymal Stem Cell Basic Research and Potential Applications

12

M. B. Gugjoo, Amar Pal, D. M. Makhdoomi,
and G. T. Sharma

Abstract

Regenerative medicine incorporating stem cells is being extensively studied to develop therapeutics for various incurable diseases. Mesenchymal stem cells (MSCs) due to their special features and easy isolation procedures are mostly being employed for therapeutic applications. These cells with or without other biomaterials have been studied under in vivo experimental and clinical trials of horse. MSCs therapeutic potential is seen to transgress their conventionally restricted healing potential for musculoskeletal system. The results of equine studies potentially can be construed for human medicine. The current chapter highlights horse MSCs sources, properties, and potential therapeutic applications.

M. B. Gugjoo (✉)

Division of Veterinary Clinical Complex, FVSc & AH, SKUAST-Kashmir,
Jammu and Kashmir, India

A. Pal

Division of Surgery, Indian Veterinary Research Institute, Izatnagar, UP, India

D. M. Makhdoomi

Division of Surgery and Radiology, FVSc & AH, SKUAST-K,
Srinagar, Jammu and Kashmir, India

G. T. Sharma

Division of Physiology and Climatology, Indian Veterinary Research Institute,
Izatnagar, UP, India

© The Editor(s) (if applicable) and The Author(s), under exclusive license to
Springer Nature Singapore Pte Ltd. 2020

M. B. Gugjoo, A. Pal (eds.), *Mesenchymal Stem Cell in Veterinary Sciences*,
https://doi.org/10.1007/978-981-15-6037-8_12

12.1 Introduction

Equine industry is one of the biggest industries be it in the form of racing, polo, or for recreational and transportation purposes. In racehorses, musculoskeletal disorders reduce their performance and even destroy their carrier (Perkins et al. 2005). Among soft tissue ailments, skin wounds, eye affections, and reproductive disorders pose a significant problem to clinicians. Various horse and human developmental and autoimmune ailments have comparable pathophysiology (Gershwin 2007). Horse and human cartilage thickness and physiological features approximate to each other (Aigner et al. 2010). The cartilage defects associated with intense physical activity too resemble (Desancé et al. 2018). Thus, horse can make an excellent model animal to provide proof-of-therapeutic principle of mesenchymal stem cells (MSCs) in human medicine (McIlwraith et al. 2011; De Schauwer et al. 2013; Gugjoo et al. 2019b). Tissue engineering employing MSCs is being studied extensively. MSCs meager population in adult tissues/organs makes their culture expansion necessary for effective utilization. Innumerable in vitro studies have piled upon equine MSCs (eMSCs). These studies have been conducted on their culture characteristics, characterization, and their therapeutic properties. The in vivo studies have supported the therapeutic potentials of MSCs, although variably (Carrade et al. 2011a, b; Spaas et al. 2012; Burk et al. 2013) with mechanism(s) behind the healing largely being unrevealed. The current chapter deliberates on the eMSCs ex vivo properties and their potential therapeutic applications.

12.2 In Vitro Studies

12.2.1 Mesenchymal Stem Cell Sources, Culturing, and Characterization

Horse MSCs (eMSCs) have diverse sources ranging from adult tissues to fetal membranes as detailed in Table 12.1. Donor tissues usually harbor small fraction of MSCs and are isolated along with the other cell types including progenitor cells (Chong et al. 2011). The characterization of eMSCs has been made as per the standard protocol of the International Society for Cell Therapy (ISCT) (Dominici et al. 2006). eMSCs and human MSCs tend to have comparable marker expression and differentiation potential (Hillmann et al. 2016). eMSCs invariably follow the plastic adherence and are multipotent (Guest et al. 2008; Ranera et al. 2011; De Schauwer et al. 2012). Their surface marker expression, however, remains controversial as these cells variably express different surface markers and lack expression of certain markers. This discrepancy in marker expression has mainly been observed in relation to the tissue source as shown in Table 12.1. eMSCs also express pluripotency markers like Oct4, Sox-2, and Nanog and show extended differentiation like neural cell-like cells, myogenic cell-like cells, tenocyte-like cells, and hepatocyte-like cells (Violini et al. 2009; Coli et al. 2011; Gomiero et al. 2016; Pennington et al. 2016; Cabezas et al. 2018).

Table 12.1 Horse mesenchymal stem cell sources and their characterization

Positive markers	Negative markers	Differentiation	References
<i>Adipose tissue MSCs (AD-MSCs)</i>			
CD29, CD44, CD105, CD90 (limited expression)	CD45	Osteogenic and adipogenic	Marycz et al. (2012)
CD29, CD44, and CD90	MHC-II, CD34	Adipogenic, chondrogenic, and osteogenic	Alipour et al. (2015)
CD29, CD44, CD105 (87%)	–	Adipogenic, chondrogenic, and osteogenic	Xie et al. (2013)
CD73, CD90, CD105		Adipogenic, chondrogenic, and osteogenic	González-Fernández et al. (2016)
CD105, CD90, CD49, CD44	CD43, CD73	Adipogenic, chondrogenic, and osteogenic	Pall et al. (2016)
CD90, CD105, CD44, CD73, CD13, CD133, CD29, OCT-4	CD45, CD34, CD31, SDF-1 receptor CXCR4	–	Angelone et al. (2017)
CD44, CD90, CD105	CD45	Adipogenic, chondrogenic, and osteogenic	Nawrocka et al. (2017)
CD29, CD44, CD90, CD105, CD166	CD34, CD45, and CD79a	Chondrogenic and osteogenic	Bundgaard et al. (2018)
CD29, CD44, CD90, CD-105, MHC-I	MHC-II, CD45, OCT4, Sox2	Adipogenic, chondrogenic, and osteogenic	Cabezas et al. (2018)
CD90, CD105, Oct4		Osteogenic	Elashry et al. (2018)
<i>Amniotic membrane MSCs (am-MSCs)</i>			
CD90, CD73, CD34, Oct4 (weak), IL-6, IL- β 1, IL-8 (weak)	MHC-I, MHC-II, CD45, Sox2, Nanog, INF- γ , TNF- α , IL-4	Adipogenic, chondrogenic, and osteogenic	Iacono et al. (2017)
CD29, CD44, CD106, CD105	CD34, MHC-II	Adipogenic, chondrogenic, osteogenic, and neurogenic	Perrini et al. (2016)
<i>Bone marrow MSCs (BM-MSCs)</i>			
CD29, CD44, CD172a	CD34, CD45	–	Fortier et al. (2010)
CD29, CD44, CD105 (64%)	–	Adipogenic, chondrogenic, and osteogenic	Xie et al. (2013)
CD11a/18, CD34, CD44, CD90		Adipogenic, chondrogenic, and osteogenic	Lombana et al. (2015)

(continued)

Table 12.1 (continued)

Positive markers	Negative markers	Differentiation	References
CD90, CD29	MHC-II, CD44, CD45RB	Adipogenic, chondrogenic, and osteogenic	Mitchell et al. (2015)
CD90, CD105, CD44, CD73	CD34, CD45	Adipogenic, chondrogenic, and osteogenic	Barrachina et al. (2016)
CD90, CD44, CD29	MHC-II, CD86	—	Clark et al. (2016)
CD73, CD90, CD105		Adipogenic, chondrogenic, and osteogenic	González-Fernández et al. (2016)
CD44, CD29, CD90, CD73 (at p3 and p4 only), CD105 (at P3 25% expression)	CD34, MHC-II, CD105 (P4)	Adipogenic, chondrogenic, and osteogenic	Branly et al. (2017)
CD29, CD44, CD90, MHC-I, Sox2	CD34, CD105, MHC-II, Nanog, Oct4, SSEA-1, -3, -4	Adipogenic, chondrogenic, and osteogenic	Zahedi et al. (2018)
CD29, CD44, CD90, CD105, CD166	CD34, CD45, and CD79a	Chondrogenic and osteogenic	Bundgaard et al. (2018)
CD29, CD90	CD34, CD45, CD14, CD73, and CD105, CD79a, DRB, NANOG, and SOX2	Adipogenic, chondrogenic, osteogenic, and tenogenic	Korchunjit et al. (2019)
CD44, CD90, MHC-I, CD105 (33.9%)	CD11a/CD18, MHC-II	Chondrogenic and osteogenic	Schrock et al. (2018)
CD29, CD44, CD90, CD105, and MHCI, MHC-II	CD45 and CD79a	Chondrogenic	Gale et al. (2019)
<i>Dental pulp MSCs (DP-MSCs)</i>			
CD11a/CD18, CD44, CD90, CD105, MHC-I, MHC-II	CD34, CD45	Adipogenic, chondrogenic, and osteogenic	Ishikawa et al. (2017)
<i>Dermal MSCs</i>			
CD90, CD105 (48%), CD29, CD44	CD34, CD45, CD79 α , CD14, MHC-II, CD73	—	Michler et al. (2018)
<i>Endometrium MSCs (end-MSCs)</i>			
CD29, CD44, CD105, CD90, Pericyte (NG2, CD146)	CD34, CD45, MHC-II	Adipogenic, chondrogenic, osteogenic, and smooth muscle	Rink et al. (2017)
CD29, CD44, CD90, CD-105, MHC-I	MHC-II, CD45, OCT4, Sox2	Adipogenic, chondrogenic, and osteogenic	Cabezas et al. (2018)

(continued)

Table 12.1 (continued)

Positive markers	Negative markers	Differentiation	References
<i>Gingiva MSCs</i>			
CD90, CD105, Vimentin	CD31	Adipogenic, chondrogenic, and osteogenic	Mensing et al. (2011)
<i>Hoof MSCs (H-MSCs)</i>			
CD44, CD29, CD105, Oct4, Sox2, keratin (K)14, K15, K19	–	Adipogenic, osteogenic, and neurogenic	Yang and Lopez (2019)
<i>Peripheral blood MSCs (PB-MSCs)</i>			
CD90, CD105 (40.5%), CD44, MHC-I (60.2%), CD11a/CD18 (42.1%)	CD34, CD45, MHC-II	Adipogenic, chondrogenic, and osteogenic	Ishikawa et al. (2017)
<i>Periodontal ligament (PL-MSCs)</i>			
CD90, CD105, Vimentin	CD31	Adipogenic, chondrogenic, and osteogenic	Mensing et al. (2011)
CD44, CD29, MHC-II			Beerts et al. (2017)
<i>Synovial fluid MSCs (SF-MSCs)</i>			
CD45, CD105, CD90, CD34, CD117, CD133, TRAI-1-81, VEGF, LY6A, OCT3/4, Nanog	–	–	Prado et al. (2015)
CD90, CD44, PCNA, Vimentin, PGP-9.5, lysozyme	–	Adipogenic, chondrogenic (weak), and osteogenic	Fülber et al. (2016)
<i>Synovial membrane MSCs (Sy-MSCs)</i>			
CD90, CD44, PCNA, Vimentin, PGP-9.5, lysozyme		Adipogenic, chondrogenic (weak), and osteogenic	Fülber et al. (2016)
CD44 and CD90, MHC-I, CD11a/18, CD105, MHC-II (weak), Sox2	CD34, CD45.	Chondrogenic, osteogenic, and tenogenic	Yamasaki et al. (2018)
<i>Umbilical cord MSCs (UC-MSCs)</i>			
CD44, CD90 (weak)	MHC-II, CD34, Oct4	Osteogenic and adipogenic	Maia et al. (2017)
<i>Wharton's jelly MSCs (WJ-MSCs)</i>			
CD90, CD73, CD34, Oct4, IL-6, IL-β1, IL-8	MHC-I, MHC-II, CD45, Sox2, Nanog, INF-γ, TNF-α, IL-4	Adipogenic, chondrogenic, and osteogenic	Iacono et al. (2016)

AD-MSCs adipose tissue MSCs, *Am-MSCs* amniotic membrane MSCs, *BM-MSCs* bone marrow MSCs, *DP-MSCs* dental pulp MSCs, *End-MSCs* endometrium MSCs, *H-MSCs* hoof MSCs, *PB-MSCs* peripheral blood MSCs, *PL-MSCs* periodontal ligament, *SF-MSCs* synovial fluid MSCs, *Sy-MSCs* synovial membrane MSCs, *UC-MSCs* umbilical cord MSCs, *WJ-MSCs* Wharton's jelly MSCs

12.2.2 Tissue Source and MSCs Features

Peripheral blood (PB) contains the lowest concentration of eMSCs (ePB-MSCs), and as such the success rate to harvest these cells from such a tissue is lower. ePB-MSCs harvesting rate too varies and had been demonstrated to range from as low as 33% (Koerner et al. 2006), 44% (Martinello et al. 2010) and to the maximum of 66% (Carvalho et al. 2013b). Their differentiation potential too had been limited in comparison to horse bone marrow-derived MSCs (eBM-MSCs) (Koerner et al. 2006). The initial cell concentration of stromal vascular fraction (SVF) and/or mononuclear cell fraction (MNC) plays an important role to achieve better MSCs yield. Horse adipose tissue (eAD)-stromal vascular fraction (SVF) had ranged from 1.47×10^6 cells to 2.71×10^6 cells per gram of tissue (Nixon et al. 2008; Tuemmers et al. 2012) while adult horse bone marrow had yielded $6.4 \pm 3.4 \times 10^6$ nucleated cells (MNCs) per ml of aspirate (Hildebrand et al. 2002; Baghavan et al. 2009). MSCs yield from horse bone marrow (BM) remains lower than the adipose tissue (AD) (Toupadakis et al. 2010; Stewart and Stewart 2011).

Proliferation potential of eMSCs varies with tissue source. Bone marrow (BM)-derived and adipose tissue (AD)-derived MSCs tend to show higher proliferation *in* comparison to umbilical cord (UC)-derived MSCs (Barberini et al. 2014). MSCs harvested from a particular tissue system may be more tenable in relevant ailments. eBM-MSCs tend to show superior musculoskeletal tissue differentiation ability as compared to eAD-MSCs (Noel et al. 2008; Vidal et al. 2008; Frisbie et al. 2009; Toupadakis et al. 2010; Branly et al. 2017). Even among similar tissue/organ system, MSCs differentiation potential may vary. Synovial (Sy)-MSCs and BM-MSCs had comparable chondrogenic potential, but the latter cell type had better osteogenic potential (Gale et al. 2019). For musculoskeletal ailments, eBM-MSCs in comparison to eAD-MSCs may give better response. Altered sensitivity of eAD-MSCs to osteogenic growth factors like transforming growth factor (TGF- β) and bone morphogenetic protein (BMP) may predispose them to reduced chondrogenic differentiation. Such a reduction in chondrogenesis may be potentiated with BMP-6 (Estes et al. 2006; Hennig et al. 2007). eMSCs from a particular tissue but from varied locations (ilium vs. sternum) may not show any observable difference (Lombana et al. 2015).

12.2.3 Donor Health Status and MSCs Properties

Individual health status affects cell viability and as such may affect clinical results. AD-MSCs harvested from metabolic syndrome (insulin resistance disease) horses may be more senescent with an insubstantial differentiation potential. Oxidative stress that promotes accumulation of toxic compounds in the mitochondria might lead to the cell senescence. Autophagic switch to counter oxidative stress might decrease MSCs differentiation potential (Marycz et al. 2016a, b). *In vitro* application of *Spirulina platensis* had restored senescent eAD-MSCs morphology and functionality and could have been possible through the reduction in oxidative stress

(Nawrocka et al. 2017). eSy-MSCs harvested from osteoarthritic/osteocondrosis and healthy tissue tend to show comparable phenotype and differentiation potential except for chondrogenesis, being better in healthy tissue-derived MSCs (Fülber et al. 2016). In clinical settings, the problem can be countered with the implantation of higher cell doses (Frisbie and Smith 2010)

12.2.4 Effect of Aspiration and Implantation on MSCs

Cell aspiration/implantation technique affects the cell viability. The compromise in cell viability may be more during aspiration than during the injection. The aspiration creates more negative pressure leading to cell stress, and as such forcible aspiration of MSCs should be prevented (Williams et al. 2016; Lang et al. 2017). Injection of cells through wide bore needles (19-gauge) had not affected eMSCs viability, while those injected through small bore (23-gauge) had become apoptotic. The current recommended needle size of 21-gauge needle balances the cell viability and needle-induced damage at the site of injection (Garvican et al. 2016). However, the consensus on these findings is lacking (Williams et al. 2016). The material of transport/storage container, plastic, or glass had nonsignificant effect on the cell viability (Espina et al. 2016).

12.2.5 Effect of Drugs/Anesthetics on MSCs

Anesthetics and/drugs affect MSCs viability based on their class (Edmonds et al. 2016; Schrock et al. 2018). Romifidine and mepivacaine had nonsignificant effect, while detomidine and butorphanol had significantly reduced MSCs viability. Xylazine had also decreased MSCs viability but insignificantly. Commonly used steroids like methylprednisolone and triamcinolone may compromise eBM-MSCs viability (Edmonds et al. 2016). Therapeutic concentrations of antibiotics like aminoglycosides (gentamicin and amikacin) had deleteriously affected eBM-MSCs, while hyaluronic acid had proven beneficial for their proliferation (Bohannon et al. 2013).

12.3 Clinical Applications of eMSCs

Stem cell therapy in horse medicine mostly remains in experimental phase due to lack of the literature incorporating uniform case controlled studies (Imamura and Tanaka 2009; Clegg and Pinchbeck 2011; Volk and Theoret 2013; Dehghan et al. 2015; Ojeh et al. 2015). In horses, the therapeutic applications of stem cells had been mainly aimed to address the issues of musculoskeletal system and first of its kind had been conducted on superficial digital flexor tendonitis (Smith et al. 2003). As the potential goes beyond, MSCs potential applications are now being evaluated

in nonmusculoskeletal tissues as well (Gugjoo et al. 2019a). Below are given the details of the in vivo studies conducted in horse.

12.3.1 Wound Repair

Horse wounds are predisposed to issues like proud flesh and/infections. The current standard therapeutic options heal only about 24% of wounds by primary healing (Wilmink et al. 2002). The limited availability of donor skin and more failures in “graft take” under grafting makes it a least opted therapeutic solution (Theoret 2009). Potential applications of stem cells in wound healing had come to the fore when a higher number of the cells exhibiting similar phenotype to that of BM-MSCs were observed in blood of burn patients (Mansilla et al. 2006; Fox et al. 2008). Since then stem cell therapies are being evaluated.

Ex vivo horse study had demonstrated that encapsulated MSCs and their conditioned media might promote the repair of skin wounds. The repair could occur through equine dermal fibroblast mobilization and increased pro-wound healing gene expression (Bussche et al. 2015). In vivo implanted eBM-MSCs tend to remain well oriented and integrated under the epithelium of repaired soft palate (Carstanjen et al. 2006). The locally injected allogeneic equine umbilical cord blood (eUCB)-MSCs had improved wound healing of hind limb wounds. The cell-injected wounds had exhibited better tissue regeneration with little inflammatory cells. The expression of healing factors (transforming growth factor- β 1 and cyclooxygenase 2) was significantly higher in cell-treated wounds as compared to the control. MSCs healing potential was unaffected by their culture technique (normoxia vs. hypoxia) (Table 12.2) (Textor et al. 2017).

In clinical studies (foal deep sore wounds and decubital ulcers), local implantation of allogeneic amnion-derived MSCs (Am-MSCs) along with carboxymethyl-cellulose (Iacono et al. 2016) or platelet-rich plasma (Table 12.3) (Iacono et al. 2012) had led to earlier and improved wound healing as compared to wounds treated without the cells. The cells had been implanted multiple times and along with the other biomaterials (Iacono et al. 2016). The combined application (involving local and parenteral injection) of PB-MSCs had improved wound healing of 4 chronic dermal wounds of adult horse metatarsals. Granulation tissue had formed significantly much earlier, while scar formation was delayed in comparison to the control (Spaas et al. 2013). Thus, MSCs may be employed to repair equine wounds. Improved healing upon the MSCs transplantation might arise through regeneration of epidermal cells and/increased angiogenesis (Krause et al. 2001; Wu et al. 2007; Borena et al. 2009). However, all these studies have mostly been uncontrolled, and as such evidence-based studies involving large significant numbers of cases are desired.

Table 12.2 In vivo preclinical experimental mesenchymal stem cell studies on cutaneous wounds in horse

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Bilateral distal forelimb full thickness wounds (3 wounds; each 2.5 × 2.5 cm ² spaced at 2.5 cm vertically aligned)	6 horses (each wound in horse treated with hypoxic- and normoxia-preconditioned cells. Other wounds were treated with above cell types laden in autologous fibrin gel. Control with one saline and other fibrin gel-treated wounds	6 weeks	Umbilical cord blood MSCs	15–20 × 10 ⁶ per wound	Histology, thermography, gene expression, and wound surface area	Cell treated irrespective of prior preconditioning improved histological scoring and wound area. No significant advantage of hypoxic conditioning. An improved transforming growth factor beta and cyclooxygenase-2 gene expression at 1 week. Histology showed wounds treated with MSCs more of pro-healing than pro-inflammatory	Textor et al. (2017)

Table 12.3 In vivo clinical mesenchymal stem cell studies on cutaneous wounds/eye affection in horse

Clinical case	Number of animals	Study type	Study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Soft palate defect	1	Case report	14 days	BM-MSCs	10×10^6	Macroscopy, histochemistry	The cells were organized in a manner similar to that in native soft palate tissue, indicating successful engraftment	Carstanjen et al. (2006)
Decubital ulcers (septic neonatal foal)	1 (multiple sores)	Case report	7 months	Amniotic fluid-derived MSCs, platelet-rich plasma (PRP)	Ulcers were treated for 15 days with mesenchymal stem cells (MSCs) plus PRP, PRP gel alone, or aloe vera gel. 5×10^6 MSCs (applied twice a week for 4 consecutive times)	Wound area measurement	MSCs along with platelet-rich plasma (PRP) gel improve decubital ulcers earlier and effectively compared to those implanted with PRP or aloe vera gel only. At 7 months, an ulcer treated with aloe gel was still not completely healed	Iacono et al. (2012)

Chronic dermal wounds of metatarsals (unresponsive to conventional therapies)	4	Case report	4 weeks and 8 weeks Uncontrolled study	PB-MSCs	5×10^5 (local), 1.25×10^5 (intravenous)	Wound-healing parameters (granulation tissue, crust formation, and scar formation)	MSCs injected locally as well as parenterally in 4 wounds showed positive visual and clinical outcome. Granulation tissue was formed within 4 weeks of the cell application, significantly earlier compared to the control. The scar formation, however, was delayed by more than 2 months as compared to the control	Spaas et al. (2013)
---	---	-------------	---	---------	--	--	---	---------------------

(continued)

Table 12.3 (continued)

Clinical case	Number of animals	Study type	Study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Deep sore wounds	7 (9 spontaneous pressure sores on the carpus (4), fetlock (2), and hock (3) of 5 hospitalized foals	Case report	Case controlled (criteria for selection of cases: Assessing classification of skin wounds, classification of the National Pressure Ulcer Advisory Panel)	Carboxymethyl/cellulose-seeded heterologous amnion-derived MSCs	5×10^5 cells/cm ² (applied twice a week for 4 consecutive times)	Wound area measurement	Cell seeded scaffold implantation led to faster dermal wound healing as compared to control (applied commercial preparation of formosulfathiazole)	Iacono et al. (2016)

Corneal ulcers and retinal detachment	4 (corneal ulcers) and 1 (bilateral retinal detachment)	Case reports	6 months	PB-MSCs	Ophthalmic artery and locally	Clinical examination, ultrasound	After cell treatment, animals were active and able to jump. PB-MSCs improved the optic nerve function and visual acuity in the left eye after 2 months. The animal could even jump and cross barriers after 6 months. However, no substantial improvement was seen in the right eye	Marfe et al. (2012)
--	---	-----------------	----------	---------	-------------------------------------	--	---	---------------------------

12.3.2 Keratitis and Other Ophthalmic Conditions

Laterally placed large eyes make horse conducive to their injuries (Wada et al. 2010; Clode and Matthews 2011). Corneal wounds, especially of ulcerative keratitis, account 68.5% (Andrew and Willis 2005) to 90.5% (Wada et al. 2010) of the ocular problems, among which infectious keratitis accounts for 35–39% of all the cases (Strubbe et al. 2000; Olivier et al. 2003). To address these issues, a study had demonstrated that a significant corneal epithelial restoration and reduction of inflammatory condition occur with conjunctival and/or ophthalmic artery injection of PB-MSCs (Table 12.3) (Spaas et al. 2011; Marfe et al. 2012).

Even in an old (2 months) case of equine bilateral retinal detachment, improvement in nerve function and visual acuity had been demonstrated in left eye with intravenous injection of PB-MSCs. However, right eye had inconsiderable improvement (Marfe et al. 2012). The variable pathophysiology involved and/or insufficient cellular concentration achieved in the right eye could have been the reasons for unfavorable results in right eye. Thus, more extensive studies are desired as per the pathophysiology with MSCs dose titration.

12.3.3 Tendon/Ligament Injuries

Tendonitis tends to lead to equine lameness (Barreira et al. 2008). This condition is managed by rest and rehabilitation, although recurrent tendonitis in 43% cases has been reported (Dyson 1997; Violini et al. 2009). Lack of basic understanding of tendon cell characteristics and their precursors in tendon healing currently challenges effective tendon healing (Bi et al. 2007). To gain insights and provide early healing of tendon, MSCs are being evaluated.

Numerous MSCs studies (experimental or clinical) on tendon or ligament injuries have been conducted as enlisted in Table 12.4 and 12.5. The models have been created either surgically (Geburek et al. 2017) or by enzymatic action (Nixon et al. 2008; Carvalho et al. 2013a). Apart from experimental models, MSCs therapeutic applications have also been evaluated in clinical tendinopathies and/desmitis (Renzi et al. 2013; Van Loon et al. 2014; Beerts et al. 2017). Most of the studies have evaluated BM-MSCs and AD-MSCs with two studies demonstrating umbilical cord (UC)-MSCs (Van Loon et al. 2014) and PB-MSCs (Beerts et al. 2017). The cells have been injected intralesionally after some delay. Some studies have additionally incorporated scaffolds like platelet-rich plasma (Renzi et al. 2013), serum (Pacini et al. 2007; Geburek et al. 2017), fibrinogen (Crovace et al. 2010), and plasma lysate (Del Bue et al. 2008). Growth factors like insulin-like growth factor (IGF-1) have also been added (Smith et al. 2003; Schnabel et al. 2009). In all these studies, a single dose of the variable cell concentration has been made barring two studies that had injected the cells twice (Van Loon et al. 2014; Vandenberghe et al. 2015).

Most of the studies have shown positive role of MSCs in tendon healing. One of the studies, however, had failed to demonstrate significant therapeutic effects of the MSCs (Caniglia et al. 2012). BM-MSCs suspended in fibrinogen (Crovace et al.

Table 12.4 In vivo preclinical experimental stem cell studies on tendinitis in horse

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Superficial digital flexor tendinitis (collagenase-induced)	8 (4 cell treated and 4 control)	6 weeks	AD-nucleated cell fraction	$13.83 \pm 3.41 \times 10^6$ cells (3 doses at 40–53 h range)	Biochemical and molecular and ultrasonography were performed	Ultrasonography revealed no difference in rate or quality of repair between groups. Histologic evaluation revealed a significant improvement in tendon fiber architecture; reductions in vascularity; inflammatory cell infiltrate; collagen type III formation; and improvements in tendon fiber density and alignment in ADNC-treated tendons. Repair sites did not differ in DNA, proteoglycan, or total collagen content. Gene expression of collagen type I and type III in treated and control tendons was similar. Gene expression of COMP was significantly increased in ADNC-injected tendons	Nixon et al. (2008)

(continued)

Table 12.4 (continued)

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Superficial digital flexor tendinitis	12 (6 BM- MSCs; 6 IGF-I gene enhanced BM- MSCs)	8 weeks	BM- MSCs, IGF-I gene enhanced BM- MSCs	10 × 10 ⁶ (local implantation)	Histological and immunohistochemical scores, ultrasound	Expression of collagen types I and III, IGF-I, cartilage oligomeric matrix protein (COMP), matrix metalloproteinase-3 (MMP-3), matrix metalloproteinase-13 (MMP-13), and aggrecanase-1 (ADAMTS-4) was similar in MSC and control tendons. Both MSC and AdIGF-MSC injection resulted in significantly improved tendon histological scores	Schnabel et al. (2009)
Superficial digital flexor tendinitis	6 (4 animals: All four limbs; 2 animals: 3 limbs)	18 weeks after treatment	BM- MSCs; BM- MNCs	5.5 × 10 ⁶ cBMSCs or 1.2 × 10 ⁸ BMMNCs (average) intralesionally	Histological and immunohistochemical scores	In cBMSC- and BMMNCs-treated tendons, a high expression of cartilage oligomeric matrix protein (COMP) and type I collagen, but low levels of type III collagen were revealed by immunohistochemistry, with a normal longitudinally oriented fiber pattern. Placebo-treated tendons expressed very low quantities of COMP and type I collagen but large numbers of randomly oriented type III collagen fibers. Both cBMSC and BMMNCs grafts resulted in a qualitatively similar healing improvement of tendon extracellular matrix, in terms of the type I/III collagen ratio, fiber orientation, and COMP expression	Crovace et al. (2010)

Superficial digital flexor tendinitis (collagenase-induced)	8 (4 animals biopsy at 60 days; 4 animals biopsy at 150 days)	60, 150 days	AD-MSCs	10×10^6 (30 days after collagenase injection)	Histological and immunohistochemical scores, clinical and ultrasonographic features	No differences occurred between the groups regarding the clinical and ultrasonographic analyses; however, histopathological evaluation revealed a significant improvement in tendon fiber organization and diminished inflammatory infiltrate, whereas immunohistochemical analysis showed increased expression of type I collagen in the treated group as compared with controls. The cellular therapy model implanted in this experiment promoted increased perivascular inflammatory infiltrate, fibroblastic density, neovascularization, and qualitative healing improvement of tendon extracellular matrix, in terms of fiber orientation and type I/III collagen ratio; moreover, it was considered to be a safe and viable process	Carvalho et al. (2011)
Superficial digital flexor (SDFT)	6 horses (2 wounds in each). One treated with MSCs and the other bone marrow supernatant	12 weeks after cell implantation	Autologous BM-MSCs	10×10^6 (ultrasound guidance)	Mass average diameter (MAD), collagen fibril index (CFI), area-dependent diameter (ADD)	MAD and CFI in addition to the bimodal distribution of collagen fibril size were significantly lower in treated and control injured animals as compared to the normal and control limbs	Caniglia et al. (2012)

(continued)

Table 12.4 (continued)

Model type	Number of animals	Model defect size/ study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Bilateral forelimb superficial digital flexor tendons (SDFT)	9 horses (2 in each horse)	22 weeks	AD-MSCs	1×10^7 injected intralesionally after 2 weeks	Ultrasonography and histology	Color Doppler signal at 2 weeks was significantly more extensive. Histology showed significantly more neovascularization after 22 weeks	Conze et al. (2014)
Bilateral forelimb superficial digital flexor tendons (SDFT)	9 horses (2 in each horse)	24 weeks	AD-MSCs in inactivated serum and inactivated serum only	Ultrasound-guided 10×10^6 cells were injected intralesionally	Ultrasonography, clinical, biochemical, and biomechanical evaluation	Statistically nonsignificant differences in two treated groups. Mature collagen crosslink hydroxylsypyrindoline was comparable to normal tendon collagen in AD-MSCs treated group, while it was lower in the other group. Stress at failure and modulus of elasticity in AD-MSCs group were significantly lower than the mature normal tendon	Geburek et al. (2017)

Table 12.5 In vivo clinical mesenchymal stem cell studies on tendinitis in horse

Clinical case	Number of animals	Study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Superficial digital flexor tendinitis	26 (11 cell treated; 15 control)	Uncontrolled study	Autologous BM-MSCs	9.5×10^6	Ultrasound and ability to return to race	Nine MSC-treated animals recovered from their clinical conditions had an excellent ultrasound image of tendons after a period ranging from 3 to 6 months and returned to racing with good or even optimal results in the previous category of competition in 9–12 months without any reinjuring event. All of them are still active more than 2 years from diagnosis. One of the 2 remaining horses received less than 1×10^6 of MSCs, and its tendon did not heal, relapsing after rehabilitation; the other was lost to follow-up. Small number of MSCs may be sufficient to repair damaged tendons without the use of scaffold support. Ultrasound scanning showed that fibers were correctly oriented in cell-treated tendons. Further, no ectopic bone deposition occurred	Pacini et al. (2007)

(continued)

Table 12.5 (continued)

Clinical case	Number of animals	Study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Tendinitis (case controlled study)	16	15–240 days	Allogeneic AD-MSCs + platelet-rich plasma	Local intralesional injection (cell concentration not given)	Ultrasonography	14 out of 16 treated horses recovered and went back to activity. Two did not recover, one among them was affected by chronic tendonitis previously treated with different therapies (infiltrations, blisters, etc.), while the other horse, after 8 weeks and a satisfactory recovery, went back to activity too early and the lesion recurred	Del Bue et al. (2008)
Tendinitis (among 23 animals, 14 had frontal right and 9 frontal left limb affected); desmitis (among 9, 5 frontal right and 4 frontal left limb cases. 1 case had bilateral SL lesion). Lesion of 15–30% of transversal tendon surface was included	33 (18 BM-MSCs treated; 13 pin firing treated)	8–15 months	Autologous BM-MSCs	2×10^6 cells in PRP per cm^2 of lesion	Clinical evaluation and ultrasonography	Gradual healing was observed on ultrasonography, and at 24 weeks the tendon was anatomically restored. 13/18 in cell-treated group returned to race competitions, while none among pin fired horses	Renzi et al. (2013)

Superficial digital flexor tendon	12 (6 animals cell treated; 6 control)	6 months	BM-MSCs, growth factors, natural mechanical stimulus	1×10^7 autologous BM-MSCs suspended in 2 mL of marrow supernatant	Morphology, biomechanical, ultrasonography, histology	Cell-treated tendons had significantly lower structural stiffness although there is no significant difference in calculated modulus of elasticity. Lower (improved) histological scoring of organization and crimp pattern, lower cellularity, DNA content, vascularity, water content, GAG content, and MMP-13 activity were seen in cell-treated tendons	Smith et al. (2013)
Tendinitis of superficial flexor tendons (SDFT) and deep digital flexor tendon (DDFT), and desmitis of superficial ligament (SL) and inferior check ligament (ICL) (first time injured)	52 animals (young: 5–9 years; medium aged: 10–14 years; >15 years)	6 months	Umbilical cord blood-derived MSCs	2– 10×10^6 (single dose) 20×10^6 (double dose in 5 horses at 4–36 weeks). 4/5 double-treated animals were from SL group	Clinical and ultrasonography	40/52 (77%) animals returned to the normal activity. 20/23 SDFT cases had positive result. 15/22 cases of SL had positive result. 4/6 DDFT and ICL had positive result. Control cases (SDFT: 1/3; SL: 2/3) reached to normal or higher performance levels after 6 months or more following proper rehabilitation programme. No significant difference in results with respect to the age was recorded	Van Loon et al. (2014)

(continued)

Table 12.5 (continued)

Clinical case	Number of animals	Study period	Biomaterial used	Cell dose/ assembly	Evaluation criteria	Overall result	References
Proximal suspensory ligament injury (treated for 4 months by NSAIDs and PRP; AAEP scale 2/5)	01 (case report)	1 year	Allogeneic tenogenically induced peripheral blood-derived MSCs	0.5×10^6 cells tenogenically differentiated; second dose at 12 weeks after the first injection allogeneic tenogenically induced MSCs + PRP	American Association of Equine Practitioners (AAEP) scale and ultrasonography, radiographic examination, computed tomography	From 4 weeks after treatment, the lameness reduced to an AAEP grade 1/5 and a clear filling of the lesion could be noticed on ultrasound. At 4 weeks after the second injection, the horse trotted sound under all circumstances with a close to total fiber alignment. The horse went back to previous performance level at 32 weeks after the first regenerative therapy and is currently still doing so (i.e., 20 weeks later or 1 year after the first stem cell treatment)	Vandenbergh et al. (2015)
Superficial ligament and superficial digital flexor tendon lesions	SL ($n = 68$); SDF ($n = 36$)	24 months	Allogeneic tenogenically induced peripheral blood MSCs	Cryopreserved $2-3 \times 10^6$ (at least 50% viability) in 1 mL PRP	Clinical, lameness, and ultrasonographic evaluation	After 12th month, 83.8% of SDFT and 79.2 of SL returned to previous performance level. At 24th month, 82.4% and 85.7% of SL and SDFT cases returned to previous performance level, respectively. Cell therapy significantly lowered reinjury rate	Beerts et al. (2017)

2010), autologous serum (Pacini et al. 2007), and plasma lysate (Del Bue et al. 2008; Renzi et al. 2013; Vandenberghe et al. 2015) had improved tendon repair especially in relation to the functional recovery.

The intralesional injected MSCs had remained viable, distributed, and integrated within the injured tissue. The cell numbers had decreased with the time, and small numbers had been identified in the peripheral blood up to 24 h and in the contralateral tendon lesions up to 24 weeks (Burk et al. 2016). Even up to 34 days, the cells had been located in the injected lesions (Guest et al. 2008). AD-MSCs tend to decrease inflammation markedly and improve alignment of collagen fibrils and tendon fibers (Nixon et al. 2008). The cell concentration, however, appears to have a role to play. One million cells had been insufficient for effective tendon healing (Smith 2008; Caniglia et al. 2012), while higher cell concentrations like ten million (Smith et al. 2009) and 20 million (Van Loon et al. 2014) had significantly improved tissue matrix approximating to the normal tendon tissue.

Delayed implantation (day 30) of AD-MSCs in superficial digital flexor tendonitis too had improved healing as better histological and immunohistochemical scores were demonstrated. However, an insignificant difference in sonographic and clinical signs had been demonstrated between the cell-treated or nontreated cases (Carvalho et al. 2011). Combined application of BM-MSCs and IGF-1 had improved healing of the superficial digital flexor tendonitis. Tendons treated with cells and IGF-1 were histologically better in comparison to those treated with BM-MSCs only. The cell-treated tendons had better histological scores as compared to the control (Schnabel et al. 2009).

Even clinical improvement with the application of MSCs together with the routine management procedures has been demonstrated. The clinical studies on ligament and tendon injuries had demonstrated that AD-MSCs and BM-MSCs had improved most of the cases (85%) and these animals had returned back to competition (Ferris et al. 2009; Leppanen et al. 2009). Animals of diverse age, gender, and breed had similar outcome (Ferris et al. 2009). Single case report of superficial digital flexor tendonitis locally treated with BM-MSCs and growth factors along with natural stimulus had improved tendon healing. The stimulus might have triggered MSCs differentiation into tendon fibroblasts (Smith et al. 2003). Apart from growth factors, platelet-rich plasma (PRP) scaffold laden allogeneic AD-MSCs (Del Bue et al. 2008) or PB-MSCs (Beerts et al. 2017) had improved tendonitis and tendon/ligament injuries, respectively. Most of these animals had returned back to the normal activity. Combined application of allogeneic PB-MSCs and platelet-rich plasma (PRP) had moderately improved suspensory ligament (SL) and superficial digital flexor tendon (SDFT) sonographic features at 6-week period that had further improved at 24 months. The reinjury rate has markedly reduced in these cell-treated cases (18%) in comparison to conventionally treated horses (44%) (Beerts et al. 2017). Similar biomaterial combination used at 4 weeks after diagnosis and repeated at 32-week period in a proximal suspensory ligament desmitis had completely restored its fiber alignment being observed under ultrasonography. These animals had improved performance almost similar to the initial levels (Vandenberghe et al. 2015). An equine tendonitis study had failed to demonstrate

significant results with AD-MSCs in comparison to the autologous inactivated serum. However, better histopathological observations were observed in the AD-MSC-treated tendons than control (Geburek et al. 2017).

One of the studies had failed to observe improved effects of MSCs on tendonitis and might have been due to the delayed cell implantation (1 month) (Caniglia et al. 2012). Cell induced pro-healing effects may be affected by inflammation at early stage, while limited results at delayed implantation might occur due to the developed fibrous tissue (Dahlgren 2009; Carvalho et al. 2011; Carvalho et al. 2013a). Further, the evaluation criteria and period of evaluation may also affect the results (Caniglia et al. 2012).

These studies favor MSCs application in tendon/ligament injuries, although variably. The evaluation criteria followed must be streamlined and the studies that incorporate various biomaterials must be compared with standard to come to the conclusion. Cells of uniform batches with respect to source, concentration, and passage must be applied through standard routes for effective comparison. Advanced techniques may also be applied to demonstrate cellular fate (Geburek et al. 2016). The proper follow-up of the studies and for extended period may be made.

12.3.4 Cartilage Injury

Cartilage healing is a challenge as it has limited intrinsic regenerative potential (Kinner et al. 2005; Kang et al. 2008; Campos et al. 2012). The healing usually occurs with mechanically inferior fibrous tissue that shows nonintegration to the native tissue (Campos et al. 2012; Gugjoo et al. 2017).

The stem cell osteochondral defect healing has been studied for various joints but mostly in the stifle joint both in preclinical experimental (Table 12.6) and in clinical (Table 12.7) cases. The critical defect size has been variable. Various MSCs sources like bone marrow, adipose tissue, peripheral blood, and synovium have been evaluated for osteochondral healing though the former two sources have been subject of most of the studies. The cell concentrations implanted in these studies have been variable ranging from five million to 20 million. Apart from simple MSCs, the chondrogenically differentiated allogeneic MSCs have also been evaluated (Broeckx et al. 2019). As MSCs fail to adhere or stick to the osteochondral defects, scaffolds like collagen repair patch (González-Fernández et al. 2016), allogeneic plasma (Broeckx et al. 2019), allogeneic PRP (Broeckx et al. 2014), fibrin glue (Raheja et al. 2011), and hyaluronan (McIlwraith et al. 2011) have been incorporated. The healing or clinical improvement in these studies has been evaluated by variable parameters including clinical examination, radiography, computed tomography (CT) and magnetic resonance imaging (MRI), arthroscopy or biomechanical testing, and histochemical examination. Most of the osteoarthritis studies have failed to demonstrate effective cartilage repair (Wilke et al. 2007; Frisbie et al. 2009; McIlwraith et al. 2011), but beneficial effect of pain reduction has been observed (Ferris et al. 2009; González-Fernández et al. 2016).

Combined application of BM-MSCs and hyaluronic acid or autologous platelet enhanced fibrin scaffolds (APEF) had been made (McIlwraith et al. 2011; Goodrich et al. 2016). In both these studies, clinical improvement had failed to occur at 12 months. Slight improvement with respect to gross morphology and arthroscopy had been reported at 12-month period (McIlwraith et al. 2011). The cobblestone-like appearance of healed tissue bearing fair to good integration at 3 months was demonstrated. However with time the tissue had degraded (Goodrich et al. 2016). Thus, extended studies are desired to see the course of disease.

In one of the comparative study, an equine meniscal tear model treated with MSCs of two sources (BM-MSCs or AD-MSCs) laden in collagen repair patch had demonstrated that cells of different sources have comparable healing effect. The defects were repaired by fibrocartilage in cell-treated animals, while in control group repair was either partial or nil (González-Fernández et al. 2016). Another comparative study had demonstrated higher pro-healing effects of BM-MSCs on osteoarthritis as compared to uncultured stromal vascular fraction (SVF). Joints treated with SVF had unwanted increase in tumor necrosis factor- α (TNF- α) concentration in synovial fluid (Frisbie et al. 2009). The combined treatment involving bone marrow aspirate implantation and microfracture too had favored cartilage regeneration in comparison to the microfracture technique alone. Microfracture enhances bone marrow mononuclear cell fraction availability for cartilage healing but may not have been sufficient (Fortier et al. 2010). One of the studies had compared autologous BM-MSCs treatment to the arthroscopy in joint affections. Out of total cases ($n = 33$) treated with intra-articular cell (15,020 million) implantation, 43% reached to previous level of work, 33% reached to work, and 24% had failed to work. In three cases, transient joint flare had been observed within 24 h of cell treatment. Joints treated with arthroscopy had significantly lower recovery rate (60–63%) (Ferris et al. 2014).

Inflammatory environment tends to reduce cell therapeutic effects. MSCs express higher adhesion molecules and have decreased migration potential under inflammation (Barrachina et al. 2016; Reesink et al. 2017). Additionally, these cells show reduced potential to secrete cartilage-specific matrices in the presence of pro-inflammatory cytokines (Taylor et al. 2007; Zayed et al. 2016) although such properties of MSCs may also be affected by tissue source (Zayed et al. 2016). Thus, efforts to reduce inflammation in the joints before application of MSCs should be made (Ferris et al. 2014). Currently, the local MSCs implantation is made at 20 million along with the scaffold (hyaluronan; González-Fernández et al. 2016).

12.3.5 Bone Injury

Bone heals naturally with healed tissue approximating to natural native tissue (Taylor et al. 2007). The healing of a fractured bone in horse is challenging due to its uncontrolled movement. In case of extensive fractures or tumors, the ability to heal becomes even more challenging. To improve healing in such conditions, tissue

Table 12.6 In vivo chondrogenic preclinical experimental mesenchymal stem cell studies in horse

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Chronic full thickness cartilage defects in femoro-patellar articulations	6 (12 defects) group I: Autogenous fibrin vehicle containing MSCs; group II: Autogenous fibrin alone in control joints	15 mm/1 month and 8 months	MSCs loaded into self-polymerizing autogenous fibrin vehicle	MSCs/ fibrinogen mixture containing 12×10^6 MSC/ mL	Histology, histochemistry, collagen type I and type II, immunohistochemistry, collagen type II in situ hybridization, and matrix biochemical assays	Arthroscopic scores in group I were significantly improved at the day 30. Biopsy showed MSC-implanted defects contained increased fibrous tissue with several defects containing predominantly type II collagen. At 8 months, there was no significant difference between stem cell-treated and control defects	Wilke et al. (2007)
Middle carpal joint osteochondral defect	24 (8 each group) group I: AD-MSCs; group II: BM-MSC; group III: Control	15 mm/70 days	AD-MSCs and BM-MSCs	16.3×10^6 (AD-MSCs) and 10.5×10^6 (BM-MSCs)	Clinical outcome, macroscopy, histopathology, articular cartilage matrix evaluation	Nonsignificant differences in healing improvement with cell treatment were seen compared to control except improvement in PGE2 levels	Frisbie et al. (2009)

Full thickness femoral condyle defects followed by microfracture	10 (20 defects in stifle joint) group I: BM-MSCs and microfracture; group II: Microfracture	10 mm/6 months and 12 months	MSCs and hyaluronan	20 × 10 ⁶ MSCs + 22 mg hyaluronan	Radiography, arthroscopy, magnetic resonance imaging and gross, histologic, histomorphometric, immunohistochemical and biochemical examinations	Nonsignificant evidence of any clinically significant improvement in the joints with BM-MSCs. Arthroscopic and gross evaluation confirmed a significant increase in repair tissue firmness and a trend for better overall repair tissue quality (cumulative score of all arthroscopic and gross grading criteria) in BM-MSC-treated joints. Immunohistochemical analysis showed significantly greater levels of aggrecan in repair tissue treated with BM-MSC injection. There were no other significant treatment effects	Mellor et al. (2011)
--	---	------------------------------	---------------------	--	---	--	----------------------

(continued)

Table 12.6 (continued)

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Chronic chondral defects in the medial femoral trochlea	4 (8 defects) group I: MSCs; group II: Control	10 mm/ months	AD-MSCs	1.35×10^7	Macroscopic, histopathological, and histochemical evaluations	The use of MSC in the treatment of chondral defects minimized joint inflammation, as confirmed by synovial fluid analysis. The treatment resulted in an improved repair tissue, verified by macroscopic examination and histochemical and histopathological analysis	Yamada et al. (2013)
Chondral defect model	12 (24 defects) group I: BM-MSCs + APEF; group II: APEF	15-mm defect in lateral trochlear ridge/3 months and 12 months	BM-MSCs along with autologous platelet enhanced fibrin scaffolds (APEF)	12×10^6	Arthroscopy, histological examination, magnetic resonance imaging (MRI), micro-computed tomography (micro-CT), and biomechanical testing	Addition of BM-MSCs to APEF did not enhance cartilage repair and stimulated bone formation in some cartilage defects	Goodrich et al. (2016)

Osteochondral defect (bilateral distal radius, 6 mm × 5 mm)	3 (right-side implanted with cells; left without cells)	9 weeks	Synovium-derived MSCs	5 × 10 ⁶ (each in right defect and radiocarpal joint)	CT and histology	Radiolucent volumes of defects calculated by analysis of postmortem CT images were decreased in implanted sites compared with control sites in all horses. The average scores for ICRS gross and histopathological grading scales in implanted sites were equal to or higher than those of the controls. These results suggest that allogeneic implantation and subsequent autologous injection of SM-MSCs might not obstruct subchondral bone formation in defects	Yamasaki et al. (2018)
---	---	---------	-----------------------	--	------------------	---	------------------------

(continued)

Table 12.6 (continued)

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Stifle injury/ femorotibial lesions (meniscal, cartilage, or ligamentous; case controlled)	39 (33 had reports available)	24 months	Autologous BM-MSCs + 20 mg of HA	15–20 × 10 ⁶	American Association of Equine Practitioners (AAEP) lameness score, graded severity of lesion	43% of horses returned to previous level of work, 33% returned to work, and 24% failed to return to work. In horses with meniscal damage (n/424), a higher percentage in the current study (75%) returned to some level of work compared to those in previous reports (60–63%) that were treated with arthroscopy alone, which resulted in a statistically significant difference between studies (P/40.038). Joint flare post-injection was reported in 3 horses (9.0%); however, no long-term effects were noted	Ferris et al. (2014)

Table 12.7 In vivo chondrogenic clinical mesenchymal stem cell studies in horse

Clinical condition/ailment	Number of animals	Study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Bilateral articular cartilage fissure defects of the medial femoral condyles and concurrent cranial cruciate ligament injury	Single case	4 months and 15 months	Autologous BM-MSCs + fibrin glue	1×10^7 implanted at 90 days, and 3 months, and 13 months period	Arthroscopic examination and performance records	At 4 months marked cartilage surface smoothing and reduction in the cartilage defect depth. Further, moderate improvement in the cranial cruciate ligament was observed. After 15 months of the initial MSC treatment, the horse returned to racing and had comparable race earning to that of preinjury records	Raheja et al. (2011)
Chronic degenerative joint disease of pastern joint	Single case	4, 8, and 12 weeks and 1 year period	Peripheral blood-derived MSCs	2.5×10^6	American Association of Equine Practitioners (AAEP), radiography and pressure plate analysis	Clinical improvement observed and pressure plate analysis showed load rate (LR) symmetry ratio increased considerably in both gaits, indicating an increased speed of loading at the walk as well as at the trot. A clear improvement in peak vertical force (PVF) and vertical impulse (VI) symmetry ratios was evident at the trot, indicating an increased symmetry of the weight-bearing function of the forelimbs	Spaas et al. (2012)

(continued)

Table 12.7 (continued)

Clinical condition/allment	Number of animals	Study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Degenerative joint disease of fetlock	20 (group I: PRP; group II: MSCs; group III: MSCs and PRP; or group IV: Chondrogenic-induced MSCs and PRP)	6 and 12 weeks and 6 and 12 months	Peripheral blood MSCs, platelet-rich plasma (PRP)	6.7×10^3 MSCs/ cm^2 cells chondrogenically induced + 200×10^6 platelets	American Association of Equine Practitioners (AAEP)	Group 4 animals generated the highest evolution scores, although it had statistically insignificant difference against group 4 in the early or late evolution score	Broeckx et al. (2014)
Bilateral meniscus defect (10 mm in length and 6 mm in depth)	6 (3 treated with BM-MSCs; 3 treated with AD-MSCs)	12 months	BM-MSCs and AD-MSCs + collagen repair patch	0.5×10^6 cells	Macroscopic assessment of meniscal repair, histochemical analysis	At 12 months after treatment, cell-treated defects were regenerated with fibrocartilaginous tissue, whereas untreated defects were partially repaired or not repaired	González-Fernández et al. (2016)
Osteochondral fragment-induced osteoarthritis	10 (cell and surgery treated, $n = 5$; surgery treated only, $n = 5$)	30, 60, and 90 days	Allogeneic BM-MSCs	1×10^7	Physical examination, osteophyte formation, racing performance, mononuclear cells in synovial fluid analysis	MSC treatment group had an improved physical examination score, better racing performance, lesser osteophyte formation, and mononuclear cells in synovial fluid than the conventional treatment group	Korchunjit et al. (2019)
Early stage fetlock degenerative joint disease	75 (50 in treated and 25 in control)	3, 6, and 18 weeks and 1 year	Chondrogenically induced allogeneic MSCs + allogeneic plasma	2×10^6 cells + $\sim 85 \times 10^6$ platelets	AAEP score system	Lameness scores ($P < 0.001$), flexion test responses ($P < 0.034$), and joint effusion scores ($P < 0.001$)	Broeckx et al. (2019)

engineering employing MSCs may be evaluated. In case of horse, very limited MSCs osteogenic studies, however, could be located.

An experimental study had been aimed to enhance the arthrodesis of pastern joint by utilizing tissue engineered construct (stabilized by locking compression plate). The engineered construct composed of BM-MSCs and scaffold and growth factor had enhanced bone formation and resulted in arthrodesis (Table 12.8) (Seo et al. 2014). In metacarpal osteotomy model, periosteum MSCs committed to osteoprogenitor cells along with fibrin glue, however, had failed to show improved radiographic and histological features in comparison to the fibrin glue alone (McDuffee et al. 2012). This questions the role of osteoprogenitor cells in bone healing, although other reasons including limited osteoprogenitor cell concentration and scaffold type involved may also affect the outcome. A single horse clinical case report had demonstrated improved hoof regeneration with AD-MSCs after 3 weeks and complete fracture healing at 3 months (Table 12.9) (Marycz et al. 2012). However, detailed in vivo osteogenic studies of eMSCs are currently unavailable.

12.3.6 Immune-Mediated, Inflammatory, and Ischemic Diseases

A lot of literature focusing on MSCs immunomodulation is available. These studies have demonstrated cellular ability to control immune reactions. MSCs have prevented such reactions under in vitro and/or in vivo experimental studies (Augello et al. 2005; Einstein et al. 2007; Sordi and Piemonti 2011). MSCs applications in equine recurrent uveitis, systemic lupus erythematosus (SLE), and endotoxemia that are immune-mediated and/inflammatory diseases may also be evaluated. Such studies can additionally form basis of therapeutics in human medicine as the condition in two species shares pathophysiology (Deeg et al. 2002; Liang et al. 2010; Sun et al. 2009; Shi et al. 2012). MSCs inhibit proliferation of B cell and memory CD8⁺ T cell while T regulatory cell proliferation is promoted (Peroni and Borjesson 2011; Im et al. 2014; Cortinovis et al. 2015).

12.3.7 Laminitis

Laminitis is an ischemic clinical condition in horse usually exhibiting loss of p63 positive epidermal stem cells (Carter et al. 2011). Application of MSCs in clinical laminitis has been demonstrated to provide add-on benefits to the traditional managemental care (Table 12.10). Route of MSCs application seems to play an important role. Intravenous injection of allogeneic BM-MSCs had shown better results as compared to their epidermal injection (Sole et al. 2012). The intra-arterial infusion had resulted in retention of MSCs in the distal limb, with a symmetrical and homogeneous distribution. Contrarily, intravenous regional limb perfusion (IVRLP) had resulted in an asymmetrical and heterogeneous distribution within the hoof. The IVRLP performed on standing, sedated horses had resulted in lower and more

Table 12.8 In vivo preclinical experimental mesenchymal stem cell studies on osteogenesis in horse

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose/assembly	Evaluation criteria	Overall result	References
4th metacarpal osteotomy	10 (20 metacarpal, 10 control, and 10 experimental)	1 cm/12 weeks	Periosteum-derived osteoprogenitor cells (osteogenic lineage differentiated)	20×10^6	Radiography and histology	MSCs along with fibrin glue-treated animals failed to show improved radiographic and histological features in comparison to the fibrin glue alone	McDuffee et al. (2012)
Pastern joint arthrodesis	6 (12; 6 control and 6 experimental)	Locking compression plate stabilization	BM-MSCs, gelatin/ β -tricalcium phosphate and BMP-2	1×10^7	Radiography, histology, computed tomography	An improved bone formation and subsequent arthrodesis in comparison to the use of locking compression plate application had occurred	Seo et al. (2014)
Splint bones defect (4 defects each in second metacarpal and metatarsal bone of right limbs and 4th metacarpal and metatarsal in left limbs)	6 animals (group A: BM-MSCs + BMP-2 in sponge; group B: BM-MSCs in sponge; group C: BMP-2 in sponge; group D: Saline)	1 cm/16 weeks	BM-MSCs, BMP-2, and gelatin β -tricalcium phosphate sponges	Autologous BM-MSCs and BMP-2 in gelatine sponge	Radiography, CT, and histology	Group A had statistically significant radiographic and histological scores compared to control, while CT scores were statistically significant than other groups	Tsuzuki et al. (2014)

Table 12.9 In vivo clinical mesenchymal stem cell studies on osteogenesis in horse

Clinical condition/ailment	Number of animals	Study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Phalanx digitalis distalis fracture	1	12 weeks after surgery	Orthopedic screw wrapped with plasma protein adhesive/ MSCs biofilm	6×10^6 in plasma protein adhesive biofilm	Clinical evaluation and radiography	After 3 months, hoof was significantly regenerated with bone completely regenerated	Marycz et al. (2012)

Table 12.10 In vivo mesenchymal stem cell applications in horse laminitis

Clinical condition/ailment	Number of animals	Study period	Biomaterial used	Cell dose	Evaluation criteria	Overall result	References
Chronic laminitis	12	14 months	Allogeneic UC-MSCs	20–25 × 10 ⁶ (3–4 treatments) (retrograde venous digital perfusion)	–	10/12 horses (83%) responded well. However, long-term success rates still need to be determined. The first “successful” case is only 14 months out from initial treatment	Morrison et al. (2014)
Chronic laminitis	30 (onset of laminitis < 30 days [10 cases], 30–60 days [4 cases], 60–90 days [2 cases], and > 90 days [14 cases])	4 months	Allogeneic UC-MSCs or BM-MSCs	20–30 × 10 ⁶ (4 treatments at monthly interval) (retrograde venous digital perfusion, using the palmar/plantar digital vein)	Radiographically	Of the 30 cases, 21 patients (70%) had a successful outcome. In the cases receiving stem cells <30 days after the onset of laminitis, the success rate was 100%. The success rate in the cases receiving stem cells >90 days after the onset of laminitis was only 50%. Statistical analysis is pending	Dryden et al. (2013)
Chronic laminitis (III, IV grade)	9	1 year	Allogeneic AD-MSCs (first dose) and autologous AD-MSCs (two doses)	15 × 10 ⁶ in 15 mL PRP (implanted thrice at 1 month interval)	Clinical observations and venography	Progressive amelioration of vascularization of foot. An improvement in structure and function of hoof. At 1 year, 7/9 horses were in activity with 2 developing recurrent laminitis. After 1 year, only two animals could be seen in activity and rest either deceased due to colic or were euthanized (recurrent laminitis cases). Two cases were not observed after 1 year	Angelone et al. (2017)

variable retention of MSCs and poor distribution within the hoof. Coronary band injection had failed to show any migration of MSCs within the hoof (Spriet et al. 2013).

MSCs may prove beneficial within 30 days of third phalanx displacement after which lamellar wedge develops (Dryden et al. 2013; Morrison et al. 2014). Post lamellar wedge development, little observable improvement with MSCs has been reported. Autologous or allogeneic MSCs from adipose tissue and umbilical cord have been used. These cells have been injected more than once along with other biomaterials like platelet-rich plasma. An overall improvement in the hoof anatomy (based on quality and shape) and laminitic foot weight bearing and mobility had been demonstrated (Angelone et al. 2017).

12.3.8 Neurological Problems

Neuron is a highly differentiated cell with almost no regenerative power. Stem cells though are present in the nervous tissue, but their role is yet to be understood. Various studies including that of horse have variably supported neural or mesenchymal stem cell differentiation into the neuron-like cell (Hoynowski et al. 2007; Jamnig and Lepperdinger 2012; Villagrán et al. 2016). Endogenous neural stem cells (NSCs) tend to generate scar tissue that limits further injury but may hamper neurological activity (Stenudd et al. 2015; Watanabe et al. 2015). Currently, stem cell pro-healing effects may be due to their ability to secrete trophic factors (Sadan et al. 2009). In vivo preliminary MSCs studies in other species appear promising as improved clinical parameters have been demonstrated post-spinal cord injury (Ra et al. 2011; Bhat et al. 2019). In case of horse, commonly encountered affections are of spinal cord, peripheral nerves, and laryngeal hemiplegia. However, the relevant studies remain to be conducted. One study utilizing BM-MSCs as therapeutic for transected portion of peripheral nerve had failed to demonstrate any observable improvement (Table 12.11) (Villagrán et al. 2016). An extensive research in the field is desired to conclude MSCs role, if any in the nerve regeneration. Apart from peripheral nerve injuries, MSCs role in neurological disorders like equine myeloencephalopathy and equine motor neuron diseases may also be evaluated.

12.3.9 Reproductive Disorders

MSCs role has been evaluated in horse endometriosis (Mambelli et al. 2013; Corradetti et al. 2014; Falomo et al. 2015; Rink et al. 2018). MSCs in utero implantation has been made like that of artificial insemination. MSCs derived from amnion (AM-MSCs) or endometrium (End-MSCs) may potentially replenish endometrium in pregnancy failure (Corradetti et al. 2014; Falomo et al. 2015). Intrauterine-implanted cells had failed to migrate to healthy endometrium while these cells had migrated endometriosed glandular and periglandular tissues (Mambelli et al. 2013;

Table 12.11 In vivo preclinical mesenchymal stem cell study on nerve regeneration in horse

Model type	Number of animals	Model defect size/study period	Biomaterial used	Cell dose/assembly	Evaluation criteria	Overall result	References
Excision of lateral and medial stumps of ramus communicans	3 horses (bilateral incision)	45 days	Allogeneic BM-MSCs	10×10^6	Clinical and histological examination	No evidence of nerve regeneration at 45 days. No difference in histology of treated and control animals	Villagrán et al. (2016)

Rink et al. 2018). These cells had shown immunomodulation (Falomo et al. 2015) and decreased inflammation (Rink et al. 2018) in endometritis.

MSCs may also play role to improve fertility by enhancing the follicular growth. MSCs tend to increase follicular number in ovary, although with an insignificant effect on dynamism (Grady et al. 2016).

12.3.10 Miscellaneous Conditions

MSCs may also be evaluated in inflammatory lung disease like recurrent airway obstruction (heaves). Stem cells tend to proliferate pulmonary vessel cells, decrease apoptotic cell number, and inhibit pulmonary vessel muscularization (Lee et al. 2015). MSCs have been trans-differentiated to other cell types like hepatocyte-like cells and myocyte-like cells (Reed and Johnson 2008; Lin et al. 2015; Pennington et al. 2016), but their in vivo therapeutic applications remain to be evaluated.

12.4 Conclusion(S)

Stem cells hold a great potential to act as an all-in-one therapeutic option for diverse ailments. MSCs generally follow principles of ISCT but may show differences in cell surface marker expression with respect to their sources. eMSCs from bone marrow, adipose tissue, or fetal tissues have mainly been evaluated for therapeutics. Numerous horse stem cell studies have demonstrated favorable results though without any definitive conclusion(s). MSCs from various tissue sources, together with variable routes, concentration, and biomaterials have been utilized in these studies. The conducted studies are uncontrolled, lacking sufficient sample size. Thus, extensive case controlled blinded studies involving uniform cell source, concentration, and biomaterials are desired in order to arrive at any conclusion.

References

- Aigner T, Cook J, Gerwin N, Glasson S, Lavery S, Little C, McIlwraith W, Kraus V (2010) Histopathology atlas of animal model systems—overview of guiding principles. *Osteoarthritis Cartil* 18:S2–S6
- Alipour F, Parham A, Kazemi Mehrjerdi H, Dehghani H (2015) Equine adipose-derived mesenchymal stem cells: phenotype and growth characteristics, gene expression profile and differentiation potentials. *Cell J* 16(4):456–465
- Andrew SA, Willis AM (2005) Diseases of the cornea and sclera. In: Gilger BC (ed) *Equine ophthalmology*. Elsevier Saunders, St. Louis, pp 157–251
- Angelone M, Conti V, Biacca C, Battaglia B, Pecorari L, Piana F, Gnudi G, Leonardi F, Ramoni R, Basini G, Dotti S, Renzi S, Ferrari M, Grolli S (2017) The contribution of adipose tissue-derived mesenchymal stem cells and platelet-rich plasma to the treatment of chronic equine laminitis: a proof of concept. *Int J Mol Sci* 18:2122
- Augello A, Tasso R, Negrini SM, Amateis A, Indiveri F, Cancedda R, Pennesi G (2005) Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. *Eur J Immunol* 35:1482–1490

- Baghavan M, Taghiyar L, Dehghan M, Fallahi F, Kazemi H (2009) Equine marrow-derived mesenchymal stem cells: isolation, differentiation and culture optimization. *Iran J Vet Res* 10:1–26
- Barberini DJ, Paiva Freitas NP, Magnoni MS, Maia L, Listoni AJ, Heckler MC, Sudano MJ, Golim MA, da Cruz Landim-Alvarenga F, Amorim RM (2014) Equine mesenchymal stem cells from bone marrow, adipose tissue and umbilical cord: immuno-phenotypic characterization and differentiation potential. *Stem Cell Res Ther* 5(1):25
- Barrachina AR, Remachaa A, Romeroa FJ, Fortier LA (2016) Effect of inflammatory environment on equine bone marrow derived mesenchymal stem cells immunogenicity and immunomodulatory properties. *Vet Immunol Immunopathol* 171:57–65
- Barreira APB, Alves ALG, Saito ME, Gómeza J, Jarrína CD, Ramírezb A, Galecioa JS (2008) Autologous implant of bone marrow mononuclear cells as treatment of induced equine tendinitis. *Int J Appl Res Vet Med* 6:46–54
- Beerts C, Suls M, Broeckx SY, Seys B, Vandenberghe A, Declercq J, Duchateau L, Vidal MA, Spaas JH (2017) Tenogenically induced allogeneic peripheral blood mesenchymal stem cells in allogeneic platelet-rich plasma: 2-year follow-up after tendon or ligament treatment in horses. *Front Vet Sci* 4:158
- Bhat IA, Sivanarayanan TB, Somal A, Pandey S, Bharti MK, Panda BSK, Indu B, Verma M, Anand J, Sonwane A et al (2019) An allogeneic therapeutic strategy for canine spinal cord injury using mesenchymal stem cells. *J Cell Physiol* 234(3):2705–2718
- Bi Y, Ehrlichou D, Kilts T et al (2007) Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. *Nature Med* 13:1219–1227
- Bohannon LK, Owens SD, Walker NJ, Carrade DD, Galuppo LD, Borjesson DL (2013) The effects of therapeutic concentrations of gentamicin, amikacin and hyaluronic acid on cultured bone marrow-derived equine mesenchymal stem cells. *Equine Vet J* 45:732–736
- Borena BM, Pawde AM, Pal A, Aithal HP, Kinjavdekar P, Singh R, Kumar D (2009) Autologous bone marrow-derived cells for healing excisional dermal wounds of rabbits. *Vet Rec* 165(19):563–568
- Branly T, Bertoni L, Contentin R, Rakic R, Gomez-Leduc T, Desancé M, Hervieu M, Legendre F, Jacquet S, Audigé F, Denoix JM, Demoor M, Galéra P (2017) Characterization and use of equine bone marrow mesenchymal stem cells in equine cartilage engineering. Study of their hyaline cartilage forming potential when cultured under hypoxia within a biomaterial in the presence of BMP-2 and TGF- β 1. *Stem Cell Rev Rep* 13(5):611–630
- Broeckx S, Zimmerman M, Crocetti S, Suls M, Marien T, Ferguson SJ, Chiers K, Duchateau L, Franco-Obregón A, Wuertz K et al (2014) Regenerative therapies for equine degenerative joint disease: a preliminary study. *PLoS One* 9(1):e85917
- Broeckx SY, Seys B, Suls M, Vandenberghe A, Marien T, Adriaensen E, Declercq J, Van Hecke L, Braun G, Hellmann K et al (2019) Equine allogeneic chondrogenic induced mesenchymal stem cells are an effective treatment for degenerative joint disease in horses. *Stem Cells Dev* 28(6):410–422
- Bundgaard L, Stensballe A, Elbæk KJ, Berg LC (2018) Mapping of equine mesenchymal stromal cell surface proteomes for identification of specific markers using proteomics and gene expression analysis: an in vitro cross-sectional study. *Stem Cell Res Ther* 9:288
- Burk J, Badylak SF, Kelly J, Brehm W (2013) Equine cellular therapy-from stall to bench to bedside? *Cytometry A* 83(1):103–113
- Burk J, Berner D, Brehm W, Hillmann A, Horstmeier C, Josten C, Paebst F, Rossi G, Schubert S, Ahrberg AB (2016) Long-term cell tracking following local injection of mesenchymal stromal cells in the equine model of induced tendon disease. *Cell Transplant* 25(12):2199–2211
- Bussche L, Harman RM, Syracuse BA, Plante EL, Lu Y-C, Curtis TM, Ma M, Van de Walle GR (2015) Microencapsulated equine mesenchymal stromal cells promote cutaneous wound healing in vitro. *Stem Cell Res Ther* 6:66
- Cabezas J, Rojas D, Navarrete F, Ortiz R, Rivera G, Saravia F, Rodriguez-Alvarez L, Castro FO (2018) Equine mesenchymal stem cells derived from endometrial or adipose tissue share significant biological properties, but have distinctive pattern of surface markers and migration. *Theriogenology* 106:93–102

- Campos DFD, Drescher W, Rath B, Tingart M, Fischer H (2012) Supporting biomaterials for articular cartilage repair. *Cartilage* 3(3):205–221
- Caniglia CJ, Schramme MC, Smith RK (2012) The effect of intralesional injection of bone marrow derived mesenchymal stem cells and bone marrow supernatant on collagen fibril size in a surgical model of equine superficial digital flexor tendonitis. *Equine Vet J* 44:587–593
- Carrade DD, Affolter VK, Outerbridge CA, Watson JL, Galuppo LD, Buerchler S, Kumar V, Walker NJ, Borjesson DL (2011a) Intradermal injections of equine allogeneic umbilical cord-derived mesenchymal stem cells are well tolerated and do not elicit immediate or delayed hypersensitivity reactions. *Cytotherapy* 13(10):1180–1192
- Carrade DD, Owens DS, Galuppo LD, Vidal MA, Ferraro GL, Librach F, Buerchler S, Friedman MS, Walker NJ, Borjesson DL (2011b) Clinicopathologic findings following intra-articular injection of auto-logous and allogeneic placentally derived equine mesenchymal stem cells in horses. *Cytotherapy* 13:419–430
- Carstanjen B, Desbois C, Hekmati M, Behr L (2006) Successful engraftment of cultured autologous mesenchymal stem cells in a surgically repaired soft palate defect in an adult horse. *Can J Vet Res* 70:143–147
- Carter RA, Engiles JB, Megee SO, Senoo M, Galantino-Homer HL (2011) Decreased expression of p63, a regulator of epidermal stem cells, in the chronic laminitic equine hoof. *Equine Vet J* 43(5):543–551
- Carvalho AM, Alves ALG, Gomes de Oliveira PG, Álvarez LEC, Renée CAH, Elenice D (2011) Use of adipose tissue-derived mesenchymal stem cells for experimental tendinitis therapy in equines. *J Equine Vet Sci* 31:26–34
- Carvalho AD, Badial PR, Alvarez LEC, Yamada AL, Borges AS, Deffune E, Hussni CA, Garcia Alves AL (2013a) Equine tendonitis therapy using mesenchymal stem cells and platelet concentrates: a randomized controlled trial. *Stem Cell Res Ther* 4(85):1–13
- Carvalho AM, Yamada ALM, Martins JRB, Maia L, Golim MA, Deffune E, Hussni CA, Alves ALG (2013b) Isolation and characterization of equine peripheral blood-derived multipotent mesenchymal stromal cells. *Pesqui Vet Bras* 33(9):1151–1154
- Chong PP, Selvaratnam L, Abbas AA, Kamarul T (2011) Human peripheral blood derived mesenchymal stem cells demonstrate similar characteristics and chondrogenic differentiation potential to bone marrow derived mesenchymal stem cells. *J Orthop Res* 30:634–642
- Clark KC, Kol A, Shahbenderian S, Granick JL, Walker NJ, Borjesson DL (2016) Canine and equine mesenchymal stem cells grown in serum free media have altered immunophenotype. *Stem Cell Rev Rep* 12:245–256
- Clegg P, Pinchbeck GL (2011) Evidence-based medicine and stem cell therapy: how do we know such technologies are safe and efficacious? *Vet Clin North Am Equine Pract* 27:373–382
- Clode AB, Matthews A (2011) Diseases and surgery of the cornea. In: Gilger BC (ed) *Equine ophthalmology*, 2nd edn. Elsevier Saunders, St. Louis, pp 181–266
- Coli A, Nocchi F, Lamanna R, Iorio M, Lapi S, Urciuoli P, Scatena F, Giannessi E, Stornelli MR, Passeri S (2011) Isolation and characterization of equine amnion mesenchymal stem cells. *Cell Biol Int Rep* 18(1):23
- Conze P, van Schie HTM, van Weeren R, Staszyc C, Conrad S, Skutella T, Hopster K, Rohn K, Stadler P, Geburek F (2014) Effect of autologous adipose tissue-derived mesenchymal stem cells on neovascularization of artificial equine tendon lesions. *Regen Med* 9(6):743–757
- Corradetti B, Correani A, Romaldini A, Marini MG, Bizzaro D, Perrini C, Cremonesi F, Lange-Consiglio A (2014) Amniotic membrane-derived mesenchymal cells and their conditioned media: potential candidates for uterine regenerative therapy in the horse. *PLoS One* 9(10):e111324
- Cortinovis M, Casiraghi F, Remuzzi G, Perico N (2015) Mesenchymal stromal cells to control donor-specific memory T cells in solid organ transplantation. *Curr Opin Organ Transplant* 20(1):79–85
- Crovace A, Lacitignola L, Rossi G, Francioso E (2010) Histological and immunohistochemical evaluation of autologous cultured bone marrow mesenchymal stem cells and bone marrow

- mononucleated cells in collagenase-induced tendinitis of equine superficial digital flexor tendon. *Vet Med Int* 2010:1
- Dahlgren LA (2009) Fat-derived mesenchymal stem cells for equine tendon repair. *Regen Med* 4(Suppl 6):2
- De Schauwer C, Piepers S, Van de Walle GR, Demeyere K, Hoogewijs MK, Govaere JL, Braeckmans K, Van Soom A, Meyer E (2012) In search for cross-reactivity to immunophenotype equine mesenchymal stromal cells by multicolor flow cytometry. *Cytometry A* 81(4):312–323
- De Schauwer C, Van de Walle GR, Soom AV, Meyer E (2013) Mesenchymal stem cell therapy in horses: useful beyond orthopedic injuries? *Vet Q* 33(4):234–241
- Deeg CA, Ehrenhofer M, Thureau SR, Reese S, Wildner G, Kaspers B (2002) Immunopathology of recurrent uveitis in spontaneously diseased horses. *Exp Eye Res* 5:127–133
- Dehghan MM, Eslaminejad MB, Motalebizadeh N, Ashrafi Halan J, Tagiyar L, Soroori S, Nikmahzar A, Pedram M, Shahverdi A, Kazemi Mehrjerdi H, Izadi S (2015) Transplantation of autologous bone marrow mesenchymal stem cells with platelet-rich plasma accelerates distraction osteogenesis in a canine model. *Cell J* 17(2):243–252
- Del Bue M, Ricco S, Ramoni R, Conti V, Gnudi G, Grolli S (2008) Equine adipose-tissue derived mesenchymal stem cells and platelet concentrates: their association in vitro and in vivo. *Vet Res Commun* 32:S51–S55
- Desancé M, Contentin R, Bertoni L, Gomez-Leduc T, Branly T, Jacquet S, Betsch J-M, Batho A, Legendre F, Audigé F, Galéra P, Demoor M (2018) Chondrogenic differentiation of defined equine mesenchymal stem cells derived from umbilical cord blood for use in cartilage repair therapy. *Int J Mol Sci* 19:537
- Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8(4):315–317
- Dryden VC, Morrison S, Bras R, Morrell SA (2013) Using stem cells in clinical cases. *J Equine Vet Sci* 33(10):872–873
- Dyson S (1997) Treatment of superficial digital flexor tendinitis: a comparison of conservative management, sodium hyaluronate, and glycosaminoglycan polysulfate. In: *Proceedings of the 43rd Annual Convention of the American Association of Equine Practitioners*, pp 297–300
- Edmonds RE, Garvican ER, Smith R KW, Dudhia J (2016) Influence of commonly used pharmaceutical agents on equine bone marrow-derived mesenchymal stem cell viability. *Equine Vet J* 49(3):352–357
- Einstein O, Fainstein N, Vaknin I, Mizrahi-Kol R, Reihartz E, Grigoriadis N, Lavon I, Baniyash M, Lassmann H, Ben-Hur T (2007) Neural precursors attenuate autoimmune encephalomyelitis by peripheral immunosuppression. *Ann Neurol* 61:209–218
- Elashry MI, Baulig N, Heimann M, Bernhardt C, Wenisch S, Arnhold S (2018) Osteogenic differentiation of equine adipose tissue derived mesenchymal stem cells using CaCl₂. *Res Vet Sci* 117:45–53
- Espina M, Jülke H, Brehm W, Ribitsch I, Winter K, Delling U (2016) Evaluation of transport conditions for autologous bone marrow-derived mesenchymal stromal cells for therapeutic application in horses. *PeerJ* 4:e1773
- Estes BT, Wu AW, Guilak F (2006) Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6. *Arthritis Rheum* 54:1222–1232
- Falomo ME, Ferroni L, Tocco I, Gardin C, Zavan B (2015) Immunomodulatory role of adipose-derived stem cells on equine endometriosis. *Biomed Res Int* 2015:1
- Ferris D, Frisbie DD, Kisiday J et al (2009) Clinical evaluation of bone marrow-derived mesenchymal stem cells in naturally occurring joint disease. *Regen Med* 4:S16
- Ferris DJ, Frisbie DD, Kisiday JD, McIlwraith CW, Hague BA, Major MD, Schneider RK, Zubrod CJ, Kawcak CE, Goodrich LR (2014) Clinical outcome after intra-articular administration of bone marrow derived mesenchymal stem cells in 33 horses with stifle injury. *Vet Surg* 43(3):255–265

- Fortier LA, Potter HG, Rickey EJ, Schnabel LV, Foo LF, Chong LR, Stokol T, Cheetham J, Nixon AJ (2010) Concentrated bone marrow aspirate improves full-thickness cartilage repair compared with microfracture in the equine model. *J Bone Joint Surg* 92:1927–1937
- Fox A, Smythe J, Fisher N, McGrouther DA, Watt SM, Harris AL (2008) Mobilization of endothelial progenitor cells into the circulation in burned patients. *Br J Surg* 95(2):244–251
- Frisbie DD, Smith RWK (2010) Clinical update on the use of mesenchymal stem cells in equine orthopaedics. *Equine Vet J* 42:86–89
- Frisbie DD, Kisiday JD, Kawcak CE, Werpy NM, McIlwraith CW (2009) Evaluation of adipose-derived stromal vascular fraction or bone marrow-derived mesenchymal stem cells for treatment of osteoarthritis. *J Orthop Res* 27:1675–1680
- Fülber J, Maria DA, Correia da Silva LCL, Massoco CO, Agreste F, Baccarin RYA (2016) Comparative study of equine mesenchymal stem cells from healthy and injured synovial tissues: an in vitro assessment. *Stem Cell Res Ther* 7:35
- Gale AL, Linardi RL, McClung G, Mammone RM, Orved KF (2019) Comparison of the chondrogenic differentiation potential of equine synovial membrane-derived and bone marrow-derived mesenchymal stem cells. *Front Vet Sci* 6:178
- Garvican ER, Cree S, Bull L, Smith RKW, Dudhia J (2016) Viability of equine mesenchymal stem cells during transport and implantation. *Stem Cell Res Ther* 5:1
- Geburek F, Mundle K, Conrad S, Hellige M, Walliser U, van Schie HT, van Weeren R, Skutella T, Stadler PM (2016) Tracking of autologous adipose tissue derived mesenchymal stromal cells with in vivo magnetic resonance imaging and histology after intralesional treatment of artificial equine tendon lesions - a pilot study. *Stem Cell Res Ther* 7:21
- Geburek F, Roggel F, van Schie HTM, Beineke A, Estrada R, Weber K, Hellige M, Rohn K, Jagodzinski M, Welke B, Hurschler C, Conrad S, Skutella T, van de Lest C, van Weeren R, Stadler PM (2017) Effect of single intralesional treatment of surgically induced equine superficial digital flexor tendon core lesions with adipose-derived mesenchymal stromal cells: a controlled experimental trial. *Stem Cell Res Ther* 8:129
- Gershwin LJ (2007) Veterinary autoimmunity: autoimmune diseases in domestic animals. *Ann N Y Acad Sci* 1109:109–116
- Gomiero C, Bertolutti G, Martinello T, Van Bruaene N, Broeckx SY, Patruno M, Spaas JH (2016) Tenogenic induction of equine mesenchymal stem cells by means of growth factors and low-level laser technology. *Vet Res Commun* 40(1):39–48
- González-Fernández ML, Pérez-Castrillo S, Sánchez-Lázaro JA, Prieto-Fernández JG, López-González ME, Lobato-Pérez S, Colaço BJ, Olivera ER, Villar-Suárez V (2016) Assessment of regeneration in meniscal lesions by use of mesenchymal stem cells derived from equine bone marrow and adipose tissue. *Am J Vet Res* 77:779–788
- Goodrich LR, Chen AC, Werpy NM, Williams AA, Kisiday JD, Su AW, Cory E, Morley PS, McIlwraith CW, Sah RL, Chu CR (2016) Addition of mesenchymal stem cells to autologous platelet-enhanced fibrin scaffolds in chondral defects: does it enhance repair? *J Bone Joint Surg* 98:23–34
- Grady ST, Watts AE, Hinrichs K (2016) Effect of mesenchymal stem cells on equine ovarian follicular development. *J Equine Vet Sci* 41:51–84
- Guest DJ, Smith MR, Allen WR (2008) Monitoring the fate of autologous and allogeneic mesenchymal progenitor cells injected into the superficial digital flexor tendon of horses: preliminary study. *Equine Vet J* 40:178–181
- Gugjoo MB, Pal A, Abdelbaset-Ismail A, Aithal HP, Kinjavdekar P, Kumar GS, Sharma GT (2017) Mesenchymal stem cells with IGF-1 and TGF- β 1 in laminin gel for osteochondral defects in rabbits. *Biomed Pharmacother* 93:1165–1174
- Gugjoo MB, Amarpal DMM, Sharma GT (2019a) Equine Mesenchymal stem cells: properties, sources, characterization, and potential therapeutic applications. *J Equine Vet* 72:16–27
- Gugjoo MB, Amarpal FMR, Gayas MA, Ahmad RA, Dhama K (2019b) Animal mesenchymal stem cell research in cartilage regenerative medicine – a review. *Vet Q* 39(1):95–120

- Hennig T, Lorenz H, Thiel A, Goetzke K, Dickhut A, Geiger F, Richter W (2007) Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGF- β receptor and BMP profile and is overcome by BMP-6. *J Cell Physiol* 21:682–691
- Hildebrand K, Jia F, Woo S (2002) Response of donor and recipient cells alter transplantation of cells to the ligament and tendon. *Microsc Res Tech* 58:34–38
- Hillmann A, Ahrberg AB, Brehm W, Heller S, Josten C, Paebst F, Burk J (2016) Comparative characterization of human and equine mesenchymal stromal cells: a basis for translational studies in the equine model. *Cell Transplant* 25:109–124
- Hoynowski SM, Fry MM, Gardner BM, Leming MT, Tucker JR, Black L, Sand T, Mitchell KE (2007) Characterization and differentiation of equine umbilical cord-derived matrix cells. *Biochem Biophys Res Commun* 362(2):347–353
- Iacono E, Merlo B, Pirrone A, Saidijam M (2012) Effects of mesenchymal stem cells isolated from amniotic fluid and platelet-rich plasma gel on severe decubitus ulcers in a septic neonatal foal. *Res Vet Sci* 93(3):1439–1440
- Iacono E, Lanci A, Merlo B, Ricci F, Pirrone A, Antonelli C, Mariella J, Castagnetti C (2016) Effects of amniotic fluid mesenchymal stem cells in carboxymethyl cellulose gel on healing of spontaneous pressure sores: clinical outcome in seven hospitalized neonatal foals. *Turk J Biol* 40:484–492
- Iacono E, Pascuccib L, Rossia B, Bazzucchi C, Lanci A, Ceccoli M, Merlo B (2017) Ultrastructural characteristics and immune profile of equine MSCs from fetal adnexa. *Reproduction* 154(4):509–519
- Im K, Park MJ, Kim N, Lim JY, Park HS, Lee SH, Nam YS, Lee ES, Lee JH, Cho ML, Cho SG (2014) Induction of mixed chimerism using combinatory cell-based immune modulation with mesenchymal stem cells and regulatory T cells for solid-organ transplant tolerance. *Stem Cells Dev* 23(19):2364–2376
- Imamura M, Tanaka J (2009) Graft-versus-leukemia effect of non-myeloablative stem cell transplantation. *Korean J Intern Med* 24(4):287–298
- Ishikawa S, Horinouchi C, Mizoguchi R, Senokuchi A, Kamikakimoto R, Murata D, Hatazoe T, Tozaki T, Misumi K, Hobo S (2017) Isolation of equine peripheral blood stem cells from a Japanese native horse. *J Equine Vet Sci* 28(4):153–158
- Jamnik A, Lepperding G (2012) From tendon to nerve: an MSC for all seasons. *Can J Physiol Pharmacol* 90:295–306
- Kang S, Bada LP, Kang C, Lee JS, Kim CH, Park JH, Kim BS (2008) Articular cartilage regeneration with microfracture and hyaluronic acid. *Biotechnol Lett* 30:435–439
- Kinner B, Capito RM, Spector M (2005) Regeneration of articular cartilage. *Adv Biochem Eng Biotechnol* 94:91–123
- Koerner J, Nesic D, Romero JD, Brehm W, Mainil-Varlet P, Grogan SP (2006) Equine peripheral blood-derived progenitors in comparison to bone marrow-derived mesenchymal stem cells. *Stem Cells* 24(6):1613–1619
- Korchunjit W, Laikul A, Taylor J, Watcharat K, Ritruethai P, Supokawej A, Wongtawan T (2019) Characterisation and allogeneic transplantation of equine bone marrow derived multipotent mesenchymal stromal cells collected from cadavers. *J Equine Vet Sci* 73:15–23
- Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ (2001) Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 105:369–377
- Lang HM, Schnabel LV, Cassano JM, Fortier LA (2017) Effect of needle diameter on the viability of equine bone marrow derived mesenchymal stem cells. *Vet Surg* 46:731–737
- Lee H, Kwon JC, Kim JH, Kim KC, Cho MS, Yang YS, Oh W, Choi SJ, Seo ES, Lee SJ, Wang TJ, Hong YM (2015) The effect of umbilical cord blood derived mesenchymal stem cells in monocrotaline-induced pulmonary artery hypertension rats. *J Korean Med Sci* 30(5):576–585
- Leppanen M, Heikkilä P, Katiskalahti T, Tulamo RM (2009) Management of equine tendon and ligament injuries with expanded autologous adipose-derived mesenchymal stem cells – a clinical study. *Regen Med* 4(2):21–22

- Liang J, Zhang H, Hua B, Wang H, Lu L, Shi S, Hou Y, Zeng X, Gilkeson GS, Sun L (2010) Allogeneic mesenchymal stem cells transplantation in refractory systemic lupus erythematosus: a pilot clinical study. *Ann Rheum Dis* 69(8):1423–1429
- Lin PC, Chiou TW, Lin ZS et al (2015) A proposed novel stem cell therapy protocol for liver cirrhosis. *Cell Transplant* 24(3):533–540
- Lombana KG, Goodrich LR, Phillips JN, Kisiday JD, Rupple-Czerniak A, McIlwraith CW (2015) An investigation of equine mesenchymal stem cell characteristics from different harvest sites: more similar than not. *Front Vet Sci* 2:67
- Maia L, Dias MC, de Moraes CN, de Paula F-D'AC, da Mota LS, Santiloni V, da Cruz Landim-Alvarenga F (2017) Conditioned medium: a new alternative for cryopreservation of equine umbilical cord mesenchymal stem cells. *Cell Biol Int* 41(3):239–248
- Mambelli LI, Winter GH, Kerkis A, Malschitzky E, Mattos RC, Kerkis I (2013) A novel strategy of mesenchymal stem cells delivery in the uterus of mares with endometriosis. *Theriogenology* 79(5):744–750
- Mansilla E, Marin GH, Drago H, Sturla F, Salas E, Gardiner C, Bossi S, Lamonega R, Guzmán A, Nuñez A, Gil MA, Piccinelli G, Ibar R, Soratti C (2006) Bloodstream cells phenotypically identical to human mesenchymal bone marrow stem cells circulate in large amounts under the influence of acute large skin damage: new evidence for their use in regenerative medicine. *Transplant Proc* 38(3):967–969
- Marfe G, Massaro-Giordano M, Ranalli M, Cozzoli E, Di Stefano C, Malafiglia V, Poletini M, Gambacurta A (2012) Blood derived stem cells: an ameliorative therapy in veterinary ophthalmology. *J Cell Physiol* 227:1250–1256
- Martinello T, Bronzini I, Maccatrozzo L, Iacopetti I, Sampaiolesi M, Mascarello F, Patruno M (2010) Cryopreservation does not affect the stem characteristics of multipotent cells isolated from equine peripheral blood. *Tissue Eng Part C* 16:771–781
- Marycz K, Grzesiak J, Wrzeszcz K, Golonka P (2012) Adipose stem cell combined with plasma-based implant bone tissue differentiation in vitro and in a horse with a phalanx digitalis distalis fracture: a case report. *VetMed* 57(11):610–617
- Marycz K, Kornicka K, Basinska K, Czyrek A (2016a) Equine metabolic syndrome affects viability, senescence, and stress factors of equine adipose-derived mesenchymal stromal stem cells: new insight into EqASCs isolated from EMS horses in the context of their aging. *Oxidative Med Cell Longev* 2016:1
- Marycz K, Kornicka K, Marędzia M, Golonka P, Nicpoń J (2016b) Equine metabolic syndrome impairs adipose stem cells osteogenic differentiation by predominance of autophagy over selective mitophagy. *J Cell Mol Med* 20(12):2384–2404
- McDuffee LA, Pack L, Lores M, Wright GM, Esparza-Gonzalez B, Masaoud E (2012) Osteoprogenitor cell therapy in an equine fracture model. *Vet Surg* 41(7):773–783
- McIlwraith CW, Frisbie DD, Rodkey WG et al (2011) Evaluation of intraarticular mesenchymal stem cells to augment healing of microfractured chondral defects. *Arthroscopy* 27:1552–1561
- McIlwraith CW, Fortier LA, Frisbie DD, Nixon AJ (2011) Equine models of articular cartilage repair. *Cartilage* 2:317–326
- Mensing N, Gasse H, Hambruch N, Haeger J-D, Pfarrer C, Staszky C (2011) Isolation and characterization of multipotent mesenchymal stromal cells from the gingiva and the periodontal ligament of the horse. *BMC Vet Res* 7:42
- Michler JK, Hillmann A, Savkovic V, Meulling CKW (2018) Horse hair follicles: a novel dermal stem cell source for equine regenerative medicine. *Cytometry A* 93(1):104–114
- Mitchell A, Rivas KA, Smith R III, Watts AE (2015) Cryopreservation of equine mesenchymal stem cells in 95% autologous serum and 5% DMSO does not alter post-thaw growth or morphology in vitro compared to fetal bovine serum or allogeneic serum at 20 or 95% and DMSO at 10 or 5%. *Stem Cell Res Ther* 6:231
- Morrison S, Dryden VC, Bras R, Morrell S (2014) How to use stem cells in clinical laminitis cases. How to choose regenerative medicine and therapies. *AAEP Proc* 60:499–503
- Nawrocka D, Kornicka K, Smieszek A, Marycz K (2017) *Spirulina platensis* improves mitochondrial function impaired by elevated oxidative stress in adipose-derived mesenchymal stromal

- cells (ASCs) and intestinal epithelial cells (IECs), and enhances insulin sensitivity in equine metabolic syndrome (EMS) horses. *MarDrugs* 15:237
- Nixon A, Dahlgren L, Haupt J, Yeager A, Ward D (2008) Effect of adipose-derived nucleated cell fractions on tendon repair in horses with collagenase-induced tendinitis. *Am J Vet Res* 69:928–937
- Noel D, Caton D, Roche S, Bony C, Lehmann S, Casteilla L, Jorgensen C, Cousin B (2008) Cell specific differences between human adipose-derived and mesenchymal-stromal cells despite similar differentiation potentials. *Exp Cell Res* 314:1575–1584
- Ojeh N, Pastar I, Tomic-Canic M, Stojadinovic O (2015) Stem cells in skin regeneration, wound healing, and their clinical applications. *Int J Mol Sci* 16(10):25476–25501
- Olivier FJ, Brooks DE, Kullberg ME, Komaromy AM, Lassaline ME, Andrew SE, Gelatt KN, Stevens GR, Blalock TD, van Setten GB, Schultz GS (2003) Evaluation of various compounds to inhibit activity of matrix metalloproteinases in the tear film of horses with ulcerative keratitis. *Am J Vet Res* 64:1081–1087
- Pacini S, Spinabella S, Trombi L, Fazzi R, Galimberti S, Dini F, Carlucci F, Petrini M (2007) Suspension of bone marrow-derived undifferentiated mesenchymal stromal cells for repair of superficial digital flexor tendon in race horses. *Tissue Eng* 13(12):2949–2955
- Pall E, Toma S, Crecan C, Cenariu M, Groza I (2016) Isolation and functional characterization of equine adipose tissue derived mesenchymal stem cells. *Agric Agric Sci Procedia* 10:412–416
- Pennington MR, Curtis TM, Divers TJ, Wagner B, Ness SL, Tennant BC, Van de Walle GR (2016) Equine mesenchymal stromal cells (MSC) from different sources efficiently differentiate into hepatocyte like cells (HLC). *Tissue Eng Part C Methods* 22(6):596–607
- Perkins N, Reid S, Morris R (2005) Profiling the New Zealand thoroughbred racing industry. 2. Conditions interfering with training and racing. *N Z Vet J* 53:59–68
- Peroni JF, Borjesson DL (2011) Anti-inflammatory and immunomodulatory activities of stem cells. *Vet Clin North Am Equine Pract* 27:351–362
- Perrini C, Strillacci MG, Bagnato A, Esposti P, Marini MG, Corradetti B, Bizzaro D, Idda A, Ledda S, Capra E, Pizzi F, Lange-Consiglio A, Cremonesi F (2016) Microvesicles secreted from equine amniotic-derived cells and their potential role in reducing inflammation in endometrial cells in an in-vitro model. *Stem Cell Res Ther* 7:169
- Prado AAF, Favaron PO, Correia da Silva LCL, Baccarin RYA, Miglino MA, Maria DA (2015) Characterization of mesenchymal stem cells derived from the equine synovial fluid and membrane. *BMC Vet Res* 11:281
- Ra JC, Shin IS, Kim SH, Kang SK, Kang BC, Lee HY, Kim YJ, Jo JY, Yoon EJ, Choi HJ, Kwon E (2011) Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans. *Stem Cells Dev* 8:1297–1308
- Raheja LF, Galuppo LD, Bowers-Lepore J, Dowd JP, Tablin F, Yelowley CE (2011) Treatment of bilateral medial femoral condyle articular cartilage fissures in a horse using bone marrow-derived multipotent mesenchymal stromal cells. *J Equine Vet Sci* 31:147–154
- Ranera B, Lyahyai J, Romero A, Vázquez FJ, Remacha AR, Bernal ML, Zaragoza P, Rodellar C, Martín-Burriel I (2011) Immunophenotype and gene expression profiles of cell surface markers of mesenchymal stem cells derived from equine bone marrow and adipose tissue. *Vet Immunol Immunopathol* 144:147–154
- Reed SA, Johnson SE (2008) Equine umbilical cord blood contains a population of stem cells that express Oct4 and differentiate into mesodermal and endodermal cell types. *J Cell Physiol* 215(2):329–336
- Reesink HL, Sutton RM, Shurer CR, Peterson RP, Tan JS, Su J, Paszek MJ, Nixon AJ (2017) Galectin-1 and galectin-3 expression in equine mesenchymal stromal cells (MSCs), synovial fibroblasts and chondrocytes, and the effect of inflammation on MSC motility. *Stem Cell Res Ther* 8:243
- Renzi S, Ricco C, Dotti S, Sesso L, Grolli S, Cornali M, Carlin S, Patruno M, Cinotti S, Ferrari M (2013) Autologous bone marrow mesenchymal stromal cells for regeneration of injured equine ligaments and tendons: a clinical report. *Res Vet Sci* 95(1):272–277

- Rink BE, Amilon KR, Esteves CL, French HM, Watson E, Aurich C, Donadeu FX (2017) Isolation and characterization of equine endometrial mesenchymal stromal cells. *Stem Cell Res Ther* 8:166
- Rink BE, Beyer T, French HM, Watson E, Aurich C, Donadeu FX (2018) The fate of autologous endometrial mesenchymal stromal cells after application in the healthy equine uterus. *Stem Cells Dev* 27(15):1046–1052
- Sadan O, Melamed E, Offen D (2009) Bone marrow-derived mesenchymal stem cell therapy for neurodegenerative diseases. *Expert Opin Biol Ther* 9:1487–1497
- Schnabel L, Lynch M, Van der Meulen M, Yeager A, Kornatowski M, Nixon A (2009) Mesenchymal stem cells and insulin-like growth factor-I gene- enhanced mesenchymal stem cells improve structural aspects of healing in equine flexor digitorum superficialis tendons. *J Orthop Res* 27:1392–1398
- Schrock C, Eydt C, Geburek F, Kaiser L, Päbst F, Burk J, Pfarrer C, Staszky C (2018) Bone marrow-derived multipotent mesenchymal stromal cells from horses after euthanasia. *Vet Med Sci* 3:239–251
- Seo J-P, Yamaga T, Tsuzuki N, Yamada K, Haneda S, Furuoka H, Tabata Y, Sasaki N (2014) Minimally invasive proximal interphalangeal joint arthrodesis using a locking compression plate and tissue engineering in horses: a pilot study. *Can Vet J* 55:1050–1056
- Shi D, Wang D, Li X, Zhang H, Che N, Lu Z, Sun L (2012) Allogeneic transplantation of umbilical cord-derived mesenchymal stem cells for diffuse alveolar hemorrhage in systemic lupus erythematosus. *Clin Rheumatol* 31:841–846
- Smith RKW (2008) Mesenchymal stem cell therapy for equine tendinopathy. *Disabil Rehabil* 30:1752–1758
- Smith RKW, Korda M, Blunn GW, Goodship AE (2003) Isolation and implantation of autologous equine mesenchymal stem cells from bone marrow into the superficial digital flexor tendon as a potential novel treatment. *Equine Vet J* 35:99–102
- Smith R, Young N, Dudhia J, Kasashima Y, Clegg PD, Goodship A (2009) Effectiveness of bone marrow-derived mesenchymal progenitor cells for naturally occurring tendinopathy in the horse. *Regen Med* 4(Suppl 6):25–26
- Smith RKW, Werling NJ, Dakin SG, Alam R, Goodship AE, Dudhia J (2013) Beneficial effects of autologous bone marrow-derived mesenchymal stem cells in naturally occurring tendinopathy. *PLoS One* 8(9):e75697
- Sole A, Spriet M, Galuppo LD, Padgett KA, Borjesson DL, Wisner ER, Brosnan RJ, Vidal MA (2012) Scintigraphic evaluation of intra-arterial and intra-venous regional limb perfusion of allogeneic bone marrow-derived mesenchymal stem cells in the normal equine distal limb using (99m) Tc-HMPAO. *Equine Vet J* 44:594–599
- Sordi V, Piemonti L (2011) Therapeutic plasticity of stem cells and allograft tolerance. *Cytotherapy* 13:647–660
- Spaas JH, Gambacurta A, Poletini M, Broeckx S, Van Hoeck F, De Schauwer C, Van De Walle GR, Van Soom A (2011) Purification and expansion of stem cells from equine peripheral blood, with clinical applications. *Vlaams Diergeneeskundig Tijdschrift* 80:129–135
- Spaas JH, Guest DJ, Van de Walle GR (2012) Tendon regeneration in human and equine athletes: Ubi Sumus-quo Vadimus (where are we and where are we going to)? *Sports Med* 42(10):871–890
- Spaas JH, Broeckx S, Van de Walle GR, Poletini M (2013) The effects of equine peripheral blood stem cells on cutaneous wound healing: a clinical evaluation in four horses. *Clin Exp Dermatol* 38:280–284
- Spriet M, Trela JM, Padgett KA, Vidal MA (2013) Scintigraphic comparison of 4 different techniques of administration of mesenchymal stem cells to the equine foot. *JEquine VetSci* 33:867
- Stenudd M, Sabelström H, Frisén J (2015) Role of endogenous neural stem cells in spinal cord injury and repair. *JAMA Neurol* 72(2):235–237
- Stewart MC, Stewart AA (2011) Mesenchymal stem cells: characteristics, sources, mechanisms of action. *Vet Clin North Am Equine Pract* 27:243–261

- Strubbe DT, Brooks DE, Schults GS et al (2000) Evaluation of tear film proteinases in horse with ulcerative keratitis. *Vet Ophthalmol* 3:111–119
- Sun L, Akiyama K, Zhang H, Yamaza T, Hou Y, Zhao S, Xu T, Le A, Shi S (2009) Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. *Stem Cells* 27:1421–1432
- Taylor SE, Smith RKW, Clegg PD (2007) Mesenchymal stem cell therapy in equine musculoskeletal disease: scientific fact or clinical fiction? *Equine Vet J* 39:172–180
- Textor JA, Clark KC, Walker NJ, Aristizobal FA, Kol A, LeJeune SS, Bledsoe A, Davidyan A, Gray SN, Bohannon-Worsley LK, Woolard KD (2017) Allogeneic stem cells alter gene expression and improve healing of distal limb wounds in horses. *Stem Cells Transl Med* 7(1):98–108
- Theoret C (2009) Tissue engineering in wound repair: the three “R”s - repair, replace, regenerate. *Vet Surg* 38:905–913
- Toupadakis CA, Wong A, Genetos DC, Cheung WK, Borjesson DL, Ferraro GL, Galuppo LD, Leach JK, Owens SD, Yellowley CE (2010) Comparison of the osteogenic potential of equine mesenchymal stem cells from bone marrow, adipose tissue, umbilical cord blood, and umbilical cord tissue. *Am J Vet Res* 71:1237–1245
- Tsuzuki N, Nakao S, Seo J, Yamada K, Haneda S, Furuoka H, Tabata Y, Sasaki N (2014) Effect of biodegradable gelatin β -tri calcium phosphate sponges containing mesenchymal stem cells and bone morphogenetic protein-2 on equine bone defect. *J Equine Vet Sci* 34(7):903–910
- Tuermers C, Rebolledo N, Aguilar R (2012) Effect of the application of stem cells for tendon injuries in sporting horses. *Arch MedVet* 44:207–215
- Van Loon VJF, Scheffer CJW, Genn HJ, Hoogendoorn AC, Greve JW (2014) Clinical follow-up of horses treated with allogeneic equine mesenchymal stem cells derived from umbilical cord blood for different tendon and ligament disorders. *Vet Q* 34:92–97
- Vandenberghe A, Broeckx SY, Beerts C, Seys B, Zimmerman M, Verweire I, Suls M, Spaas JH (2015) Tenogenically induced allogeneic mesenchymal stem cells for the treatment of proximal suspensory ligament desmitis in a horse. *Front Vet Sci* 2:49
- Vidal MA, Robinson SO, Lopez MJ, Paulsen DB, Borkhsenius O, Johnson JR, Moore RM, Gimble JM (2008) Comparison of chondrogenic potential in equine mesenchymal stromal cells derived from adipose tissue and bone marrow. *Vet Surg* 37:713–724
- Villagrán CC, Schumacher J, Donnell R, Dhar MS (2016) A novel model for acute peripheral nerve injury in the horse and evaluation of the effect of mesenchymal stromal cells applied in situ on nerve regeneration: a preliminary study. *Front Vet Sci* 3:80
- Violini S, Ramelli P, Pisani L, Gorni C, Mariani P (2009) Horse bone marrow mesenchymal stem cells express embryo stem cell markers and show the ability for tenogenic differentiation by in vitro exposure to BMP-12. *BMC Cell Biol* 10:29
- Volk SW, Theoret C (2013) Translating stem cell therapies: the role of companion animals in regenerative medicine. *Wound Repair Regen* 21(3):382–394
- Wada S, Hobo S, Niwa H (2010) Ulcerative keratitis in thoroughbred racehorses in Japan from 1997 to 2008. *Vet Ophthalmol* 13(2):99–105
- Watanabe S, Uchida K, Nakajima H, Matsuo H, Sugita D, Yoshida A, Honjoh K, Johnson WE, Baba H (2015) Early transplantation of mesenchymal stem cells after spinal cord injury relieves pain hypersensitivity through suppression of pain-related signaling cascades and reduced inflammatory cell recruitment. *Stem Cells* 33(6):1902–1914
- Wilke MM, Nydam DV, Nixon AJ (2007) Enhanced early chondrogenesis in articular defects following arthroscopic mesenchymal stem cell implantation in an equine model. *J Orthop Res* 25:913–925
- Williams LB, Russell KA, Koenig JB, Koch TG (2016) Aspiration, but not injection, decreases cultured equine mesenchymal stromal cell viability. *BMC Vet Res* 12:45
- Wilmink JM, van Herten J, van Weeren PR, Barneveld A (2002) Retrospective study of primary intention healing and sequestrum formation in horses compared to ponies under clinical circumstances. *Equine Vet J* 34:270–273
- Wu Y, Chen L, Scott PG, Tredget EE (2007) Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells* 25:2648–2659

- Xie L, Zhang N, Marsano A, Vunjak-Novakovic G, Zhang Y, Lopez MJ (2013) In vitro mesenchymal trilineage differentiation and extracellular matrix production by adipose and bone marrow derived adult equine multipotent stromal cells on a collagen scaffold. *Stem Cell Rev Rep* 9:858–872
- Yamada ALM, Carvalho AD, Moroz A, Watanabe MJ, Hussni CA, Rodrigues CA, Garcia Alves AL (2013) Mesenchymal stem cell enhances chondral defects healing in horses. *Stem Cell Discov* 3(4):218–225
- Yamasaki A, Omura T, Murata D, Kobayashi M, Sunaga T, Kusano K, Ueno Y, Kuramoto T, Hobo S, Misumi K (2018) A pilot study of regenerative therapy by implanting synovium-derived mesenchymal stromal cells in equine osteochondral defect models. *J Equine Sci* 29(4):117–122
- Yang Q, Lopez MJ (2019) The equine hoof: laminitis, progenitor (stem) cells, and therapy development. *Toxicol Pathol*:019262331988046
- Zahedi M, Parham A, Dehghani H, Mehrjerdi HK (2018) Equine bone marrow-derived mesenchymal stem cells: optimization of cell density in primary culture. *Stem Cell Investig* 5:31
- Zayed MN, Schumacher J, Misk N, Dhar MS (2016) Effects of pro-inflammatory cytokines on chondrogenesis of equine mesenchymal stromal cells derived from bone marrow or synovial fluid. *Vet J* 217:26–32



Future of Mesenchymal Stem Cell Research

13

M. B. Gugjoo and Amar Pal

Abstract

Mesenchymal stem cells due to their characteristic features are considered to provide a single stop solution to diverse problems. The cells have been harvested from almost all the adult body tissues and fetal membranes. These tissues harbor cells in differential concentration and with variable properties. The main impediment in their direct clinical applications is the weak understanding of their basic physiological properties. The cells have been studied in various in vivo experimental models and in clinical trials of dog, horse, and cat. Most of these studies are non-uniform and lack case control and as such no conclusive understanding has been achieved. The current chapter details MSCs current status and their future prospectus in veterinary sciences.

13.1 Introduction

Stem cell application remains one of the most researched and sought-in options for therapeutics. Among all the stem cell types, currently mesenchymal stem cells (MSCs) mainly contribute to the preclinical and clinical studies. The cell per se is considered to provide therapeutics in diverse clinical ailments. The cell has innumerable sources, easy harvesting/ isolation, and established characterization procedures. The cell also has minimal teratogenic effects and lack ethical issue as is

M. B. Gugjoo (✉)

Division of Veterinary Clinical Complex, FVSc & AH, SKUAST-Kashmir,
Jammu and Kashmir, India

A. Pal

Division of Surgery, Indian Veterinary Research Institute, Izatnagar, UP, India

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2020

M. B. Gugjoo, A. Pal (eds.), *Mesenchymal Stem Cell in Veterinary Sciences*,
https://doi.org/10.1007/978-981-15-6037-8_13

333

usually associated with the pluripotent stem cell, especially in humans. There are various features attributed to the cell some of which are well established and characterized while others remain to be studied and confirmed. Below are the details of MSCs features, their applications, and associated complications.

13.2 In Vitro MSCs Characteristics

Stem cells are characterized by the atypical stemness properties of self-renewal and differentiation properties. The self-renewal property however, is currently questioned. These cells have finite culture life as evidenced by decreased population doubling potential and morphological changes that ensue with extended culturing. This culture life span also varies with the species type with feline MSCs relatively bearing limited culture life span. Some of the studies although have shown that MSCs are able to maintain their pool through the “self-renewal” and “differentiate” into more advanced and lineage specific cell type. The viral vectors, however, may be used to induce immortality. However, there is dearth in understanding of the basic mechanism(s) behind these properties. MSCs have been isolated from almost all the tissue types including the fetal membranes. Among, MSCs derived from bone marrow, adipose tissue and fetal membranes have been studied extensively. The cells are usually being cultured along with the fetal bovine serum (FBS). Due to the associated limitations of disease transmission, changed cell behavior due to xenogeneic proteins and variability in preparations, its alternatives are being studied. Alternatives like allogeneic platelet lysate, autologous/allogeneic serum, or commercially available serum substitute have provided little success to culture MSCs in veterinary species, unlike that of human MSCs.

There is lack of a single definition or availability of a specific quantitative assay to identify MSCs in mixed cell population. Animal source MSCs like humans are being characterized based upon the recommendations of the International Society for Cellular Therapy (ISCT). The cells that are plastic adherent, express some surface markers and lack hematopoietic markers (CD105+, CD90+, CD70+, MHC-I+; CD34-, CD45-, MHC-II-), and are able to differentiate into at least three lineages (adipogenic, chondrogenic, and osteogenic) are characterized as MSCs. Although, the cells are plastic adherent and show tri-lineage differentiation but discrepancies have been demonstrated in their surface marker expression and need to be evaluated. MSCs are considered to be multipotent but recent studies have demonstrated their extended differentiation potential. The cellular plasticity goes beyond as the cells also express the pluripotency markers like Oct4, Sox-2, and Nanog, and also tend to differentiate into germinal cell-like cells, neurocyte-like cells, myocyte-like cells, and hepatocyte-like cells, among others. Thus, the cellular mechanisms behind such properties are required to be elucidated. Apart from these properties, these cells are able to “migrate” and “home” into the distant tissues, and bring about “anti-inflammatory and/ immuno-modulatory” actions. There are numerous studies that have demonstrated their immune-modulatory and migration/homing properties but consensus

lacks among the studies upon the mechanisms involved. Thus, further studies are desired aimed at the mechanisms involved in these properties.

MSCs concentration varies in tissue sources; peripheral blood harbors minimal cell concentration, and adipose tissue harbors better cell concentration than the bone marrow. The cell from the particular source but at different locations may or may not have comparable culture characteristics. Even the mechanisms involved in their differentiation may also vary with tissue source. Age of the donor also affects their properties especially population doubling and differentiation properties. The population doubling and differentiation potential of MSCs decrease with aging of donor. The donor health status also has bearing on cellular characteristics. Diseases like equine metabolic syndrome (insulin resistance disease in equines) and feline foamy virus affect MSCs properties that become senescent and carry limited differentiation potential. Prion disease in sheep and osteoarthritis in horse though do not affect MSCs growth but may decrease their neurogenic and chondrogenic potential, respectively. Even the reproductive cyclicity may also affect properties of the endometrium derived MSCs. It becomes imperative to elucidate the mechanisms behind such discrepancies for their effective utilization.

MSCs aspiration and injection method also affect their viability. The vigorous aspiration appears more damaging than that of the injection. Even needle bore size affects the MSCs viability. The large bore needle tends to have limited adverse effect on cell viability as compared to the small bore needles. The type of storage container whether glass or plastic although has no obvious effect on cell viability. The storage type affects MSCs viability with cryopreservation better able to preserve cell viability for longer time as compared to the biological fluids. For transit storage of shorter duration (8–12 hrs) phosphate buffer saline appears useful clinically as cells do not show any adverse effect of residual proteins of foreign materials like FBS or dimethylsulfoxide (DMSO). Sedatives (detomidine and butorphanol), antibiotics (aminoglycosides), and steroids (methylprednisolone and triamcinolone) at therapeutic concentrations tend to deleteriously affect MSCs viability.

Environmental factors involving different scaffolds, growth factors, and cell type into the culture environment affect MSCs proliferation and determine their fate. Apart from these factors mechanics also have bearing on their proliferation and differentiation. Currently, the effect of these factors has been studied under isolated experiments. The results under in vivo environment may be altogether variable as the in vivo conditions have simultaneous effect on various factors. Thus, ex vivo systems should ape the environment available under in vivo conditions.

13.3 In Vivo MSCs Characteristics

The ultimate aim of harvesting and culture expansion of MSCs is to utilize them in research and under clinical settings. MSCs under preclinical studies and clinical trials have variably improved the outcome without any major adverse effect except few minor reactions. There are various mechanism attributed to their therapeutic applications. The undesirable reactions reported including limited inflammation

and pulmonary parenchymal edema and hemorrhage are self-limiting. Such effects however, may be controlled by proper care and applications of the anticoagulants like heparin may be required in disseminated intravascular coagulation. Currently, MSCs are considered to provide therapeutic benefits mainly through their paracrine secretion, although their *in vivo* survival and subsequent differentiation if achieved may provide enhanced and effective results. Immuno-compromised and/ or immuno-modulatory properties have made their allogeneic and or xenogeneic applications possible. Mostly no observable immune reactions to the allogeneic/ xenogeneic cells have been reported, barring few studies and thus, further studies for their safety are desired before cells are utilized in clinical settings.

The cells have been evaluated in both musculoskeletal as well as non-musculoskeletal tissues for their therapeutic effects. However, MSCs application mostly remains under experimental phase. The limited understanding of their basic physiological properties, poorly understood cellular interactions with respect to the microenvironment restricts their definitive clinical applications. Further, variable culture conditions make understanding more complicated. The combined use of cells and other biomaterials like scaffolds and growth factor makes understanding even more complex.

In case of musculoskeletal system, MSCs have been studied both in preclinical experimental models (sheep, goat, dog, cat, horse, and cattle) as well as in clinical trials (dog, cat, and horse). MSCs irrespective of the source (allogeneic or autologous) have been utilized without any fatality. Applications of MSCs in musculoskeletal affections like osteoarthritis, bone fractures, muscle damage, cardiac infarctions, periodontal defects, ligaments and tendon injuries have been studied with an overall positive response.

In case of osteoarthritis, the cells may show improvement with respect to the pain relief. However, no obvious and actual hyaline cartilage seems to develop that integrates to the native hyaline tissue. Inflammation induced MSC's increased expression of adhesion molecules together with their decreased production of glycosaminoglycans may be one of the reasons for their insignificant positive results. To compensate these lacunas MSCs may be genetically engineered.

Bone regenerative medicine may be required in case of extensive damages that otherwise has good healing potential. In case of extensive bone fractures, or other relevant problems like delayed/non-union, regenerative medicine may be employed involving MSCs and other biomaterials. In general MSCs, along with other factors like scaffolds and growth/humoral factors promotes bone healing in shorter time interval. However, the cellular dosology, scaffold type, and growth factors incorporated remain to be standardized.

Muscle damage/injury is a problem as post-injury muscles are repaired by fibrous tissue. The preliminary reports on the application of MSCs in these ailments favor their application but intricacies in definitive therapy for the management of such ailments is yet to be elucidated.

In case of ligament/tendon injuries, MSCs especially in equines have been fruitful as represented by ability of animals to return to the normal activity. However, the

sole MSCs application may not be the reason behind but along with the other standard managemental practices.

Cardiac ailments like infarction is extensively studied area in the stem cell research. The preclinical experimental studies conducted on sheep/goat favor MSCs application. A lot more needs to be studied to confirm their feasibility, safety, and beneficial effect on cardiac infarctions. However, no beneficial effect on dilatation cardiomyopathy has been demonstrated in a dog study.

In relation to the neurological system, preliminary reports show MSCs beneficial effects to some extent. But the cells under in vivo conditions have not been reported to actually differentiate into the neurons and the possible beneficial effect may be through the release of trophic factors. Currently, the study area is in its infancy.

Apart from the musculoskeletal tissues, the cells have been utilized in non-musculoskeletal tissues like dermal wounds, vocal fold injuries, kerato-conjunctivitis sicca (KCS), anal fistula, inflammatory bowel disease (IBD), chronic kidney disease (CKD), asthma, feline eosinophilic keratitis (FEK). MSCs in general promote healing in dermal wounds, vocal fold injuries, and anal fistula. However, some of the reports show recurrence in cases like anal fistula which need to be studied. In case of KCS, asthma, FEK, CKD, and IBD in dogs and cats, improved results have variably been reported following the MSCs application. Apart from these conditions, studies on MSCs have also been started to evaluate effect on diabetes, liver affections, tumors, etc.

Available in vivo literature in veterinary science has frequently relied on study designs that do not incorporate blinded randomized control trials to act as evidence based medicine. The experimental power of these studies also suffers due to the limited sample size enhancing inter-animal variability. Furthermore, these studies lack sufficient follow-up period to determine long term effects.

13.4 Conclusion(S)

MSCs are adult stem cells being harvested from almost all the body tissues including fetal membranes. These cells lack any specific marker and are characterized on the basis of certain characteristic features being recommended by ISCT. The cell lines from different sources have shown variations in relation to the surface markers and differentiation properties. The cells from aged and unhealthy donors tend to be less effective. These variations need further incites for their effective utilization. The cells are being cultured and cryopreserved in FBS that may pose adverse reactions and as such FBS alternatives are being evaluated. MSCs therapeutic effects are being evaluated in various models and even in clinical trials in dog, cat, and horse. However, further extensive studies aimed at their actual involved mechanisms need to be recognized. The current lack of blinded randomized control trials and insufficient sample size make their application even more complicated. The studies should be aimed at the MSCs posology that can help to determine their dose, route, and frequency of application.