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Advances in stem cell therapy in veterinary medicine: Basic research to clinical practice

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Abstract
Application of stem cells in regenerative and reparative therapies is emerging worldwide. Stem cells are derived from embryonic and adult tissue, has ability to divide for indefinite period in the culture and give rise to specialized cells. Stem cells can be totipotent, pluripotent, multipotent and unipotent on the basis of their potential of differentiation. Multipotent stem cells, subset of adult stem cells capable of producing bone, cartilage and fat cells and supports the formation of blood cells. Mesenchymal stem cells (MSCs) are non-blood adult stem cells obtained from bone marrow and adipose tissue. They are excellent candidate for cell therapy. Stem cells can be prepared through various methods. However, numerous factors such as age and physical status of the donor, tissue source i.e., fat and bone marrow; method of harvesting, manipulation, handling and storage of the sample, may affect the number and function of the stem cells. Stem cell therapy has been studied in various conditions such as soft tissue injury, wound healing, screening of drugs, tissue engineering, in regenerative medicine for treating several incurable ailments, in regeneration of tissue, repair of cardiovascular muscle and vascular tissue, rheumatoid arthritis, osteoarthritis and burns. This review describes the clinical application of stem cells in various unresolving and complicated conditions.

Keywords: Stem cells, regenerative therapy, wound healing, bone repair, hepatic disease

Introduction
Stem cells are the cells with the ability to divide for indefinite period in culture and give rise to specialized cells. These cells are derived from embryonic stem cells and adult stem cells. Embryonic stem cells are also known as pre-natal stem cells. Embryonic stem cells are the primitive or undifferentiated cells which are derived from a 5-day old embryo, capable of dividing without differentiating for a prolonged period in culture. They are known to develop into cells and tissues of the three primary germ layers (Fossum, 2013) [35]. They are derived from morulae, intact blastocysts, inner cell mass, single blastomeres, parthenogenetic embryos (Sritanaudomchai *et al.*, 2007; Chung *et al.*, 2006; Strelchenko *et al.*, 2004) [101, 23, 104]. Similarly, adult stem cells are also known as bone marrow cells or post-natal stem cells. These adult stem cells are a population of cells found in bone marrow. The subset of adult stem cells are multipotent stem cells which are capable of producing bone, cartilage and fat cells and have ability to support the formation of blood cells. These cells are obtained from different sources, mainly bone marrow and adipose tissue and are used for treatment of animal diseases around the world (Marx *et al.*, 2014; Torricelli *et al.*, 2011) [73, 107]. However, bone marrow serves as a source of both hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs).
Mesenchymal stem cells (MSCs) are a non-blood adult stem cells obtained from different germ layers such as ectoderm, mesoderm and endoderm. They are distributed in all the vascularized adult tissues, i.e., adipose tissue, skin, heart, brain, vessels, bones, cartilage, umbilical cord blood, amniotic fluid, dental pulp, tendons, synovial membrane, skeletal muscle, Wharton's jelly, neurons and hepatocytes (Gade *et al.*, 2012; Pratheesh *et al.*, 2013; Isern *et al.*, 2014) [37, 90, 49]. Although, it is not clear that MSCs obtained from different tissues are similar (Fossum, 2013) [35]. Mesenchymal stem cells remain quiescent or non-dividing for long periods of time until they are activated by disease or tissue injury or by a normal need for additional cells to maintain the tissues. They are easily accessible from fat, bone marrow and skin. Isolation is straightforward, can be greatly expanded as many cells can be derived from a single donor. They can be bio-preserved with minimal loss of potency. There are no significant adverse reactions to allogeneic versus autologous MSCs transplants. They have potent immunosuppressive properties, which allow them to modulate the function of all major immune cell populations, thus impeding the immune responses.

Therefore, they are excellent candidate for cell therapy. Mesenchymal stem cells are immune-privileged with low MHC I and no MHC II expression, therefore reducing the risk of rejection and complications for transplantation (Uccelli *et al*., 2006) [108]. They are hypoimmunogenic and prevents T-cell response. They also modulate the dendritic cells and disrupts the natural killer cells & modulate CD8+, CD4+ T cell function (Di Nicola *et al.,* 2002) [31]. However, as the MSCs differentiate, this immune privileged characteristic is lost leading to a gradual host response to the implanted cells (Li *et al.,* 2013) [70].

On the basis of their differentiation potential, stem cells are classified as totipotent, pluripotent, multipotent and unipotent stem cells. Totipotent cells are the cells which have the ability to differentiate into all possible cell types. Examples are the zygote formed at egg fertilization and the first few cells that result from the division of the zygote. Pluripotent cells are the cells which have the ability to differentiate into almost all the cell types. Examples include embryonic stem cells and cells derived from the mesoderm, endoderm and ectoderm germ layers that are formed in the beginning stages of the differentiation of embryonic stem cell. Multipotent cells are the cells having ability to differentiate into a closely related family of cells. Examples include hematopoietic (adult) stem cells that can become red and white blood cells or platelets.

History

The concept of stem cells was introduced over a century ago by Alexander Maximow. After 1963, modern research had begun on it. Stem cells are isolated first as colony-forming unit fibroblasts from the murine bone marrow by Friedenstein *et al.,* 1970 [36]. After this, embryonic stem cells are identified. Human embryonic stem cells were isolated by Thomson & Gearhart (1998). Induced pluripotent stem cells are identified by inserting four key genes in ordinary adult cells by Yamanaka in 2006. In 2007, Nobel Prize was awarded to Evans, Mario Capecchi and Oliver Smithies for the work on genetics and embryonic stem cells. In 2012, Nobel Prize was awarded to Yamanaka and John Gurdon for creating the induced pluripotent stem cells.

Preparation of stem cells

For obtaining the embryonic stem cells, first blastocysts are obtained from a donor dog and embryonic stem cells are established and expanded indefinitely. These cells are differentiated into the tissue of choice and used for tissue replacement therapy in a recipient dog, also used for *in-vitro* research including drug and toxicology studies (Schneider *et al*., 2010) [96]. Three basic methods are given below for the preparation of stem cells. The first technique is the simplest one used for the production of stem cells for veterinary patients. In this technique, fat or bone marrow is collected and is centrifuged to concentrate the cells. This produces a mixture of both stem and non-stem cells, results in a relatively low yield of stem cells (1 in 10,000-1,000,000 of the nucleated cells are stem cells). CD34, a surface marker found on the hematopoietic cells, is frequently used to identify and remove the non-mesenchymal cells from a marrow culture.

The second technique include isolation of cells, followed by treatment with growth factors/cytokines for differentiation into different cell type of interest (i.e., chondrocyte, cardiac myocyte). Similarly, for osteogenic differentiation, MSCs can be induced *in vitro* by treating a monolayer culture with a dexamethasone, ascorbic acid-2-phosphate and beta glycerophosphate. Mesenchymal stem cells can be isolated, propagated and characterized into differentiated osteoblasts in canines by utilizing specific laboratory techniques (Dobhal, 2015) [32]. Chondrogenic differentiation can be induced by bovine insulin, transferrin, selenious acid, linoleic acid, bovine serum albumin, sodium pyruvate, proline, L-glutamine or TGF-beta 1. Mesenchymal stem cells also have ability to form muscle cells and cardiomyocytes when treated with the demethylating agent 5-azacytidine. However, this technique still results in a relatively low yield of stem cells (pre-differentiated cells). It has been shown that the proliferation and differentiation capabilities of chondrogenic cells of a 4-month-old dog are higher than those in a 12-month-old dog (Li *et al*., 2009) [68]. Studies on isolation, propagation and characterization of bone marrow derived mesenchymal stem cells and their differentiation into chondrocytes in dogs are also reported (Joshi, 2017) [55]. Mesenchymal stem cells can be isolated, propagated, characterized and differentiated into cardiac myocytes in canines (Mubarik, 2017) [79]. Similarly, mesenchymal stem cells can be differentiated into neurocytes in canines (Verma, 2018) [110]. Isolation, expansion and characterization of bone marrow derived mesenchymal stem cells and their differentiation into hepatocytes in canines are also reported (Khanum, 2018) [59]. Studies on isolation, expansion and characterization of bone marrow derived mesenchymal stem cells and their differentiation into skeletal myocytes in canines by using specific laboratory techniques are also reported (Sandhu *et al.,* 2020) [93].

The third technique is the most complex technique, is also considered as gold standard technique. In this technique, billions of mesenchymal stem cells can be produced from a small bone marrow aspirate. First cells are obtained, followed by expansion of cell population in the culture, followed by treatment with the growth factors.

When required, these stem cells may differentiate into-

1. Hematopoietic stem cells, give rise to all the types of blood cells (RBCs, WBCs) that regulate function of immune system.
2. Mesenchymal stem cells, give rise to variety of cell types such as osteocytes, chondrocytes, adipocytes and other connective tissue cells such as tendon.
3. Neural stem cells, give three major cell types, i.e., neurons, astrocytes and oligodendrocytes.
4. Epithelial stem cells, give rise to absorptive cells, goblet cells, paneth cells and enteroendocrine cells.
5. Skin stem cells, which forms basal layer of the epidermis and hair follicles.
6. Epidermal stem cells, forms keratinocytes.
7. Follicular stem cells form hair follicle and epidermis.

However, numerous factors may affect the number and function of the stem cells includes age and physical status of the donor, source of tissue (fat, bone marrow), method of harvesting, manipulation, handling and storage of the sample.

Clinical application of stem cells

- For therapeutic applications, stem cells are generally harvested from bone marrow i.e., femur of dogs and cats, sternum of horses or adipose tissue from the inguinal region of the dogs and cats and dorsal surface of the gluteus maximus of horses. Stem cells have been used successfully during the acute inflammatory stage of an injury, but once bone has remodelled or tissue has matured, the utility of stem cell therapy falls

significantly.

- Stem cell therapy has been studied in varied conditions which includes cancer, heart disease, immune disorders, Alzheimer and Parkinson diseases, blindness and diabetes. In the veterinary world, stem cell therapy has been used primarily in soft tissue injury and wound healing.
- Embryonic, adult and induced pluripotent stem cells are valuable for application in drug screening and tissue engineering (Gurtner *et al.*, 2007) [45].
- In farm animals, embryonic stem cells (ESCs) may be helpful in generating transgenic animals (Brevini *et al.*, 2008) [17].
- The stem cell-based approach is also used in treating type I diabetes via islet transplantation and is well documented in human medicine (Street *et al.*, 2004) [103].
- Stem cells has been used in the area of regenerative medicine in the treatment of several incurable ailments. Adipose derived stem cell (AD-SC) is effective in tissue regeneration, repair of cardiovascular muscle and vascular tissue, rheumatoid arthritis, diabetes, osteoarthritis (hips, stifle, elbow, shoulder) and burns.
- Stem cells possess a significant potential for tissue regeneration (Mansilla *et al.*, 2005) [72].
- Adipose tissue-derived mesenchymal stem cell therapy administered intra-articularly improved the function, range of motion and quality of life.

Wound healing

In the process of wound healing, there are three phases i.e., inflammatory phase, proliferation phase and remodelling phase, occurs during wound healing. In inflammatory phase which lasted for 1-3 days, suppression of tumour necrosis factor (TNF), production of interleukin-4 (IL-4), IL-10 and blocking of T-cell proliferation occurs. During proliferation phase, production of vascular endothelial growth factor (VEGF), human growth factor (HGF) and platelet derived growth factor (PDGF) along with recruitment of keratinocytes, dermal fibroblasts and host stem cell occurs. During remodelling which lasted for up to 2 years, production of transforming growth factor-β3 (TGF-β3), keratinocyte growth factor (KGF), regulation of matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMPs) and collagen deposition occurs, which contribute in the wound healing (Maxson *et al.*, 2012) [74].

Adult bone marrow cells give rise to epidermal keratinocytes, follicular epithelial cells, sebaceous gland cells, dendritic cells after their transplantation in mice (Krause *et al.*, 2001) [65]. Autologous bone marrow derived nucleated cells have been transplanted in experimental rabbits and clinical cases for the evaluation of their tissue regeneration potential in full thickness wounds (Borena *et al.*, 2009) [16], burn wounds (Oloumi *et al.*, 2008) [85] and corneal alkali burn wounds (Ye *et al.*, 2006) [119]. Autologous bone marrow derived nucleated cells also have been transplanted in experimental rabbits and clinical cases for the evaluation of their potential of tissue regeneration in corneal wounds (Dauthal *et al.*, 2019) [28]. In patients with chronic non-healing wound i.e., in case of diabetic ulcer, a combination of autologous graft (autologous skin fibroblasts on biodegradable collagen membrane) with autologous bone marrow derived MSC showed a steady decrease in the wound size, increase in the vascularity of the dermis and dermal thickness of the wound bed after 29 days of combined treatment (Jan *et al.*, 2006) [52]. Bone marrow derived mesenchymal stem cells (BM-MSCs) have been shown to promote the healing of diabetic wounds (Gade *et al.*, 2012) [37]. Wounds treated with BM-MSC significantly exhibited faster wound closure with increased re-epithelialization, cellularity and angiogenesis. Mesenchymal stem cells have enhanced the capacity of tissue regeneration of incisional wound in 4 days especially in older populations of mice (Li *et al.*, 2017) [69]. In caprine, Wharton's jelly mesenchymal stem cells (WJMSCs) of the umbilical cord were used to treat the cutaneous wounds (Azari *et al.*, 2011) [10]. Application of mesenchymal stem cells for the treatment of surgical and chronic wounds in dogs also results in better and faster healing (Bhatt *et al.*, 2018) [22].

Clinical evidence also shows that autologous adipose-derived stem cells (AD-SCs) can dramatically improve the healing of injuries and decrease the degenerative processes (Alderman & Alexander, 2011) [6]. Embryonic stem cells (ESCs) accelerate wound healing in chronic wound of diabetic mice with the evidence of beneficial effects both histopathologically and immunohistochemically. Human umbilical cord derived MSCs (hUCB-MSCs) shows higher cell proliferation and collagen synthesis as compared to fibroblasts on *in-vitro* treatment of diabetic wounds (Jung *et al*., 2018) [56]. Bhatt *et al.* (2021) [14] had also compared the efficacy of single and twice application of mesenchymal stem cells in full thickness cutaneous wound healing.

Nanofiber expanded cord blood-derived CD34+ cell therapy might be a potential candidate to treat the chronic wounds to resolve inflammation, facilitate angiogenesis and extracellular matrix (ECM) formation for accelerated healing (Kanji *et al*., 2019) [58].

Table 1: Phases of wound healing

Phases of wound healing	Time period	Role of mesenchymal stem cells
Inflammation	1-3 days	Suppression of TNF Production of IL-10, IL-4 Inhibition of T-cell proliferation
Proliferation	2 weeks	Production of VEGF, PDGF and HGF Recruitment of keratinocytes, dermal fibroblast and host stem cells
Remodelling	Up to 2 years	Production of TGF-β3, KGF Regulation of collagen deposition Regulation of MMPs and TIMP

Role of Mesenchymal stem cell in each phase of the wound-healing process

Antimicrobial action of mesenchymal stem cells

Mesenchymal stem cells also exhibit antimicrobial activity directly or indirectly via two mechanisms. It directly secretes antimicrobial factors such as LL-37 (Krasnodembskaya *et al*., 2010) [63]. It indirectly secretes immunomodulatory factors which upregulate the bacterial killing and phagocytosis by the immune cells (Mei *et al.*, 2010) [75]. Seema (2014) [97] has studied the therapeutic potential of haematopoietic stem cells in immunodeficient canine patients.

Bone repair

Mesenchymal stem cells can undergo differentiation into osteoblasts, adipocytes and chondrocytes after exposure to specific factors in the microenvironment (Udehiya *et al.*, 2013; Fitzsimmons *et al*., 2018) [109, 34]. Mesenchymal stem cells stimulate the formation of new bone after infiltrating in the adjacent host bone. It stimulates the host bone to regenerate new bone (Kraus & Kirker, 2006) [64]. Adipose

tissue derived and bone marrow derived MSCs are the most common cells used in clinical practice (Fitzsimmons *et al*., 2018) [34]. Collection of bone marrow from the donor can be painful, resulting in bleeding and infection. On the other hand, adipose derived stem cells (ADSCs) are abundant, their collection procedure is much less painful as compared to other sources of the stem cell (Zare *et al.*, 2018) [121]. Harvested mesenchymal stem cells can be delivered to the injured area of the bone through systematic or local injections and engineering techniques. Local injection of MSCs is more effective than systemic injections since all MSCs are lost when they are trapped in the lungs after systemic infusion (Oryan *et al.*, 2017) [87]. Local injection of MSCs is a non-invasive procedure, is more advisable for a single injury whereas systemic injection of MSCs is advisable for complex fractures (Abazari *et al*., 2019) [1].

Segmental bone defects in canine were treated with bone marrow derived MSCs loaded onto porous ceramic cylinder provide good regeneration and bone healing (Brodke *et al*., 2006) [18]. In sheep, BM-MSC in conjunction with hydroxyapatite ceramic (HAC)-based carriers result in faster bone repair compared to hydroxyapatite ceramic (HAC) alone (Kon *et al.*, 2000) [60]. Bone marrow derived mesenchymal stem cells when injected 7th day post-fracture in delayed fracture healing and in non-union in case of C57 murine, promotes accelerated healing of fracture with improved quantity of callus and quality of bone. The SDF-1-CXCR4 pathway plays an important role in the fracture healing process (Wang *et al.*, 2018) [112]. There are minimal chances of rejection or immunogenic reaction when allogenic mesenchymal stem cells are used and are as effective as autogenic stem cells in the repair of experimental bone defects (Udehiya *et al.*, 2013) [109].

Chronic osteoarthritis of the hip joint treated with adipose-derived stem cells had reduced the lameness, pain and improved the range of motion (Black *et al.*, 2007) [15]. Autologous adipose derived stem cells (ADSCs) (3.2×10^7 cells) seeded on a scaffold made from hydroxyapatite (HA) and chitosan (CH) fibres has been successfully used for the treatment of non-union of radius or ulna in a crossbred dog (Lee *et al.*, 2009) [66]. Autologous adipose-derived stem cells have been studied in a disc injury model in dogs and have been shown to be effective in promoting disc regeneration (Ganey *et al*., 2009) [38]. AM-MSCs (amniotic membrane MSCs) and UC-MSCs (umbilical cord MSCs) contain higher osteogenic differentiating potential and are good sources for bone reconstruction tissue engineering (Shen *et al*., 2019) [98]. Clinical, histological and radiographical changes in cultured allogenic bone marrow derived mesenchymal stem cell (BMMSCs) implant with insulin therapy have been studied in case of bone gap defects in diabetic rabbits. Cultured allogenic bone marrow derived mesenchymal stem cells in hydroxyapatite scaffold along with local insulin therapy produces faster and better healing in alloxan-induced diabetic rabbits as compared to healthy control, untreated diabetic and local insulin treated groups (Jaiswal *et al.*, 2020a; Jaiswal *et al.*, 2020b) [50, 51]. Bhatt *et al.* (2020) [13] had standardized the isolation protocol of bone marrow derived MSCs along with their *in-vitro* differentiation into osteoprogenitor cells followed by its characterization. MSCs isolated with Ficoll-Paque density gradient centrifugation technique showed satisfactory yield. Differentiated cells showed stained deposited mineral in developing osteoblasts by using Alizarin Red S stain. These osteoblasts were further used for fracture healing in rabbit radius segmental defect.

Cartilage repair

Johnstone *et al.* (1998) [54] evaluated first MSCs for chondrogenesis under *in vitro* conditions using a specific medium. Hence, MSCs can be differentiated into chondrogenic lineage and can be utilized to treat cartilage defects (Dennis *et al.*, 2002) [30]. Under *in vivo* condition, MSCs have a potential to differentiate into chondrocytes, stimulated by the signals arising from the micro-environment of the cartilage (Steck *et al.*, 2009) [102]. The induction can occur by stimulation of cell surface receptor, growth factors, extracellular matrix or the direct interaction with the surface proteins of other resident cells (chondrocytes) (Solchaga *et al.*, 2011) [100]. Xiang *et al.* (2006) [116] reported that canine MSCs seeded in type-I collagen glycosaminoglycan (CG) matrices were used for the repair of cartilage defects of knee joints in dogs. In rabbits, repair of full thickness defects of articular cartilage was observed after the transplantation of autologous MSCs dispersed in a type-I collagen gel (Yan and Yu, 2007) [118]. In horse, bone marrow-derived MSCs had superior chondrogenic potential as compared to adipose-derived MSCs (or ASCs) in the presence of stimulatory growth factors (Vidal *et al.*, 2008) [111]. Feasible hydrostatic pressure pretreated BMSCs/PRF construct effectively promote the proliferation and chondrogenic differentiation, showed a superior capacity for the cartilage regeneration and integration of neocartilage (Zhang *et al*., 2019) [122]. TGF-ß1, PDGF, IGF-1 and EGF, all have the function of promoting the differentiation of stem cells into chondrocytes (Morscheid *et al*., 2019) [78]. In an equine model, delivery of bone marrow concentrate to acute full thickness cartilage defects has the clinical potential for improving the cartilage healing (Gonzalez-Fernandez *et al.*, 2016) [40]. In rabbits, infrapatellar fat pad derived mesenchymal stem cells were used for the treatment of osteoarthritis (Toghraie *et al.*, 2011) [106]. In induced femur-patellar defect in case of rat, mesenchymal stem cells engrafted intraarticularly within the cartilage lesion *in vitro*, promote regeneration of cartilage as evidenced by histology and immunofluorescent collagen staining (Satue *et al.*, 2019) [95].

Tendon and ligament repair

Awad *et al.* (1999) [9] reported Achilles tendon repair in a rabbit model by the use of MSC-collagen composites. In case of ligament healing in rats, MSCs differentiate into fibroblast like cells at the site of ligament injury and these cells survived up to 28 days (Guimaraes *et al.*, 2011) [41]. In adult New Zealand White rabbits, administration of MSCs along with collagen gel significantly improved the biomechanical properties after 4 weeks in the surgically induced patellar tendon defect (Krampera *et al.*, 2006) [62]. Nixon *et al.* (2008) [83] reported that re-injury rate in horses is greatly reduced due to stem cell therapy when natural mechanical stimulus along with both bone marrow and adipose derived stem cells are used. The regeneration of tendon tissue is promoted and it restores the natural movement of the body. Significant improvement in the architecture of tendon fiber, reductions in vascularity, inflammatory cell infiltrate and improvement in tendon fiber density and alignment in adipose derived stem cell treated tendons were reported by Alderman & Alexander (2011) [6]. Induction of differentiation of mesenchymal stem cells into tendon and ligament for a potential clinical application has been investigated by Gade *et al.* (2012) [37]. Adhikari *et al.* (2014) [3] have studied the application of *ex-*

vivo expanded autologous bone marrow derived mesenchymal stem cells for the repair of transected tendon in goats and concluded that autologous bone marrow derived mesenchymal stem cells enhances the tendon healing. Hence, this therapy can be used in cases of tendon injuries. Adhikari & Jadon (2015) [4] have also evaluated histopathological and electron microscopic findings during assessment of the potential of bone marrow derived mesenchymal stem cells in augmenting the tendon repair in goats. Beerts *et al.* (2017) [11] have reported that allogeneic Pb-MSCs (2-3× 10^6/ml) supplemented with PRP (platelets rich plasma) induced in injured tendon site, results in safe and long-term efficacious effect at the injured site with no side effects and low re-injury rate up to 2 years (18-44%). Expression of COL3 was upregulated in tendinopathy in equine whereas miR-29a expression was significantly downregulated in tendinopathy (Watts *et al*., 2017) [113]. Equine adipose derived stem cells (ASCs) when seeded on decellularized tendon matrix under static and 2% cyclic strain with the different stimulation regime, showed upregulation of expression of osteopontin, COL3 and DCN and downregulation of COL1 in all the groups compared to that of the monolayer control group. Although, the expression of SCX at the last point was upregulated slightly (Burk *et al*., 2016) [19]. Emerging cell sources for tendon repair include peripheral blood MSC, umbilical cord blood-mesenchymal stem cells (UCB-MSCs) and periodontal ligament cells (Liu *et al*., 2017) [71]. Periodontal ligament derived stem/progenitor cells obtained from patients undergoing orthodontic treatment, improved the healing of a full thickness achilles tendon defect as compared to an untreated defect and this resulted in similar efficacy as compared to achilles' tendon-derived cells (Hsieh *et al*., 2016) [47]. Wu *et al*. (2016) [114] reported that combining multiple stem cells in one treatment also enhances the repair of tendon. Implantation of cell sheets formed using BM-MSCs and tendon stem/progenitor cells (TSPCs) into a rat patellar tendon window defect resulted in a significant improvement in the tendon healing as compared to the defects treated with BM-MSCs or TSPCs alone. On the other hand, pretreating the MSCs with transforming growth factor (TGF) and connective tissue growth factor before implantation, led to better structural and mechanical properties in a rat patellar tendon repair model (Yin *et al*., 2016) [120]. In reconstruction of anterior cruciate ligament in a rabbit model, BM-MSCs combined with PRP in fibrin glue wrapped around a hamstring tendon, enhanced the healing of tendon-bone as compared to PRP alone (Teng *et al*., 2016) [105]. In dogs with naturally-occurring supraspinatus tendinopathy, injection of adipose derived stem cells (ADSCs) suspended in PRP into the diseased supraspinatus tendon led to improved pathology which was assessed by ultrasound and the functional improvement in gait were observed at 90 days following the treatment (Canapp *et al*., 2016) [20].

Spinal cord injuries

As nervous tissue has limited regeneration capacity, stem cells also play an important role in the improvement of regeneration of the injuries related to the spinal cord. MSCs can be differentiated into oligodendrocytes and other cell types which is needed to restore the neuronal function in the injured spinal cord (Harris, 2008) [46]. Embryonic stem cells (ESCs), neuronal stem cells (NSCs) and mesenchymal stem cells (MSCs) are usually used to accelerate the axonal regeneration and growth. Myelin regeneration is closely related to oligodendrocytes derived from neuronal stem cells (Assinck *et al*., 2017) [7]. Dasari *et al*. (2008) [27] assessed the differentiating potential of stem cells and concluded that they not only differentiate but also integrated into axonal pathways. Thus, it aids in the regeneration of the injured nerves. In addition to trans-differentiation, they may secrete growth factors that could support the neuroprotection and regeneration of axon (Harris, 2008) [46]. Shroff *et al*. (2017) [99] reported that ESCs (Embryonic stem cells) are pluripotent cells that differentiate into all tissue cells including neurons, is considered a highly potent replacer of neuronal cell in the treatment of neuronal diseases. The combined application of MSCs with biopolymer matrices provide a safe and effective approach for cell transplantation in humans in case of spinal cord injury (Mukhamedshina *et al*., 2018; Sabino *et al*., 2018) [80, 91]. Gupta *et al*. (2014) [44] have studied the neurogenic potential of autologous bone marrow derived mononucleated cells with and without TGF-β1 in rabbits and reported better healing in groups treated with BMSCs along with TGF-β1.

Adel and Gabr (2007) [2] reported that intrathecal implantation of autologous bone marrow derived MSCs improved the locomotor activity significantly in dogs within one week. Along with this, improvement in nerve conduction velocity and distinct structural consistency of the nerve cell bodies was observed in lesions treated with MSCs (Kramer *et al*., 2012) [61]. In acute spinal cord injury, A-MSCs (Adipose derived MSCs) when injected into the fourth ventricle of dogs, observed a significant improvement in the movement of hind limb with no side effects (Oh *et al*., 2016) [84]. Mukhamedshina *et al*. (2019) [81] also reported similar findings in rats and pigs. Sanluis Verdes *et al*. (2017) [94] reported that AE-MSCs (Amniotic epithelial MSCs) are also very effective for the treatment of spinal cord injury in regenerative medicine. They have many characteristics of MSCs, therefore, have been used as seed cells for the regeneration of spinal cord injury.

Ischaemic brain injury

A major cause of death and disability in humans is the damage caused by stroke injury to the central nervous system (CNS). Mesenchymal stem cells (MSCs) were also found useful for the treatment of cerebral infarction (Jeong *et al*., 2003) [53], ischemia (Chen *et al*., 2001) [22], myocardial infarction (Orlic *et al*., 2001) [86] and autoimmune disorders (Cristofanilli *et al*., 2011) [25] in experimental models. Transplantation of mesenchymal stem cells directly into the brain of adult rodent was found safe and it reduced the functional deficits associated with the stroke (Harris, 2008) [46]. Variety of stem cells treat the neurological impairment after traumatic brain injury (TBI) including mesenchymal stem cells (MSCs), neural stem cells (NSCs), multipotent adult progenitor cells (MAPCs) and endothelial progenitor cells (EPCs) (Cox, 2018) [24]. Adibi *et al*. (2016) [5] reported that MSCs can down-regulate the expression of inflammatory proteins and accelerate the repair of intracranial aneurysms. In mice, treatment with MSCs promote the recovery of neurological function in traumatic brain injury (TBI), improves learning and memory ability and reduces the neuronal apoptosis. It may be due to MSCs which promote the expression of vascular endothelial growth factor (VEGF) and Ang1 and microangiogenesis (Guo *et al*., 2017) [42].

Myocardial infarcts

Cardiac diseases cause significant morbidity and mortality in

dogs. Mesenchymal stem cells improve the function of the heart in case of acute myocardial infarction (Guo *et al*., 2020) [43]. Mesenchymal stem cells can generate various signalling molecules that are cardio-protective and can differentiate into a myocyte as well as into the lineage of the vascular system (Lehrke *et al*., 2006; Perin *et al*., 2008; Udehiya *et al*., 2013) [67, 89, 109]. The stem cell therapy would minimize the loss of cardiomyocytes by reducing the cell death, hibernating the myocardium to normal function, stimulate revascularization of the damaged region by enhancing angiogenesis, regenerating the viable cardiomyocytes, thereby, preserving the contractile function and reducing the opportunity for scarring (Caspi *et al*., 2007) [21]. Kainuma *et al*. (2018) [57] reported that transplantation of the skeletal myoblast cell sheet combined with transparency peritoneoscopy and omentopexy, promoted arteriogenesis and improved the physiology of coronary microcirculation in a 6-week myocardial infarction in rat model. It was also reported that angiotensin II receptor blocker (irbesartan) abolish the effects of rat ADSC-derived cell sheet on the attenuation of cardiac dysfunction and remodelling in a 5-week acute myocardial infarction in rat model (Yamamoto *et al*., 2018) [117]. Local release of vascular endothelial growth factor enhanced the transplantation efficiency of the layered cardiomyocyte sheet, which could be related to vascularization (Nagase *et al*., 2017) [82]. Godiyal, (2019) [39] has reported the isolation and propagation of muscle-derived mesenchymal cells and assessed their regenerative potential in skeletal muscle injuries in mice.

Hepatic disease

The effectiveness of systemically administered mesenchymal stem cells in the repair and regeneration of liver tissue has been studied most extensively in the carbon tetrachloride (CCl_4) model of progressive liver fibrosis in mice (Oyagi *et al*., 2006) [88]. Aurich *et al*. (2009) [8] reported that mesenchymal stem cells derived from human adipose tissue *in vitro*, differentiated into hepatocytes, promotes hepatic integration *in vivo*. The administration of bone marrow derived stem cells may enhance the liver regeneration in chronic liver disease after portal vein embolization and could facilitate the regeneration after partial hepatic resection (Fossum, 2013) [35]. Hepatocyte growth factor (HGF) discovered as a mitogen of hepatocytes, produced by stromal cells, stimulates many properties of the epithelial cell including proliferation, motility, morphogenesis and angiogenesis via tyrosine phosphorylation of its receptor, tyrosine-protein kinase met (c-Met) (Dally *et al*., 2017) [26]. Wu *et al*. (2018) [115] reported that transplantation of stem cells in the treatment of end-stage liver disease and diabetes mellitus has emerged as an effective therapeutic alternative in clinical practice. Saini *et al*. (2019) [92] reported that combination of hepatocytic stem cells and pepcid-C can be used safely for treating various hepatic disorders in canine patients.

MSCs, Biomaterials, growth factors in the tissue engineering approach

Combination of mesenchymal stem cells, scaffolds and growth factors are also effective in tissue regeneration. If fracture defects do not exceed 50 mm length, it can be repaired with autologous bone grafting (Dumic-Cule *et al*., 2015) [33]. Similarly, alternative tissue engineering strategies combining biomaterials/scaffolds, mesenchymal stem cells and growth factors are effective in improving bone repair if the fracture defects exceed 50 mm length (Decambron *et al*., 2017) [29]. Growth factors such as bone morphogenetic proteins (BMPs), platelet rich plasma, VEGF, TGF-β, PDGF, IGF-1 and fibroblast growth factors (FGFs) have frequently been included as scaffold in bone repair (Iaquinta *et al*., 2019) [48]. Mohammed *et al*. (2019) [77] reported that amniotic fluid derived MSCs (AF-MSCs) loaded on gel-foam scaffolds performed better during *in vivo* bone healing than BM-MSCs when regenerative potential of AF-MSCs and BM-MSCs were compared *in vitro* and *in vivo*.

Generation of transgenic animals

Isolation of totipotent stem cell from embryos and subsequent incorporation of the desired DNA into the embryo of the host results in generation of chimeric animals. A type of adult stem cells, spermatogonial stem cells (SSCs) can differentiate in the niche of a testis, which are used for the generation of transgenic animals by either transplantation or by directly injecting in the seminiferous tubules (Miao, 2013) [76]. Blastocyst injection using transgenic pluripotent stem cells is also an alternative approach for the generation of transgenic animals.

Conclusion

Regenerative medicine is an alternative, provide additional therapeutic options to potentially improve the wound healing and restore the normal skin architecture. Stem cell therapy in companion animals is most often used for the degenerative disorders, osteoarthritis of the hips, elbows, stifles and shoulders, treating spinal injuries, cardiac defects, gastrointestinal issues, renal conditions and bone repair. Stem cell therapy in regenerative veterinary medicine is a viable option for the equine as well as the small animal veterinarian, offering a safe and clinically effective tool for the clinician to assist in the treatment of the animal with difficult wounds or unresolved musculoskeletal or joint pain.

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