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Stem Cell Therapeutics in veterinary medicine in India

MUDASIR BASHIR GUGJOO[1]✉, FAJAR FAROOQ[1], QUMAILA SAKEENA[1], EJAZ RASOOL DAR[1], SHARUN KHAN[2], AMARPAL[2], JALAL-UD DIN PARRAH[1], DIL MOHAMMAD MAKHDOOMI[1], KULDEEP DHAMA[2] and GUTULLA TARU SHARMA[2]

Sher-e-Kashmir University of Agricultural Sciences and Technology, Shuhama, Srinagar, Jammu and Kashmir 190 025 India

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ABSTRACT

Stem cell, a wonder cell, acts as a basic unit for an individual development in early prenatal life, and repairs and regenerates the tissue and/ organ in post-natal life. The stem cell research although conducted extensively is still in its infancy for standardized therapeutics. Among various stem cells types, multi-potential mesenchymal stem cell (MSC) is mainly evaluated for therapeutic applications. These cells have been isolated from almost all the body organs/ tissues and fetal membranes and are culture expanded for higher concentrations. Like human, MSCs harvested from veterinary species are characterized on the basis of International Society for Cellular Therapy (ISCT). Extensive literature on their therapeutic applications in musculoskeletal and non-musculoskeletal systems evidences their potential utility under clinical settings. Currently, limited understanding in their physiological mechanisms and availability of limited non-uniform *in vivo* studies restrict their definitive therapeutic applications. Lack of regulatory set up in India makes MSCs research in veterinary medicine a more complicated field. This review details the current status and possible ways to improve MSCs therapeutic applications in veterinary medicine, in general and in Indian system, in particular.

Keywords: Clinical studies, Mesenchymal stem cell, Therapeutic applications, Veterinary Species

Since antiquity, humankind has always looked to improve human and animal health and their output and production. As such there is always a scope for improvement to effectuate improved healing and development. To ensure effective healing of ailed and ageing tissue in a shortest possible time, regenerative medicine has taken birth. The regenerative medicine essentially employs stem cells due to their special characteristics. Stem cell exhibits stemness property through self-renewal, multiplication, and differentiation. There are various stem cell types with variable differentiation potential. Stem cell (like Zygote) at the initial developmental stage show totipotency which decreases to the pluripotency (like embryonic stem cells (ESCs) of inner cell mass of trophoblast) followed by the multipotency (like adult stem cells including mesenchymal stem cells (MSCs) from adult tissues and fetal membranes) and finally the unipotency (like muscle stem cell) down the developmental ontogeny. As such, stem cell differentiation potential clearly becomes restricted after each developmental stage (Gugjoo *et al.* 2019a, Gugjoo *et al.* 2020a). Apart from the developmental ontogeny perspective of stem cells, these cells may also be developed through dedifferentiation (like induced pluripotent stem cells, iPSCs) or arise through environmental dysregulation (cancer stem cells) (Gugjoo *et al.* 2015a). iPSCs are developed through incorporation of pluripotency genes like Oct4, Sox2, Klf4 and cMYC in adult somatic cells. These cells have been developed from almost all the veterinary species including goat (Chen *et al.* 2017), sheep (German *et al.* 2015), cattle (Zhao *et al.* 2017), dog (Baird *et al.* 2015), cat (Dutton *et al.* 2019) and equine (Whitworth *et al.* 2014). Pluripotent stem cells like ESCs or iPSCs carry higher differentiation potential as compared to the adult stem cells. However, their teratogenic potential and associated ethical concerns especially of the ESCs currently restrict their therapeutic applications (Zuk *et al.* 2001, Gugjoo and Amarpal 2018, Aboul-Soud *et al.* 2021). In place, MSCs harvested from adult tissues or fetal membranes are mainly being evaluated for their therapeutic applications in regenerative medicine both in human and veterinary medicine (Amarpal *et al.* 2013, Gugjoo *et al.* 2019b, Gugjoo and Amarpal 2020a, Gugjoo *et al.* 2020a).

Why mesenchymal stem cell

MSCs have a maximum share in stem cell therapy in human medicine, in general and veterinary medicine, in particular. In veterinary medicine, MSCs required to maintain normal cell and tissue matrix turn-over in an individual are being harvested from almost all the body

Present address: [1]Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Kashmir, Shuhama, Srinagar, Jammu and Kashmir. [2]Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh. ✉Corresponding author email: mbgugjoo@gmail.com

tissues besides, the fetal membranes (Gugjoo and Amarpal 2018b, Gugjoo *et al.* 2019b, 2020b). Further, these cells have also been harvested from pluripotent stem cells like iPSCs (Chow *et al.* 2017). However, currently MSCs from adipose tissue and bone marrow and more recently fetal membranes have taken lead in the stem cell research for therapeutics (Gugjoo *et al.* 2020a, Dar *et al.* 2021). MSCs isolation and culture techniques are well established. These cells are being characterized as per the criteria set by International Society for Cellular Therapy (ISCT) for human MSCs. The criteria set for characterization includes the plastic adherence property, expression of certain surface markers (CD44, CD73, CD90, CD105, CD166) and simultaneous lack of hematopoietic markers (CD34, CD45) and their ability to differentiate into at least three lineages of adipogenic, chondrogenic and osteogenic (tri-lineage differentiation) (Dominici *et al.* 2006, Gugjoo *et al.* 2015b, Dar *et al.* 2021). However, across animal species and their tissue sources, variability in the characterization can be seen (Gugjoo *et al.* 2020a). MSCs generally meet the set criteria of plastic adherence and pluripotency differentiation across the species and tissue sources. A lack in consensus upon surface marker expression is usually reported (Gugjoo *et al.* 2019, Gugjoo *et al.* 2020a). Differences in tissue sources, harvesting methods and antibodies used may lead to the differential reporting of the expression of surface markers. Even cell detaching agent (like trypsin) may impair receptors on cell surface. MSCs dynamic immunophenotype may too impart alterations in their biological features (Colleoni *et al.* 2009, Ranera *et al.* 2011, De Schauwer *et al.* 2012, Iacono *et al.* 2012, Kang *et al.* 2013, Paebst *et al.* 2014, Tessier *et al.* 2015, Gugjoo *et al.* 2020c).

Extended differentiation potential

MSCs though categorized as multipotent have differentiation potential that goes beyond. MSCs plasticity extends to the germinal cell-like cells (Ghasemzadeh-Hasankolaei *et al.* 2015), neurocyte-like cells (Lu *et al.* 2014, Xiong *et al.* 2014, Mediano *et al.* 2015), myocyte-like cells (Vieira *et al.* 2010, Oh *et al.* 2011), islet like cells (Xiong *et al.* 2014, Peng *et al.* 2017) and hepatocyte-like cells (Xiong *et al.* 2014, Ji *et al.* 2016, Ma *et al.* 2017), among others. These cells express lineage specific markers in specific differentiation media and also express pluripotency markers like Oct4, Sox-2 and Nanog (Gugjoo *et al.* 2020d). MSCs ability to 'migrate' and 'home' into the distant tissues makes them potential candidates for peripheral application.

Ex vivo system shows MSCs trans-differentiation ability into diverse cell lineages, determined by cues arising from the available microenvironment/ niche. However, *in vivo* therapeutic action is largely considered to occur through release of immuno-modulatory, anti-apoptotic factors and chemotactic agents. MSCs give rise to the microvesicles and secrete numerous pro-healing factors in their culture medium (conditioned medium). MSCs therapeutic actions may therefore occur by the autocrine or paracrine effects. It involves: secretion of various proteins/peptides and hormones; transfer of mitochondria by way of tunnelling nanotubes or microvesicles and/ or transfer of exosomes or microvesicles (Spees *et al.* 2016, Gugjoo *et al.* 2018b). These cells secrete plethora of growth factors that aid in revascularization and matrix secretion. Their immuno-evasive nature (lack of MHC-II and co-stimulatory molecules), 'anti-inflammatory and/ immuno-modulatory' actions make them suitable candidates as allogeneic agent for scar less regeneration (Fig. 1) (Gugjoo *et al.* 2020b).

Factors affecting MSCs properties

MSCs are available in limited and variable concentration in a particular tissue and are therefore culture expanded for effective utilization. Among numerous sources, peripheral blood harbors minimal cell concentration while simultaneously adipose tissue harbors better cell concentration than that of the bone marrow (Koerner *et al.* 2006, Martinello *et al.* 2010, Carvalho *et al.* 2013). Their differentiation potential may also vary with respect to the source (Malagola *et al.* 2016, Sasaki *et al.* 2018). Particular cell source taken from different locations may or may not affect cell concentration and other characteristics (Lombana *et al.* 2015, Bahamondes *et al.* 2017). However, source based differences in MSCs therapeutic mechanisms is often reported. Even ageing affects the cell concentration and characteristics (Volk *et al.* 2012, Lee *et al.* 2017). Diseased conditions like equine metabolic syndrome (insulin resistance disease in equines) (Marycz *et al.* 2016a,b, Nawrocka *et al.* 2017), prion disease in sheep (Mediano *et al.* 2015) and feline foamy virus (Arzi *et al.* 2015) may have bearing upon cellular properties. Additionally, physiological phases like reproductive cyclicity may affect properties of the MSCs derived from reproductive organs like endometrium (Cabezas *et al.* 2014) and thus, should be

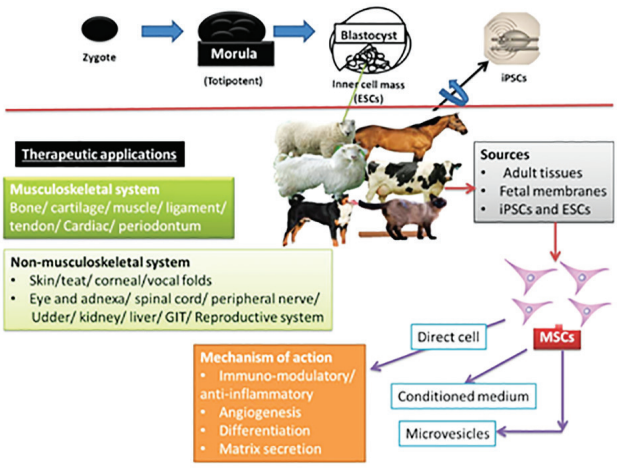

Fig. 1. Stem cells through different developmental stages of an individual. Mesenchymal stem cell sources, their products, mechanism of action and therapeutic applications. *Source:* Mesenchymal Stem Cell in Veterinary Sciences, Springer Nature (modified).

considered when utilized for therapeutic or other research purposes.

Transportation of tissue source and cells may impact MSCs isolation and their viability. MSCs for transit storage of 8-12 hrs may be carried out in biological fluids while for long term storage MSCs may be cryopreserved (Mitchell *et al.* 2015, Garvican *et al.* 2016). Storage of MSCs may be carried out in plastic or glass containers (Espina *et al.* 2016). MSCs viability may be affected by the aspiration and injection techniques as forceful aspiration and injection may lead to cell death. Needle bore size for injection or aspiration also affects MSCs viability and thus large bore needle complying with the *in vivo* transplantation may be used (Garvican *et al.* 2016). Some of the sedatives (detomidine and butorphanol) (Edmonds *et al.* 2016), antibiotics (aminoglycosides) (Bohannon *et al.* 2013, Edmonds *et al.* 2016) and steroids (methylprednisolone and triamcinolone) (Marycz *et al.* 2014) may deleteriously affect MSCs viability and thus, should be avoided.

Therapeutic applications

The main aim behind isolation and culture expansion of MSCs remains to utilize them in research and as therapeutics. Although extensive research has been conducted on MSCs but their use in veterinary practice is still restricted to experiments and to some extent to initial phases of clinical trials (Dias *et al.* 2019, Gugjoo *et al.* 2019, Gugjoo and Pal 2020). Initially, MSCs were mainly evaluated for musculoskeletal applications especially in horses (Smith *et al.* 2003) but their applications have been extended to the non-musculoskeletal tissues as well (Fig. 1). MSCs have been implanted either locally or systemically. Transplantation through the latter route may lead to some reactions and may be required in large doses to achieve therapeutic concentrations. Besides, tissues (like cartilage) without direct blood supply may not be well covered (Spaas *et al.* 2013, Broeckx *et al.* 2019). Their local or peripheral application mostly remains safe with few reports of local or systemic reactions. The reactions including local inflammation and/ or pulmonary parenchymal edema and haemorrhage mostly self-limit (Park *et al.* 2012, Kang and Park 2014). Report of disseminated intravascular coagulation in goats has been controlled through aspirin therapy (Liao *et al.* 2017).

Species wise preferences in undertaking the MSCs therapeutics under *in vivo* conditions are visible in the available literature. In pet animals and horse, experimental and clinical applications have been evaluated while in small ruminants like sheep and goat and large ruminants like cattle and in buffalo mostly experimental studies have been conducted (Gugjoo *et al.* 2020a, Gugjoo *et al.* 2020c). Small ruminants have mostly been utilized as translational animal models. MSCs therapeutic effect in musculoskeletal ailments like dog, rabbit and horse osteoarthritis/ cartilage defects (Kriston-Pál *et al.* 2017, Broeckx *et al.* 2019, Gugjoo *et al.* 2019, Huňáková *et al.* 2020), dog bone affections (Tsuzuki *et al.* 2014, Song *et al.* 2017), dog periodontal defects (Takewaki *et al.* 2017), dog and horse muscle/ tendon tears/ injuries (Beerts *et al.* 2017; Gibson *et al.* 2017, Taroni *et al.* 2017, Depuydt *et al.* 2021) and dog myocardial affections (Pogue *et al.* 2013, Yang *et al.* 2021) have been evaluated. Under non-musculoskeletal tissue ailments, MSCs applications in dog, cattle and horse skin/teat/ corneal/vocal folds injuries/ ulcers (Madhu *et al.* 2014, Zubin *et al.* 2015, Iacono *et al.* 2016, Iravani *et al.* 2017, Khashjoori *et al.* 2019, Mund *et al.* 2020), dog keratoconjunctivitis sicca (KCS) (Villatoro *et al.* 2015, Bittencourt *et al.* 2016), dog spinal cord injuries (SCI) (Escalhão *et al.* 2017, Bhat *et al.* 2019, Sharun *et al.* 2021), dog sciatic nerve injuries (Ding *et al.* 2010), dog meningoencephalomyelitis of unknown origin (MUO) (Zeira *et al.* 2015), dog hepatocutaneous syndrome (Nam *et al.* 2017), dog and cat acute kidney injury (Lee *et al.* 2017b), dog intervertebral disc diseases (Lee *et al.* 2009, Sharun *et al.* 2020), horse reproductive ailments (endometriosis, anestrus, testis) (Grady *et al.* 2019, Navarrete *et al.* 2020, de Papa *et al.* 2020) and cattle mastitis (Lange-Consiglio *et al.* 2019, Ting *et al.* 2020) have been evaluated. Furthermore, MSCs therapeutic effects have been evaluated in some of the inflammatory/immune-mediated and/ ischaemic diseases like dog and inflammatory bowel disease (IBD) (Pérez-Merino *et al.* 2015, Webb and Webb 2015), feline eosinophilic keratitis (FEK) (Villatoro *et al.* 2018), dog atopic dermatitis (Famos *et al.* 2020), dog anal fistula (Ferrer *et al.* 2016) and equine laminitis (Marycz *et al.* 2021). Recently, extended evaluation to canine diabetes, liver affections, tumors, etc have also been started (Gugjoo *et al.* 2020b).

Overall, improved outcome has been reported in majority of the studies related to the MSCs therapeutic applications in these animal studies. Some of the studies although have reported no adverse effects but simultaneously without any positive outcome like canine atopic dermatitis (Famos *et al.* 2020) and dog cardiomyopathy (Pogue *et al.* 2013, Yang *et al.* 2021). There has been variability in design, cell type (source and passage number) and other biomaterials, their dosage and route, besides the frequency of implantation. Furthermore the long term effect remains to be seen in majority of the studies. MSCs engraftment, survival and mechanism of action remains to be evaluated extensively. There are very limited animal numbers and short duration studies that fail to provide evidence based medicine. The concerns with the cell based therapy are risk of immunological concerns, pulmonary embolism, inefficient homing of MSCs to the target site, risk of tumorigenesis, and the need for rigorous quality control limits their use (Phan *et al.* 2018, Watanabe *et al.* 2021). Recently, alternatives like MSCs conditioned medium (CM)/ extracellular vesicles (EVs) are being evaluated (Ragni *et al.* 2017, Vikartovska *et al.* 2020, Morente-López *et al.* 2021). MSCs CM/ EVs contain proteins, lipids, mRNA, and miRNA that may play a significant therapeutic role (Gomzikova *et al.* 2019).

Stem cell research in veterinary medicine in India

In India, stem cell research and therapy is in infancy and has mainly been conducted in research institutes and/ universities like Indian Veterinary Research Institute (IVRI), Izatnagar; Central Institute for Research on Buffaloes (CIRG), Hisar; Central Institute for Research on Goats, Makhdoom and National Research Centre for Equines, Hisar; and Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai and Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K). Other institutes and private companies are also interested in this field. Below are details of the MSCs relevant studies conducted in veterinary science in India.

In vitro *MSCs studies in India:* MSCs have been derived from almost all the body tissues and foetal membranes in all the animals worldwide. In India, the main animal sources evaluated for MSCs are bone marrow, adipose tissue and foetal membranes as detailed in the Tables 1 and 2. Additionally, feline adipose tissue derived MSCs and canine endometrial MSCs have also been isolated and characterized from TANUVAS, Chennai (Nissar *et al.* 2018) and College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar (Sahoo *et al.* 2017), respectively. MSCs from liquids like bone marrow have been isolated by density gradient method or whole bone marrow method while MSCs from solid tissues like umbilical cord have been isolated through the enzymatic or tissue explant techniques (Rathore *et al.* 2018, Dar *et al.* 2021).

MSCs from these sources have mainly been evaluated for their ability to adhere to plastic surface, express specific surface markers, lack hematopoietic markers and differentiate into three lineages (adipogenic, osteogenic and chondrogenic). The surface markers have been evaluated up to the transcription level (Akram *et al.* 2017, Dar *et al.* 2021) or to the translational levels (Gade *et al.* 2013, Gulati *et al.* 2013, Pratheesh *et al.* 2014, Mohanty *et al.* 2016). Apart from the tri-lineage differentiation, equine Amniotic Fluid-MSCs have also been differentiated into the tenogenic lineage with the culture addition of the bone morphogenetic protein-12 (BMP-12) (Gulati *et al.* 2013). Comparing the bone marrow and adipose tissue, MSCs from the latter source may harbor higher cellular concentrations. The cellular proliferation may also be affected by the animal physiological status as the MSCs from term pregnant animal had higher proliferation potential than non-pregnant male animals (Dar *et al.* 2021).

In India, these studies are only preliminary without any details on the cultured MSCs phenotype, culture conditions and passage number, and their comparative differences with respect to the species, tissue source and age. There are questions on the self-renewal property of MSCs. Unlike ESCs/ iPSCs, there is limited expansion potential characterized by senescent morphology, surface marker attenuation, hampered differentiation and ultimately the proliferation arrest with extended passaging and thus, need to be studied. Apart from that MSCs' other stemness properties remain to be understood in India and abroad as MSCs stemness markers are being supported by handful of genes without any actual molecular basis (Kubo *et al.* 2009). Further, effect of the microenvironment/niche including effect of hypoxia, available extracellular matrices, growth/ humoral factors, among others is also lacking in India and thus, relevant studies are desired (Gugjoo and Pal 2020a).

In vivo *MSCs studies in India:* MSCs or their conditioned medium (CM) has been evaluated under various experimental models like rabbit sciatic nerve injury (Tiwary 2011), rabbit bone defect (Udehiya *et al.* 2013, Peer *et al.* 2022), rabbit osteochondral defect (Gugjoo *et al.* 2017, Gugjoo *et al.* 2020) and rat and guinea pig skin wound (Borena *et al.* 2009, Ansari *et al.* 2013, Joseph *et al.* 2020, Bhat *et al.* 2021). Most of these studies have been conducted as randomized placebo controlled studies. Besides, MSCs therapeutic effects have been evaluated in

Table 1. Small ruminants mesenchymal stem cell sources and their characterization in India

Source	Markers	Differentiation	References
gBM-MSCs	CD105+CD90+CD45−CD34−	-	Akram *et al.* 2017
gAF-MSCs	CD-73+, CD-90+, CD-105+, Oct-4+, Nanog+, Sox-2+, SSEA-1+, SSEA-4+, CD34-	Adipogenic, chondrogenic and osteogenic	Pratheesh *et al.* 2013
gBM-MSCs/	CD73+, CD105+, Stro-1+, CD34-	Adipogenic, chondrogenic, osteogenic	Pratheesh *et al.* 2017
gBM-MSCs	CD73+, CD90+, CD34-, CD45-	Adipogenic, chondrogenic, osteogenic	Dar *et al.* 2021
gWj-MSCs	CD-73+, STRO-1+, CD-105+, CD34	Adipogenic, chondrogenic and osteogenic	Pratheesh *et al.* 2014
gAF-MSCs/ gAm-MSCs/ gAS-MSCs/ gWj-MSCs	CD73+, CD90+ and CD105+, Oct4+, Sox2+, Nanog+, KLF+, cMyc+, FoxD3+, CD34-	Adipogenic, chondrogenic, osteogenic	Somal *et al.* 2016, Somal *et al.* 2017
gUC-MSCs	CD73+, Thy-1+, CD105+, CD34-	Adipogenic, osteogenic	Kumar *et al.* 2016
sAD-MSCs	CD73+, CD90+, CD34-, CD45-	Adipogenic, chondrogenic, osteogenic	Dar *et al.* 2021
sBM-MSCs	CD73+, CD90+, CD34-, CD45-	Adipogenic, chondrogenic, osteogenic	Dar *et al.* 2021

Note: g, Goat; s, Sheep; MSCs, Mesenchymal stem cells; AD, Adipose tissue; BM, Bone marrow derived; AF, Amniotic fluid derived; Wj, Wharton's jelly derived; Am, Amniotic membrane derived; AS, Amniotic sac derived; UC, Umbilical cord derived.

Table 2. Studies on large ruminant and equine mesenchymal stem cell sources and their characterization in India

Source	Markers	Differentiation	References
cUC-MSCs	CD105+, CD90+, CD73+, CD45	Adipogenic, chondrogenic, osteogenic	Debberma *et al.* 2020
bufAD-MSCs	CD105+, CD90+, CD73+, Oct-4+, CD44+, CD34-, CD45-, CD79A-	Adipogenic, Chondrogenic, Osteogenic	Hepsibha *et al.* 2011, Sampaio *et al.* 2015
bufAF-MSCs	Oct-4+, Nanog+, Sox-2+	-	Yadav *et al.* 2011
bufAM-MSCs	CD29+, CD44+, CD73+, CD90+, CD105, CD166+, TERT+, Oct-4+, Sox-2+, Nanog+, REX-1+, SSEA-1+, SSEA-4+ and TRA-1-81+, CD34-	Adipogenic, Chondrogenic, Osteogenic, Neurogenic	Dev *et al.* 2012, Ghosh *et al.* 2015
bufBM-MSCs	CD73+, CD90+, CD105+, Nanog+, Oct-4+, STRO-1+, Sox2+, CD31-, CD34-	Adipogenic, Chondrogenic, Osteogenic	Gade *et al.* 2013
bufUC-MSCs	OCT4+, NANOG+, SOX2+	Adipogenic, osteogenic	Singh *et al.* 2013
bufWj-MSCs	CD-73+, CD-105+, CD-90+, Stro-1+, Oct-4+, Nanog+, Sox-2+, CD34-	Adipogenic, Chondrogenic, Osteogenic	Sreekumar *et al.* 2014
eAF-MSCs	CD29+, CD44+, CD73+, CD90+, CD34-, CD45-, CD14-	Adipogenic, Chondrogenic, Osteogenic, Tenogenic	Gulati *et al.* 2013
eUC-MSCs	CD29+, CD44+, CD73+, CD90+, CD34-, CD45-	Adipogenic, Chondrogenic, Osteogenic	Mohanty *et al.* 2016

Note: c, Cattle; buf, Buffalo; e, Equine; MSCs, Mesenchymal stem cells; AD, Adipose tissue; BM, Bone marrow derived; Wj, Wharton's jelly derived; Am, Amniotic membrane derived; UC, Umbilical cord derived.

open trial clinical studies on dog wounds (Madhu *et al.* 2014, Rafee *et al.* 2017, Bhatt *et al.* 2018), and spinal cord injuries (Bhat *et al.* 2019, Sharun *et al.* 2020, Sharun *et al.* 2021), besides cattle teat injuries (Dar 2021).

Experimental studies

In rabbit sciatic nerve injury model, bone marrow mononuclear cells (BM-MNCs) have been evaluated for their therapeutic effects. The healing had been evaluated through neurological examination, histopathology and scanning electron microscopy (SEM) at various time intervals (30^{th}, 60^{th} and 90^{th} day). The study concluded with the positive remarks on nerve repair with the BM-MNCs application. However, complete regeneration was lacking (Tiwari 2011). In our own similar unpublished study, BM-MSCs and AD-MSCs too show positive impact on return of the clinical parameters however, complete regeneration to the extent of full clinical recovery was lacking.

In case of rabbit bone defect model, implantation of BM-MSCs and BM-MNCs were demonstrated to have ability to induce faster bone healing with or without the growth factors (TGF-ß1 and IGF-1). BM-MSCs had led to the faster healing as compared to the BM-MNCs evaluated through the radiography, histopathology and SEM. Allogeneic implantation was comparable to that of the autologous therapy (Udehiya *et al.* 2013). BM-MNCs contain MSCs in some proportion with additional growth factors. Comparing the bone defect healing in guinea pig model, MSCs and osteoinducted MSCs show improved and overall a comparable bone healing potential. Further, allogeneic MSCs improve bone healing better than the xenogeneic (Peer *et al.* 2022).

In case of the rabbit, osteochondral defect model, implantation of MSCs loaded on laminin (Gugjoo *et al.* 2017) and/ collagen I (Gugjoo *et al.* 2020) with or without the growth factors (TGF-ß1 and IGF-1) had led to the improved healing, although the complete healing with hyaline like tissue was lacking. MSCs without the scaffold was found less effective and might have been due to the lack of *in situ* availability of the cells at the defect site. Implantation of the growth factors together with MSCs had led to the significantly better healing than that of the MSCs (Gugjoo *et al.* 2017, Gugjoo *et al.* 2020).

In case of skin defect models, Implantation of MSCs/ MNCs had enhanced skin wound healing. The healing had been earlier with very less inflammatory response (Borena *et al.* 2009, Ansari *et al.* 2013, Joseph *et al.* 2020, Bhat *et al.* 2021). Comparing the single dose to the repeated dose of MSCs, the latter option was proven more effective than the former in skin wound healing (Bhat *et al.* 2021). All these experimental studies prove useful although the conclusive statement on cell passage number, dose, route, timing and repetition needs to be standardized.

Clinical studies

MSCs clinical studies have been conducted on the skin

wounds and the spinal cord injury in India**.** In a single case report on large skin wound treated by caudal superficial epigastric axial pattern flap followed by implantation of MSCs (5 million BM-MSCs given twice at 12 days interval), the wound had healed completely by 50th day post operatively (Madhu *et al.* 2014). In case of other study (case series, n=6), MSCs (2.5 million injected twice at wound periphery) had enhanced wound healing in dogs without any complication (Rafee *et al.* 2017). In another study, implantation of 01 million allogeneic BM-MSCs in dogs in skin wounds too had improved wound healing. In the same study, infected wounds were implanted with same dose but thrice at weekly intervals for effective healing (Bhatt *et al.* 2018). In case controlled study on teat fistula (mucocutaneous wounds of varying in length and shape), MSCs appear to help in early clinical wound closure with less fibrous tissue formation based on clinical and sonographic findings (Dar 2021). Thus, MSCs improve skin or mucocutaneous wound healing, although dose titration and frequency remains to be standardized. Besides there is need to grade the wounds as per the location, area, infection and above all the species for conclusive results.

In case of spinal cord injuries, MSCs too have been demonstrated to show clinical recovery. In case of thoracic and lumbar vertebral fractures (n=6), intraspinal implantation of MSCs (1 million) has been correlated with the improvement in neurological signs (Sharun *et al.* 2020). In case of Hansen type I intervertebral disc disease (IVDD) at T12-T13, 4 doses of MSCs (1 million) implanted too had led to the clinical improvement with animal showing signs of weight bearing immediately after two doses (Sharun *et al.* 2021). In a more large animal case study (n=44), different body reflexes and recovery score were significantly improved upon intraspinal implantation of MSCs (Bhat *et al.* 2019). Thus, MSCs appear promising in spinal cord injuries as well but in animals with intact and stable spinal cord. However, an overall recovery may also be affected by the type of lesion, aetiology of spinal cord injury and surgical protocol followed for treatment of the spinal fractures or IVDDs.

As MSCs prove to be effective in various experimental and clinical studies in animals (especially the dog) in India, further studies in this regard are desired. Besides, various other clinical ailments in dog and other species as mentioned above may be undertaken. Currently, there is lack of appropriate controlled studies with variation in stem cell isolation procedures, inadvertent inclusion of other biological agents. The optimal posology involving cell treatment time, their dosage and route of administration as per the clinical condition remains to be standardized. The availability of limited sample size, clinical case non-uniformity, the lack of well scrutinized data and blind folded randomized control trials especially in veterinary medicine in India or abroad make standardization even more complicated.

Stem cell regulatory concerns in India

As mentioned above, the stem cell therapy is under investigation and is thus, not approved for any specific condition except some human blood disorders. It is therefore improper to advertise MSCs therapy for any specific condition for stem cell tourism. In India human stem cell research/ therapy is being regulated by ICMR and DBT (ICMR and DBT 2017). Stem cell or its product clinical trials (substantial manipulated stem cells) shall be regulated by Central Drug Standards Control Organization (CDSCO) with due approval required from Drug Control General of India (DCGI) (Times of India report Dated: 16 April, 2018). For Indian veterinary medicine, no such regulatory guidelines are available that could control and regulate stem cell therapy for veterinary species. Lack of such guidelines not only provides break to the determined clinical oriented research but also devoids animals of their possible beneficial applicability in the near future. Due to lack of rigorous supervision of the stem cell therapy in veterinary practice by regulatory bodies, applications are being made without any basis of pre-clinical studies. As various regulatory bodies worldwide like United States Food and Drug Administration (FDA), European Medicine Agency's (EMA) and Korea (EMA 2017, EMA 2018, USFDA 2018) have provided guidelines for cell-based products for veterinary purpose, the regulatory bodies in India may formulate regulatory guidelines on such basis with due consideration on local regulatory guidelines.

CONCLUSION

Stem cells are wonder cells characterized by the self-renewal and differentiation properties. Their differentiation properties restrict with each developmental stage in an individual. Utilization of pluripotent stem cells like ESCs and iPSCs for therapeutics is currently restricted by their teratogenic and/ ethical tendencies. MSCs with an extended multi-lineage differentiation potential are devoid of such limitations. Their characteristics like self-renewal (although questioned), differentiation, migration, homing and immuno-modulatory/anti-inflammatory, besides other paracrine properties make them suitable candidates for therapeutics. Current lag in understanding of their basic properties and critical *in vivo* niche effect puts a break to their definitive therapeutic applications. These cells are affected by various factors including tissue source, age, donor health condition, processing and storage and thus, should be kept in consideration while instituting them for therapeutics. In veterinary sciences, these cells have been evaluated in musculoskeletal and non-musculoskeletal ailments, in addition to inflammatory/immune-mediated diseases. All these studies support MSCs therapeutic applications. There are several issues with respect to the cell viability post transplantation, their delivery mode, number and frequency that remain to be evaluated. Clinical trials need to be controlled with a sufficient animal size to make stem cell application, an evidence based medicine.

Under Indian environment, the studies are very limited both in relation to the cellular physiological features and their *in vivo* applications and thus, may be undertaken. In addition, regulations from concerned quarters in India need to be drafted and implemented in letter and spirit.

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