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Goat Mesenchymal Stem cell Basic Research and Potential Applications

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Highlights

- Properties of the stem cells and why currently Mesenchymal Stem Cells (MSCs) contribute most of the preclinical and clinical studies.
- Sources of MSCs and their relevance with respect to clinical applications.
- Isolation, culture and characterization of the cells.
- Potential applications of the cells and their translational application in human medicine.
- 5. What are the challenges ahead to meet the desired results?

Abstract

Stem cell, one of the most exciting research areas of 21st century, is considered to have broaden the horizons of the field of biology, in general and medicine, in particular. Stem cells, owing to their unique characteristics like self-renewal, multiplication and differentiation are considered to provide an 'all-in-one-solution' for diverse clinical problems. Among various stem cell types, mesenchymal stem cells (MSCs) are being made the subject of the most of the studies on their therapeutic applications. It is attributable to their readily available sources, ability to immunemodulate and differentiate into

mesodermal and extra-mesodermal tissues. MSCs in contrast to pluripotent stem cells carry minimal risk of teratoma formation and are free from any ethical concern. MSCs have been evaluated under innumerable studies but the definitive applications of these cells are slow, plausibly due to the incomplete understanding of their cellular physiology. Goat MSCs (gMSCs) have been isolated, culture expanded, characterized from various tissue sources and studied for their potential *in vivo* applications mostly in relation to human translational models. The current review throws some light on gMSC sources, characterization and potential therapeutic applications.

Keywords: Goat; Mesenchymal stem cells; Stem cells

Introduction

The stem cells constitute an important element in the discipline of regenerative medicine that aims to address numerous incurable diseases through tissue regeneration (Gugjoo and Amarpal, 2018; Gugjoo et al. 2019). The specialized properties of self-renewal, multiplication and differentiation of stem cells make them potential candidates for an all-in-one therapeutic solution to diverse clinical ailments. Among various stem cell types, mesenchymal stem cells (MSCs) make subject of most of the therapeutic applications (Gugjoo et al. 2019). MSCs are available in almost all the tissue types and have easy isolation and culture processes. The cells are plastic in nature, and have immuno-modulatory and/ anti-inflammatory properties with minimal risk of teratogenicity and associated ethical issues unlike that of embryonic stem cells (Song et al. 2013; Gugjoo et al. 2019).

A considerable body of literature demonstrates MSCs characteristic features and their *in vivo* applicability. MSCs are able to trans-differentiate but the cells are believed to provide therapeutic benefits largely through their paracrine actions involving immuno-modulatory, anti-inflammatory, anti-apoptotic and chemotactic effects (Wu and Tao, 2012;

Gugjoo et al. 2019b). They can inhibit the release of pro-inflammatory cytokines and promote the survival of damaged cells (Uccelli et al 2008) and repair the damaged tissue (Ma et al. 2014; Gao et al. 2016). The molecular basis including the transcription factors involved in stemness is however, yet to be fully comprehended (Kubo et al. 2009; Zheng, 2018; Gugjoo et al. 2018; Gugjoo et al. 2019a). Goat being the common model for many human translational research studies, and thus understanding of the stem cell features from such an animal may help provide further incites in human stem cell studies. Further, it can also pave the way for the stem cell therapy in veterinary medicine. The current review thus, focuses on the *in vitro* characteristics and the potential *in vivo* applications of the goat mesenchymal stem cells (gMSCs).

In vitro studies

Sources of gMSCs

gMSCs have been explored from limited sources as compared to the other ruminants. gMSCs have been harvested from adipose tissue, amniotic fluid, amniotic sac, bone marrow, cord blood, deciduous teeth, endothelium, skeletal muscle, umbilical cord and Wharton's Jelly.

Isolation and expansion of gMSCs

A standard comprehensive procedure to harvest, isolate, characterize and expand MSCs is the key to their successful utilization for regenerative medicine (Akram et al. 2017). The techniques employed for their processing, however, involve variable procedures. Isolation of MSCs from liquid sources like bone marrow, peripheral blood and umbilical cord blood directly involves density gradient separation of mononuclear cell (MNC) fraction while solid tissues like adipose tissue are first collagenase digested to harvest stromal vascular fraction (SVF). Thereafter, MNCs and SVF are cultured in serum supplemented Dulbecco's Modified Eagles Medium (DMEM) (Knippenberg et al. 2005;

Eslaminejad et al. 2009; Wu and Tao 2012). Among various available cell types only MSCs adhere to the culture plates while rest afloat and are washed off with subsequent change of the growth media (Gugjoo et al. 2019b).

Fetal bovine serum (FBS) is the commonly used serum supplement for culture expansion of MSCs. It offers drawbacks in the form of non-uniform culture ingredients and carries xenoproteins that could change cell behaviour and pose potential threat of disease transmission (Gugjoo et al. 2019b). As such FBS alternatives like allogeneic platelet lysate, autologous/allogeneic serum have been studied. These FBS alternatives though have been fruitful to culture expand human MSCs but most of the animal studies (dogs and horses) have failed to achieve the success (Russell et al. 2015; Clark et al. 2016). In goats such studies are desired.

gMSCs, in optimal culture conditions, are generally able to proliferate up to 20 passages without any effect on their characteristics. The population doubling time (PDT) increases with extended passaging (Mohamad-Fauzi et al. 2015). PDT for goat umbilical cord (UC)-MSCs was 33.49 hr and 34.91 hr at passage 5 and 12, respectively (Qiu et al. 2012). The cellular proliferation is affected by the tissue source type. The mean population doubling time (PDT) of goat bone marrow (BM) MSCs was 24.94±2.67 hr (Eslaminejad et al. 2009); goat amniotic fluid (AF) MSCs was 33.1 hr (Pratheesh et al. 2013); and goat Wharton's jelly (WJ) MSCs was 36.06±1.2 hr (Pratheesh et al. 2014) at 3rd passage. On comparing fetal adnexa derived MSCs, gWJ-MSCs and goat cord blood (CB)-MSCs had significantly ($P<0.05$) higher clonogenic potency, faster growth rate and low population doubling time (PDT) as compared to gAF-MSCs and gAS-MSCs (Somal et al. 2016). Some of the sources like gAD-MSCs were more deleteriously affected at each passage as compared to that of others like gBM-MSCs (Wang et al. 2017). This could be due to their sensitivity to culture conditions that might change their methylation pattern (Wang et al.

2017). Self renewal properties of MSCs under *in vitro* culture conditions may be affected by epigenetic modifications (Schop et al. 2009).

Culture expansion of gBM-MSCs leads to the accumulation of metabolites like ammonia that affects their growth and proliferation. To prevent adverse effect of such metabolites prolonged proliferation of gMSCs had been maintained by adding 30% medium (containing fresh nutrients and Cytodex 1 microcarriers) to culture system after every 3 days. Such a culture feeding dilutes metabolites and provides new energy sources (glucose and glutamine) and additional surface area to the cells (Schop et al. 2008). Furthermore, the cellular proliferation may also be promoted by the higher concentration of fetal bovine serum (FBS). Umbilical cord derived MSCs (UC-MSCs) faster growth is usually achieved with higher FBS concentration (20%) than at lower concentration (10%) (Martins et al. 2017). Therefore, MSCs sources, culture conditions including ingredients of the media and passage time should be given due consideration during extended passaging.

Reproductive cyclicity and gMSCs

MSCs derived from reproductive organs at different phases of reproductive cycle may have variable population doubling time. Passage 4 endometrium derived MSCs (En-MSCs) from anestrus and cyclic goats have 40.6hr and 53hr PDT, respectively (Tamadon et al. 2017). This enhanced growth of En-MSCs at anestrus period may be due to the accumulation of growth factors like basic fibroblast growth factor (bFGF), transforming growth factor -β (TGF-β), etc in endometrial tissue during anestrus phase (Tamada et al. 2000; Tamadon et al. 2017).

Characterization of gMSCs

Goat MSCs (gMSCs), have been characterized as per the criteria laid down by International Society for Cellular Therapy (ISCT). According to the criteria, MSCs are plastic adherent, express certain mesenchymal cell surface markers; lack haematopoetic

markers and at least undergo tri-lineage differentiation (Dominici et al. 2006). gMSCs have typical spindle shaped fibroblast morphology with an ability to grow for numerous passages (Knippenberg et al. 2005; Eslaminejad et al. 2009; Pratheesh et al. 2013; Mohamad-Fauzi et al. 2015; Pratheesh et al. 2017). However, UC-MSCs may have different spindle cell sizes; long and short, that express variable surface markers (Martins et al. 2017). In general, gMSCs express markers like CD105, CD166, CD90, CD73, CD44, CD29, Vimentina and Ck-pan, and lack expression of CD34, CD45, CD71, CD14, CD79a and HLA-DR similar to that of human MSCs. Some of the studies however, demonstrated weak expression of CD90 (Ghaneialvar et al. 2018), CD166 and CD105 (Knippenberg et al. 2005; Ghaneialvar et al. 2018). Such variability in surface marker expression may be explained by the differences in tissue types, harvesting methods and antibodies used as has been reported for other species (Colleoni et al. 2009; Radcliffe et al. 2010; Ranera et al. 2011; De Schauwer et al. 2012; Martignani et al. 2014). Even detaching agent like trypsin can impair certain cell surface receptor membrane proteins (Ranera et al. 2011; De Schauwer et al. 2012). Furthermore, immunophenotype of MSCs changes over the course of culture, which may cause alterations in their biological features (Mosna et al. 2010; Strioga et al. 2012).

MSCs express embryonic stem cell markers like Oct-3/4, Nanog, cell proliferation markers, Pcna that varies with respect to the donor tissue types (Dominici et al. 2006; Tripathi et al. 2010; Huang et al. 2013; da Silva Filho et al. 2014; Pratheesh et al. 2014; Mohamad-Fauzi et al. 2015; Wang et al. 2017). In case of caprine fetal adnexa derived MSCs, gWJ-MSCs had expressed significantly higher mesenchymal stem cell surface markers (CD73, CD90 and CD105) and pluripotency markers (Oct4, Klf, cMyc) as compared to other cell lines (amniotic sac-, amniotic fluid- and cord blood derived MSCs). gWJ-MSCs and gAF-MSCs though had a comparable Klf and cMyc markers expression (Somal et al. 2017).

Differentiation of gMSCs

gMSCs undergo tri-lineage differentiation including adipogenesis, chondrogenesis and osteogenesis. Further, gMSCs under particular culture conditions express proteins specific to the particular lineages like myogenic-like cells (Tripathi et al. 2010), neural like cells (Wang et al. 2018), epithelial lineage (Reza et al. 2014) and germ cells-like cells (Yan et al. 2015; Li et al. 2017; Zhang et al. 2019). Neural like cell trans-differentiation of gAD-MSCs may be achieved by incorporation of BIX-01294, a specific inhibitor of methytransferase G9a responsible for methylation of histone H3 lysine 9 (H3K9). Such a methytransferase inhibitor may rely on the Nanog regulatory network to promote gAD-MSCs trans-differentiation (Wang et al. 2018). The epithelial lineage differentiation had been induced in mammary fat pad adipose stem cells by initial mixture of insulin, hydrocortisone and epidermal growth factor and subsequent treatment with keratinocyte growth factor (Reza et al. 2014).

Transfected BM-MSCs upon over expression of germ cell specific genes [Stra8 (regulates meiotic initiation during gametogenesis), Boule (rescues meiotic defects), Dazl (regulates transcription of key transcripts)] trans-differentiated into the germ cell-like cells. However, a very limited concentration of such trans-differentiated cells was achieved. The cells that co-overexpressed all the 3 genes had more resemblance to germ cells than the cells expressing such a single gene (Zhang et al. 2019). Follicular fluid may too guide MSCs towards the germinal cell lineage. Lower concentration (2-10%, especially 5%), of follicular fluid in culture had promoted gUC-MSCs proliferation while higher concentration of 20% had promoted cellular trans-differentiation into oocyte like cells (Qui et al. 2012). Platelet plasma too had promoted the proliferation of gBM-MSCs but inhibited their osteogenic differentiation (Cheng et al. 2007). To attribute the trans-differentiation properties to MSCs,

it becomes imperative to perform the *in vivo* studies that fully support their actual differentiation into the functional particular lineage.

Cryopreservation of gMSCs

In order to keep cells readily available and maintain consistent supply, the cells are cryopreserved. The post-cryopreservation cell viability had remained almost same irrespective of the cell sources (Knippenberg et al. 2005). An evaluation of post-thaw viability of cryopreserved goat fetal adnexa MSCs had depicted that all the cells were revived successfully. The post thaw PDT varied among the cells from various sources with gWJ-MSCs having lower while gAS-MSCs having highest PDT (Somal et al. 2017), similar to that of fresh cells (Somal et al. 2016). The cells were able to express surface antigens (CD73, CD90 and CD105) and pluripotency markers (Sox2, Nanog, Oct4, KLF, FoxD3 and cMyc) (Somal et al. 2017).

Apart from the cells, tissue engineered (TE) cell-scaffold assembly may also be cryopreserved to provide an off the shelf TE constructs. In one such goat study that cryopreserved a tissue engineered scaffold (fibre meshes based on a starch and poly(caprolactone) gBM-MSCs were viable with scaffold properties being maintained (Costa et al. 2012). However, other scaffold designs and structural properties may be affected by cryopreservation and thus, individual tissue engineered constructs need to be evaluated.

Effect of source on gMSCs

The concentration, differentiation potential and immunemodulation of MSCs vary among donor tissue types. Ilium bone chip was able to provide higher gMSCs yield (approx 3000 times) in comparison to the bone marrow (Akram et al. 2017). The adipogenic differentiation potential of gAD-MSCs was superior to that of gBM-MSCs (Mohamad-Fauzi et al. 2015) while as the osteogenic differentiation potential of gBM-MSCs was

superior to gAD-MSCs (Mohamad-Fauzi et al. 2015). Such a source related difference of MSCs differentiation potential may be attributed to the upregulation of variable osteogenic pathways like p38 MAPK for gBM-MSCs and p44/42 MAPK for gAD-MSCs (Elkhenany et al. 2016). This may occur by intrinsic epigenetic differences that prime MSCs to differentiate into the surrounding tissues. This is consistent with the finding that MSCs derived from adipose tissue, bone marrow and muscle have similar but not identical promoter methylation profiles (Sorensen et al. 2010).

MSCs immuno-modulatory properties are affected by inflammatory environment. Stimulation of MSCs with pro-inflammatory cytokines (INF-γ and TNF-α) had modulated their expression of different growth factors. Among various sources of MSCs from fetal adnexa, gWJ-MSCs carried maximum potential to inhibit peripheral blood mononuclear cells followed by gAS-MSCs. Such an immuno-modulation might have been achieved by indoleamine 2,3-dioxygenase (IDO) in case of gWJ-MSCs and inducible nitric oxide synthase (iNOS) in case of gAS-MSCs (Somal et al. 2016a).

Effect of Microenvironment on gMSCs

Like other species, gMSCs characteristics are influenced by microenvironment/niche composed of resident cells, matrices and guiding signals in the form of humoral or growth factors. As *in vitro* culture system provides restricted environment to MSCs, the cellular characteristics obtained thereof may not be recapitulated under *in vivo* environment wherein the cells at specific point of time are simultaneously under the influence of numerous stimuli (Hwang et al. 2011).

Effect of resident cells

One of the components of microenvironment is the resident cells. An *in vitro* study had demonstrated that muscle derived cells (MDCs) had affected gBM-MSCs properties in a culture system. gBM-MSCs had achieved myogenic phyenotype upon fusion with MDCs

and gave rise to myotubes. Such a myogenic phenotype though had failed to develop if only MDC soluble factors were added. These soluble factors, however, did increase gBM-MSCs migration potential (Kulesza et al. 2016). Similarly, culture of infrapatellar fat pad derived MSCs along with the chondrocytes additively promoted their secretion of cartilage specific matrix (Arora et al. 2017). Based upon these evidences, effect of local resident cells have definitive role in MSCs differentiation towards the specific lineage. Thus, available cell type tends to specify the differentiation lineage of MSCs.

Influence of scaffold

Scaffold type, design and its constituents may also determine the fate of stem cells including their proliferation and differentiation. Even the spatial distribution of the cells is affected by the nature and composition of the scaffold (Prins et al. 2016). gBM-MSCs upon culture in cartilage extracellular matrix like polyethylene glycol hydrogels, collagen I, II, hyaluronic acid hydrogel and chondroitin sulphate had undergone chondrogenic differentiation and secreted functional cartilage matrix (Varghese et al. 2008; Hwang et al. 2011; Toh et al. 2012; Toh et al. 2013; Yin et al. 2016). Even addition of polyethylene glycol to the chondroitin sulphate had prevented the hypertrophic chondrocyte formation (Varghese et al. 2008). Scaffolds like silica-coated bioactive ceramic, hydroxyappatite and hyaluronic acid, starch and poly-caprolactone (SPCL) had enhanced osteogenic differentiation of gBM-MSCs (Nair et al. 2009; Nair et al. 2009a; Hwang et al. 2011; Rodrigues et al. 2014). An assembly of porous tantalum along with injectable fibrin sealant, and collagen membrane had promoted adhesion and growth of BM-MSCs. Porous tantalum even promoted differentiation of BM-MSCs into osteoblasts (Wei et al. 2019). In comparison to hydroxyappatite, silica-coated bioactive ceramic had further enhanced osteogenic potential of BM-MSCs (Nair et al. 2009; Nair et al. 2009a).

The matrix under *in vivo* condition is intricately designed and formed. As such the effect of matrix design on characteristics of MSCs has not been evaluated extensively. However, cross-linking of hydrogels of hyaluronic acid and Tyramine (HA-Ty) conjugate may have a dramatic impact on spatial organization of gBM-MSCs, and their matrix biosynthesis and overall cartilage tissue histogenesis (Toh et al. 2012). Also, scaffold cross-linking affects the cellular condensation and their spatial organization that ultimately determines fate of the cells. gMSCs cultured on tricalcium phosphate had remained interconnected and aligned but those cultured on hydroxyaptite had remained scattered (Prins et al. 2016). Collagen type II and glycosaminoglycan scaffolds that are loosely cross linked to allow cell mediated contraction had promoted chondrogenesis of gBM-MSCs while those highly cross linked and resist contraction had inhibited MSCs chondrogenesis (Vickers et al. 2010).

The technique of scaffold synthesis and its composition may affect MSCs growth and differentiation. Chitosan (prepared by freeze gelation and particle leaching out technique) supports the osteogenic differentiation, in addition to adhesion and proliferation of gBM-MSCs (Garcı´a Cruz et al. 2010). Oxidized graphene and synthesized methacrylate-endcapped caprolactone networks support the gBM-MSCs proliferation and osteogenic differentiation (Ivirico et al. 2009; Elkhenany et al. 2015). Even hydrophilicity of the scaffold may affect the differentiation of the gBM-MSCs as more hydration content (50%) suppresses the alkaline phosphatase activity (decreased osteogenesis) (Ivirico et al. 2009). Natural scaffolds (small intestine submucosa and goat acellular lung scaffold crosslinked with quercetin and nanohydroxyapatite) too favoured the osteogenic differentiation of gBM-MSCs (Li et al. 2006; Gupta et al. 2017). The biodegradability of the scaffold or implant has to be studied as degraded products may change local pH that may affect MSC characteristics (Liu 2011; Johnson et al. 2012). Extracellular matrix particle size and density

too has bearing upon gMSCs differentiation. gBM-MSCs loaded on porous small size (212 to 300-*m*m) hydroxyapatite had formed better bone tissue as compared to dense and large sized hydroxyapatite (500- to 706-*m*m) (Fischer et al. 2004).

Influence of humoral/growth factors

In addition to the physical factors, the growth or humoral factors form an essential component of microenvironment that affects the MSC characteristics. gAD-MSCs under the influence of 1,25-dihydroxyvitamin-D3 (1,25(OH)2D3) had undergo osteogenic differentiation. This may occur by polyamine metabolism as evidenced by gene expression of the polyamine-modulated transcription factor-1 (PMF-1) and spermidine/ spermine N (1)-acetyltransferase (SSAT) (Tjabringa et al. 2008). Supplementation of the microenvironment with growth factors like BMP-2 had promoted osteogenic differentiation of gAD-MSCs while BMP-7 had promoted their chondrogenic differentiation. A short period (15 min) of incubation with such factors (10ng/ml) was sufficient to induce differentiation whereas long term addition did not promote such a differentiation (Knippenberg et al. 2006; Zhang and Jiang 2006).

Mechanical factors that may be available in the microenvironment have direct bearing upon growth and differentiation of MSCs (Versari et al. 2007). The cells are mechano-sensitive as gAD-MSCs under pulsatile fluid flow had undergone osteogenic differentiation (Knippenberg et al. 2005). gBM-MSCs embedded in hydrogels along with TGF-β1 upon mechanical compression were quantitatively directed towards the chondrogenic differentiation; as exhibited by gene expression of cartilage related markers. However, such a compression had inhibited their differentiation if TGF-β1 was not added. This illustrated that the directed differentiation of MSCs may be hastened by the mechanical compression under favourable conditions (Terraciano et al. 2007).

The most of the above *in vitro* studies have recorded the effects of the scaffold/mechanical factors or humoral factors in isolation or in combination of 2-3 factors. However, under *in vivo* conditions, numerous factors are in play and thus may have different resultant effect on MSCs. Therefore, further studies need to be designed so that combined effects of all possible factors can be assessed.

Effect of donor age, health status and antibiotics/anaesthetics on gMSCs

Disease conditions of an individual also affect MSC characteristics. MSCs obtained from osteoporotic goat, are less proliferative under *in vitro* conditions and have limited osteogenic potential. However, culturing the cells with scaffolds like β-TCP had enhanced such a potential and also directed their osteogenic differentiation (Cao et al. 2012). Although, age is generally considered to decrease MSCs proliferation and differentiation potential but a study on gMSCs had shown insignificant variation in cell concentration and their proliferation with change in the donor age (Vertenten et al. 2009). Various horse and dog studies have shown that anaesthetics and antibiotics affect the growth and proliferation of MSCs, although no such study has been conducted on gMSCs (Edmonds et al. 2016). For better understanding such studies on gMSCs are desirable.

Effect of transportation and handling

Various factors like transportation media and implantation techniques may have a bearing on MSCs viability as has been demonstrated in dog and horse MSCs (Bronzini et al. 2012; Garvican et al. 2014). Equine MSCs (eMSCs) are cryopreserved for long storage period but for shorter duration storage eMSCs are preferably carried in autologus serum/ plasma. For shorter duration transit MSCs are preferably kept in phosphate buffer saline to prevent internalization of any foreign protein (Bronzini et al. 2012; Garvican et al. 2014). However, gMSCs studies are lacking in this aspect.

The cells are preferably are injected through wide bore needles to prevent any stress on the cells. The current recommended needle size is 21 guage considering the eMSCs viability (Lang et al. 2017). The transport container type (plastic or glass) may not have any bearing on cellular viability as has been reported for equine MSCs (Espina et al. 2016). However, such studies are desired for gMSCs.

***In vivo* studies**

MSCs are being extensively studied for their therapeutic application. The preliminary reports are very promising but the mechanism behind the repair is not fully understood. Despite their potential to differentiate into diverse cell lineages, currently MSCs are considered to provide therapeutic effect mainly by the release of immune-modulatory, anti-apoptotic factors and chemotactic agents (Kubo et al. 2009; Gugjoo and Amarpal, 2018; Gugjoo et al. 2019). This may be facilitated via: paracrine secretion of proteins/peptides and hormones; mitochondrial transfer by way of tunneling nanotubes; and/ or transfer of exosomes/ microvesicles containing RNA and other molecules (Spees et al. 2016**)**.

Goat makes an important human translational model for various ailments of different tissues/organs including bone, cartilage, and others. Therefore, stem cell studies in such a species may be doubly rewarding. It may help to develop therapeutics in veterinary medicine and may provide proof-of-therapeutic principle for human medicine. Demands from various quarters like NGOs, animal ethics groups, etc. to develop animal substitute models for evaluating disease pathophysiology; safety and efficacy of different medicines and/ cells including MSCs, are continuously growing. An *ex vivo* 3D organ culture system is one of the way to study physiology and pathophysiology of the disease. One of the studies on gAD-MSCs had been conducted under organ culture systems to study their spatial and temporal cellular behaviour and was demonstrated to be comparable to that of *in*

vivo studies (Peeters et al. 2015). The current review below focuses on the potential *in vivo* applications of gMSCs.

MSCs have been implanted by intravenous or local routes. In case of intravenous routes, the cells tend to reach to the desired location by their properties of migration and homing. However, intravenous injection of gMSCs carries potential to develop disseminated intravascular coagulation. In goats, intravenous injection of gMSCs ($1x10^6$) had induced hypercoagulable state within 2 hr but the condition was normalized within 24 hr (Liao et al. 2017). This might have happened due to the expression of procoagulant factors like tissue factor (TF), collagen1A and fibronectin1 by gMSCs under *in vitro* culture system (Tatsumi et al. 2013). Expression of the procoagulant factors tends to increase with the increase in the number of cell passages. However, heparin therapy appeared to be helpful to prevent such hypercoagulable state (Liao et al. 2017). Below are given the potential applications of gMSCs for veterinary practice or for the human translational research.

Cutaneous wounds

Cutaneous wound healing is an integration of the complex biological and molecular events. It involves cell migration and proliferation, extracellular matrix deposition, angiogenesis and remodelling. This orderly integrated healing process is impaired in many chronic diseases that demand external interference (Wu et al. 2007). Sometimes extensive tissue damage result in delayed healing and thus, compromise with the quality of life. MSCs tend to ameliorate tissue damage in response to injury and disease (Phinney and Prockop 2007) and are thus, seen as potential candidates to improve healing. In caprine wound healing models, local implantation of WJ-MSCs that remained viable up to 12 days had lead to complete re-epithelialisation in 7 days. The healed tissue had shown limited inflammation, thinner granulation tissue and minimal scar tissue formation (Azari et al.

2008; Azari et al. 2011). These promising results encourage their utilization in chronic non healing wounds, ulcers and/ burn injuries.

Osteochondral defects

Cartilage healing is limited due to its lack of direct blood and lymphatic network, and nerve supply. and absence of, Additionally, less proliferative resident chondrocytes are present in limited numbers (Gugjoo et al. 2017; Gugjoo et al. 2019c). Various conservative and invasive surgical techniques have so far failed to address the issue. The healing has been marred by secretion of less resilient, mechanically weaker fibrous tissue, hypertrophic cartilage formation (collagen X) and lack of integration of the healed tissue with the native cartilage (Gugjoo et al. 2017). Thus, newer advanced techniques like tissue engineering using MSCs are being considered and believed to carry immense potential to address the issues. Recent tissue engineering techniques like non-viral transfection of gMSCs with anti-angiogenesis factors have been utilized that may help in regeneration of the avascular tissues like cartilage (Sun et al. 2009) without affecting the general viability of the cells and their chondrogenic potential (Jeng et al. 2010). Among various animal species goat knee cartilage thickness (0.7-1.5 mm) approximates to that of human (2.2-2.5 mm), and thus may act as an excellent human translational model for cartilage studies (Frisbie et al. 2006).

In various goat osteochondral defect models, application of MSCs has been demonstrated to improve healing. In a goat osteochondral defect model (medial meniscus excision and resection of the anterior cruciate ligament) intra-articular implantation of gBM-MSCs had retarded degeneration of the articular cartilage, osteophytic remodelling, and subchondral bone sclerosis (Murphy et al. 2003). Apart from BM-MSCs, goat medial femoral condyle and trochlear groove osteochondral defect model treated with AD-MSCs or SVF had given rise to the improved cartilage healing as compared to the scaffold (collagen I/III) treated animals after 4 months. The cell treated animals had shown healed tissue with

improved collagen type II, increased glycosaminoglycan content, and formation of hyaline-like cartilage, in addition to its higher elastic modulus comparable to the native tissue (Jurgens et al. 2013).

The modifications of MSCs or addition of scaffolds have been demonstrated to improve MSCs induced cartilage healing (Wei et al. 2019). In a caprine femoral condyle defect model, implantation of cartilage extracellular matrix seeded with gWJ-MSCs had lead to the better repair of the cartilage and subchondral bone as compared to microfracture technique. However, a comparable inflammatory response in MSC treated animals to that of microfracture technique treated animals was demonstrated after 9 months. Overall, the regenerated tissue in MSC treated animals had shown more cartilage extracellular matrix, lacunas and collagen type II (Zhang et al. 2018). In another caprine medial femoral condyle defect model comparative studies, implantation of BM-MSCs had resulted in its improved repair as compared to bone marrow stimulation and microfracture. The BM-MSCs treated animals were awarded with better ICRS and O'Driscoll scores with increased glycosaminoglycan content and cartilage specific gene expression profiles (Bekkers et al. 2013; Nam et al. 2013).

In a comparative study on goat mandibular condyle defect model, implantation of NEL like molecule-1 (Nell-1) modified gBM-MSCs/Poly Lactic Co-Glycolic acid (PLGA) had repaired the defect by fibrocartilage at 6 weeks and complete healing with native articular cartilage by 24th week. In contrast, unmodified gBM-MSCs/PLGA had repaired the defect by fibrocartilage at 24 weeks (Zhu et al. 2011). Nell-1 is a novel growth factor that specifically targets cells committed to osteochondral lineage. More similar modifications of the cells may be attempted for effective utilization of gBM-MSCs. However, exogenous implantation of gBM-MSCs had failed to enhance meniscal healing potential of fibrin clot implantation in a goat meniscal model (Port et al. 1996). In an osteochondral defect model,

implantation of tissue engineered osteochondral graft cultured in bioreactor along with gBM-MSCs had resulted in better repaired tissue as compared to control (graftless). The mechanical stimulation of the graft and/ cell assembly had further potentiated the repair of the osteochondral defect (Pei et al. 2014).

In all these studies preclinical stuides, variable cell source, concentration, passage number and model type have been employed. Thus, gMSCs may be studied in clinical cases to determine their actual utility and clinical feasibility.

Bone defects

Bone tissue engineering is extensively carried out with about 2.2 million human bone-grafting procedures being performed annually, worldwide (Costa-Pinto et al. 2011). The cellular component forms an essential part of bone tissue engineering. MSCs ability to trans-differentiate and secrete paracrine factors make them a suitable option for both trauma related ailments as well as for the disease conditions like osteoporosis (Cao et al. 2012). The use of goat as an orthopaedic research animal model for human has increased during last few years (Proffen et al. 2012). Goat and human have comparable body features including their long bone size (Anderson et al. 1999; Van Der Donk et al. 2001). The macrostructure, although not microstructure, of the long bones of human and goat/sheep is more comparable. In addition, goat had been utilized to study bone turn over markers (proteins to detect bone metabolism) in fracture healing for human as these carry similarities in biomechanics, biochemistry and bone histology (Sousa et al. 2015).

To study the effect of MSCs, various goat translational bone defect models for human have been created. The critical defect size in these studies varied from bone to bone and study to study. Ilium size was kept at 17 mm (Anderson et al. 1999), femoral defect at 20 mm (Li et al. 2014) and tibial defects at 25mm (Liu et al. 2010) and 30 mm (Wang et al. 2010). In addition, pathological conditions like osteoporosis of the goat bones are

considered to suitably mimic human conditions (Cao et al. 2012). Bone is always under stress and as such may have effect on its healing outcome. A study had compared healing potential of dynamic perfusion bioreactor cultured scaffolds along with BM-MSCs to that of static cultured scaffolds along with BM-MSCs. It was shown that implantation of dynamic cultured biomaterial assembly had lead to the better healing as compared to that of the static cultured biomaterial assembly (Cao et al. 2012; Gardel et al. 2013).

Various bone tissue engineering techniques have been used in goats involving bones like mandible, femur and tibia (Tang et al. 2007; Nair et al. 2009a; Vertenten et al. 2009; Lippens et al. 2010; Liu et al. 2010; Zou et al. 2012; Loozen et al. 2015; Li et al. 2014; Li et al. 2015; El Hadidi et al. 2016; Zhao et al. 2017). In majority of the studies, BM-MSCs had been utilized along with scaffolds like calcium phosphate (Zou et al. 2012), triphasic ceramic-coated hydroxypatite (Nair et al. 2009a), hydrogel carriers (Lippens et al. 2010), nano-hydroxyapatite/collagen/poly (L-lactic acid) (PLLA)/chitin fibres (nHACP/CF) (Liu et al. 2010), methacrylate-endcapped poly(D,L-lactide-co-epsilon-caprolactone) (Vertenten et al. 2009), α-tricalcium phosphate (Vertenten et al. 2009), beta tri-calcium phosphate (β-TCP) (Tang et al. 2007) and alginate beads loaded onto fibrin gel (Hou et al. 2008). Overall, better repair in terms of bone quality and quantity upon implantation of combination of MSCs and scaffolds had been reported as compared to the scaffold alone. Healing in cell laden scaffold group had been evident by the better lamellar bone organization throughout the defect while scaffold implantation only managed to generate immature woven bony bridges still intermingled with scattered small remnants of the scaffold material (Nair et al. 2009a). In addition, the osteo-integration of the healed tissue has been better compared to the acellular scaffold treated animals.The obvious repair has been demonstrated as early as 8 weeks (Liu et al. 2010) to 4 months (Nair et al. 2009a). Although, BM-MSCs had carried good compatibility with methacrylate-endcapped poly(D,L-lactide-co-epsilon-caprolactone)

under culture conditions, implantation of such scaffolds along with BM-MSCs in goat tibial defects however, had failed to show osteo-conductive characteristics in the first week post implantation. Thus, it is important to mention that MSCs compatible scaffolds may not give desired results upon *in vivo* implantation.

In addition to the use of suitable scaffolds, MSCs may be genetically modified for improved outcome. Human bone morphogenetic protein (hBMP) gene (Tang et al. 2007; Loozen et al. 2015) and beta-galactosidase (gal)-gene-transduced gBM-MSCs (Tang et al. 2007) upon implantation into bone defect models had lead to the better healing of bone defects as compared to untransduced cell or acellular scaffold treated defects. The BMP-2 transduced gBM-MSCs treated animals had higher bone volume with better compressive strength and Young's modulus of the repaired tissue approximating normal tissue in comparison to beta-galactosidase gene transduced gBM-MSCs (Tang et al. 2007). Further modifications are being evaluated like addition of the antibiotics (Hou et al. 2008) and implantation of MSCs in distraction osteogenesis (Shen et al. 2006) for enhanced bone healing.

Numerous preclinical studies employing MSCs with bone tissue engineering scaffolds have shown positive outcomes. Uniform cell studies with similar cell sources, concentration, passage number and implantation type however, are desired to be studied both preclinically as well as under clinical settings. Further, uniform scaffolds and gene transfection studies have to be conducted for better outcome in order to establish standard operational procedures for bone defect repair using stem cells.

Intervertebral disc disease

Goat has also been used as an intervertebral disc disease model for human. Goat lumbar discs are large in size (~5mm disc height), and have similarities in mechanical properties and biochemical composition to that of the human discs (Beckstein et al. 2008).

Studies on intervertebral disc regeneration using gMSCs are only preliminary and need further extensive evaluation, keeping in view the importance of the condition and potential application in human beings. In goats, the preliminary studies involving the tissue engineered constructs employing MSCs have shown positive results (Gullbrand et al. 2017; Xu et al. 2017). In a goat annulus fibrosus defect model, implantation of gelatin sponge loaded with gBM-MSCs and platelet rich plasma had lead to a gradual healing from 3 weeks to 12 weeks of time as evidenced by histological examination (Xu et al. 2017). Other factors like the basement membrane molecules have also been observed to play a role in nucleus pulposus chondrogenesis and cartilage regeneration (Toh et al. 2013) and thus, need to be evaluated.

Periodontal tissue

MSCs applications have been well studied in canine periodontal defects for human translational studies (Khorsand et al. 2013; Tobita et al. 2013; Takewaki et al. 2017) but the studies in goats are nominal. In a caprine model of periodontal defect, implantation of the tissue engineered construct employing poly(DL-Lactide-co-Glycolide) scaffold along with gBM-MSCs had promoted periodontal tissue regeneration involving cementum, bone, and periodontal ligament. Such healing was not observed in animals implanted with scaffold only (Marei et al. 2009). Therefore, further goat studies are desired in larger number of animals.

Cardiovascular system

Sheep and/ goats have been used for the cardiovascular disease models for humans. In a goat left anterior descending coronary artery ligation model, implantation of autologous gBM-MSCs along with small intestinal submucosal film had prevented dilatation of the left ventricular chamber and improved the contractile ability of the myocardium, cardiac function, and collateral perfusion (Liao et al. 2006). However, very limited studies have

been conducted in goats while in sheep much more extensive research has been conducted. To understand the mechanisms involved and develop any conclusive statement, further, extensive studies are desired.

Broncho pleural fistula

Lung cancer is one of the leading causes of cancer deaths worldwide (Wakelee et al. 2007). Patients suffering from limited lung disease are operated for surgical resection. However, post-resectional bronchopleural fistula (BPF) may develop in 1% to 4% patients with the mortality rate of 12.5% to 71.2% (Sonobe et al. 2000). In a goat model, implantation of the gBM-MSCs in induced bronchopleural fistula had lead to the healing of fistula by 28th day as evident by the presence of proliferative extraluminal fibroblasts and collagenous matrix development (Petrella et al. 2014). The study appears to be quite promising, and many more studies involving different aspects of healing of pleural tissues need to be conducted to arrive at logical conclusion(s).

Reproduction

Mesenchymal stem cells may be utilized in Nuclear Transfer (NT) technology to produce cloned animals and tissues/organs for regenerative medicine. Currently, overall efficiency of NT in producing clones is low and one of the limiting factors that influence the viability of clones is the nuclear karyoplast (Kwong et al. 2014). To improve the viability of clones, MSCs as donor karyoplast appear to be promising. gBM-MSCs used as donor karyoplast had lead to the production of cloned caprine embryos capable of developing up to the hatched blastocyst stage. MSCs produced clones had shown better development up to blastocyst stage and hatching as compared to that of somatic cell (ear fibroblasts). An overall better proliferation rates, growth capacity, transfection efficiency and convergence and cleavage was demonstrated using MSCs as compared to somatic cells (Kwong et al. 2014; Ren et al. 2014). However, one of the studies had reported comparability in the fusion

and cleavage rate between somatic cells and gBM-MSCs (Kwong et al. 2014), demanding further incites in the area.

Miscellaneous conditions

Stem cell studies have been harnessed on mammary gland. Mammary stem cells (MaSC) provide for net growth, renewal and turnover of mammary epithelial cells, and are therefore potential targets for strategies to increase production efficiency (Martignani et al. 2009; Capuco et al. 2012; Martignani et al. 2014). As the MSCs have potential to trans-differentiate and secrete factors that can repair or regenerate the damaged tissue, these cells can be studied for mammary tissue regeneration.

The goat has been demonstrated to act as a good model for human stress urinary incontinence disorder (Burdzinska et al. 2017). As such the studies that deliberate on the stem cell application for the regeneration or repair of urinary system may be designed.

Conclusion(s)

A wide array of research is being conducted on gMSCs for human translational studies and goat disease conditions. Extensive literature available on gMSCs though shows positive results but without any definite conclusion(s). gMSCs can be harvested from numerous sources but due to small number they need to be culture expanded for clinical application. In goats, MSCs from bone marrow, adipose tissues or fetal adnexa are mainly used for preclinical studies. The lack of uniformity in cell source, isolation and culture techniques result in variable outcome in different studies. The optimal cell passage number, concentration and type of implantation and re-implantation period need to be studied. The inability to control *in vivo* expression and differentiation of MSCs is what currently limits their definitive application and thus, demands further extensive research in the area. Application of different biomaterials like scaffolds or growth factors along with gMSCs may be studied for better outcome. There is a need to identify the important areas for

potential application by meta-analysis of the results and scrutinizing the available data to determine the future course of the studies. Apart from experimental studies, randomised blind folded clinical studies are desired to establish actual clinical efficacy and utility of gMSCs.

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Conflict of Interest:

Authors declare there is no conflict of interest

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