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REVIEW

Mesenchymal stem cells for cartilage regeneration in dogs

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Abstract

Articular cartilage damage and osteoarthritis (OA) are common orthopedic diseases in both humans and dogs. Once damaged, the articular cartilage seldom undergoes spontaneous repair because of its avascular, aneural, and alymphatic state, and the damage progresses to a chronic and painful situation. Dogs have distinctive characteristics compared to other laboratory animal species in that they share an OA pathology with humans. Dogs can also require treatment for naturally developed OA; therefore, effective treatment methods for OA are desired in veterinary medicine as well as in human medicine. Recently, interest has grown in regenerative medicine that includes the use of mesenchymal stem cells (MSCs). In cartilage repair, MSCs are a promising therapeutic tool due to their self-renewal capacity, ability to differentiate into cartilage, potential for trophic factor production, and capacity for immunomodulation. The MSCs from dogs (canine MSCs; cMSCs) share various characteristics with MSCs from other animal species, but they show some deviations, particularly in their differentiation ability and surface epitope expression. *In vivo* studies of cMSCs have demonstrated that intraarticular cMSC injection into cartilage lesions results in excellent hyaline cartilage regeneration. In clinical situations, cMSCs have shown great therapeutic effects, including amelioration of pain and lameness in dogs suffering from OA. However, some issues remain, such as a lack of regulations or guidelines and a need for unified methods for the use of cMSCs. This review summarizes what is known about cMSCs, including their *in vitro* characteristics, their therapeutic effects in cartilage lesion treatment in preclinical *in vivo* studies, their clinical efficacy for treatment of naturally developed OA in dogs, and the current limitations of cMSC studies.

Key words: Mesenchymal stem cell; Dog; Cartilage; Osteoarthritis; Regenerative medicine; Veterinary medicine

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Core tip: Mesenchymal stem cells (MSCs) are promising therapeutic tools for treatment of cartilage damage and osteoarthritis (OA). This review summarizes the current knowledge of MSCs from dogs, including *in vitro* characteristics, *in vivo* cartilage regenerative potential, and therapeutic effects for naturally developed OA in dogs. MSCs from dogs share many *in vivo* characteristics with MSCs from other animal species and are reported to have excellent cartilage repair potential in experimental and clinical situations. The article also describes the current limitations of MSC use in dogs that remain to be resolved in the future.

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INTRODUCTION

Articular cartilage is critical for the normal functioning of the synovial joint. The ability of cartilage to withstand high cyclic loads and to minimize surface friction on articular surfaces allows it to act as a shock absorber and a lubricator in synovial joints[1-3]. Nevertheless, articular cartilage is still subject to damage by a variety of factors, including acute trauma, degeneration, or joint diseases. Once damaged, hyaline articular cartilage shows very limited intrinsic repair potential because of its' avascular, aneural, and alymphatic state. The unrepaired cartilage defects lead to pain, swelling, and deterioration in mobility and will eventually progress to osteoarthritis (OA)[4,5].

OA is a common degenerative, progressive, and painful disease in both humans[6] and dogs[7]. Nevertheless, an adequate therapy for the repair of cartilage lesions has yet to be established. A variety of surgical interventions are available in human medicine, including microfracture[8], osteochondral autografts[9], and autologous chondrocyte implantation[10]. Following microfracture, the regenerated tissue occurs as a mixed fibrocartilage tissue that is inferior in its mechanical properties to native hyaline cartilage and has poor long-term results[11]. Similarly, osteochondral autografts have limitations of graft availability and risk of donor site morbidity[12,13]. Autologous chondrocyte implantation, although it can achieve hyaline-like cartilage repair, also has some issues, including loss of the chondrocytic phenotype in monolayer culture, limited availability of chondrocytes and inadequate chondrocyte proliferative potential[14,15].

One attractive alternative source of cells for cartilage regeneration is mesenchymal stem cells (MSCs), which are readily available, possess great proliferation ability, and can differentiate into many different cell types in the body[16,17]. MSCs have been used in various applications in both human and veterinary medicine. For cartilage lesion studies, dogs are a particularly distinctive animal species as they can serve as an experimental animal model, a clinical model for human medicine, and a treatment subject. This review summarizes the *in vitro* data, preclinical *in vivo* studies, and clinical studies involving the use of MSCs for cartilage regeneration in dogs (Figure 1).

IMPORTANCE OF STUDIES ON CARTILAGE REPAIR IN DOGS

Dogs can serve as experimental or clinical models for studies on cartilage repair in humans. Although the articular cartilage is thinner in dogs than that in humans (range in humans: 1.0-2.6 mm)[18-20], this thickness is greater in medium to large breed dogs (range: 0.95-1.3 mm) than in rodent and rabbit models. This greater thickness allows the experimental creation of articular cartilage defects without causing detrimental changes in the subchondral bone[20]. In addition, unlike the other experimental animals, dogs can accept various human-type postoperative managements, including bandaging, splinting, or exercise involving (leash) walking or training on treadmills[20-22], which can prevent OA or regulate its progression after the

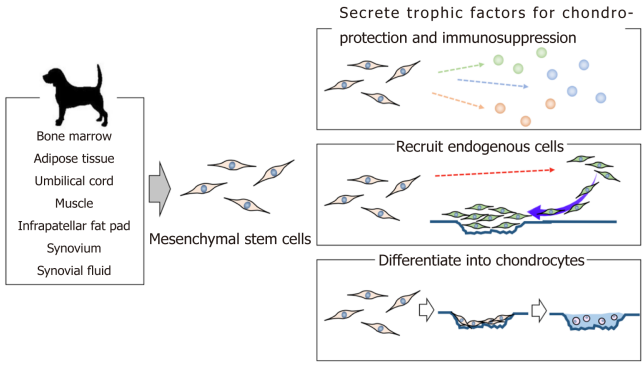

Figure 1 Schematic representation of the role of mesenchymal stem cells for cartilage regeneration. Mesenchymal stem cells (MSCs) can be isolated from various types of tissues in dogs. MSCs are an attractive therapeutic tool for cartilage regeneration as they can secrete trophic factors for chondroprotection and immunosuppression, recruit endogenous cells to the damaged lesion, and differentiate into chondrocytes, thereby ameliorating the cartilage injury.

experimental interventions.

However, the use of companion animals such as dogs as experimental models for human medicine raises ethical considerations. By contrast, the use of farm animals, such as goats, sheep and pigs, as experimental animal models is less controversial[20,23,24]. These farm animals have the further advantage that their articular cartilage thicknesses are closer to those of humans and are adequate for the creation of partial- and full-thickness defects[20,23,24]. For these reasons, dogs might be superseded by goats or pigs as experimental animal models.

Nevertheless, despite the disadvantages of using dogs as experimental animal models for preclinical studies of cartilage repair, dogs have one distinctive feature that differentiates them from other animals as they naturally develop diseases that share a close analogy with human diseases, including OA[25,26] (Figure 2). Therefore, clinical studies that are performed in dogs with naturally occurring OA can provide valuable information for the treatment of human OA patients. A variety of ante mortem diagnostic monitoring protocols, such as gait and kinematic analyses[27], arthroscopic evaluation[28], and magnetic resonance imaging[29], are feasible in dogs and allow evaluation of the therapeutic effect from a variety of viewpoints. In addition, while sharing cartilage pathologies with humans, dogs also share the lifestyles of their owners, including home and exercise habits. The life expectancy of dogs is also relatively long, and dogs are subject to similar natural and environmental factors that lead to the occurrence of OA in humans, such as traumatic injuries, obesity, and aging. Many adult dogs suffer from OA, which creates a large pool of client-owned dogs that could be available for enrollment in clinical trials of potential OA treatments[7]. Although considerable interindividual variability is evident in these clinical cases when compared to purpose-bred dogs, the results are still suitable for extrapolation to human patients.

The clinical study of dogs with OA is worthwhile for both human and veterinary medicine. Dogs suffer from many cartilage pathologies that can lead to OA, such as osteochondritis dissecans and cartilage injury caused from cranial cruciate ligament failure[30,31]; therefore, the need is increasing for cartilage lesion treatment methods that can regenerate hyaline cartilage and allow recovery of the normal mechanical function of the articular cartilage. Thus, studies on cartilage regeneration in dogs have important roles in the improvement of cartilage repair treatments and can have great significance in both human and veterinary medicine.

MSC USE IN DOGS

MSCs are cells that display self-renewal capacity and have the potential for differentiation under specific conditions into other cell types, including cartilage, bone, and adipose tissue. MSCs were first identified in bone marrow derived from mice[32], but were subsequently isolated from other tissues, including adipose tissue[33], umbilical cord[34], dental pulp[35], infrapatellar fat pad[36], and synovial membrane[37]. MSCs can also be isolated and cultured from tissues obtained from various animal species, including rats[38], rabbits[39], dogs[40], cats[41], pigs[42], and horses[43], as well as

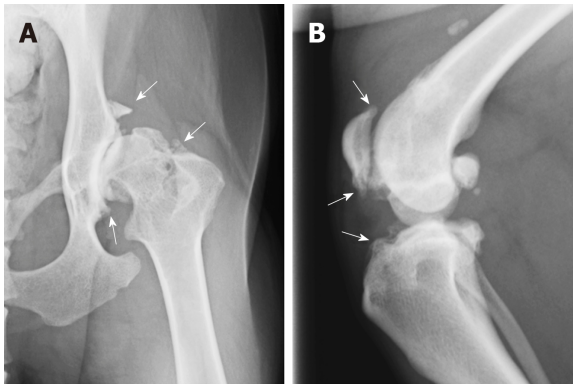

Figure 2 Radiographic findings of osteoarthritis in dogs. A: A radiographic image of the hip joint in a 8 years old Labrador retriever suffering from osteoarthritis (OA) which results from hip dysplasia; B: A radiographic image of the stifle joint in a 4 years old Shiba suffering from OA which results from cranial cruciate ligament rupture. White arrow: Osteophytes.

humans[44].

The International Society for Cellular Therapy (ISCT) proposed minimal criteria to define the characteristics of human MSCs[45]: (1) Adherence to plastic in standard culture conditions; (2) Specific surface antigen expression of CD105, CD73, CD90 and lack of expression of other hematopoietic cell surface markers, including CD45, CD34, CD14 or CD11b, CD79α or CD19, HLA-DR; and (3) Multipotent differentiation potential under specific *in vitro* culture conditions. These criteria also serve, to a certain extent, as parameters for MSCs derived from various animal species; however, the characteristics of MSCs differ somewhat among various animal species or tissues. Therefore, the criteria for human MSCs cannot be completely extrapolated to MSCs from veterinary species.

Isolation, expansion, and characterization of cMSCs

MSCs from dogs (canine MSCs: cMSCs) can be isolated from wide variety of tissue sources, including bone marrow[46,47], adipose tissue[48,49], umbilical cord[50,51], muscle[52], infrapatellar fat pad[40,53], synovium[40,53,54], and synovial fluid[55]. For research and therapeutic purposes, MSCs are needed in large numbers, so MSCs must have a high proliferation ability. Some studies have demonstrated a higher proliferation ability for cMSCs derived from adipose tissue (AD-cMSCs) than from bone marrow (BM-cMSCs)[50,56,57]. Furthermore, the colony-forming potential and proliferation ability is higher in cMSCs from synovium (Sy-cMSCs) and from infrapatellar fat pads (IF-cMSCs) than in AD-cMSCs and BM-cMSCs[40,53]. Notably, further differences are observed depending on the age of the donor dogs or the passage number of cMSCs in terms of proliferation capacity[58,59]. For example, Bertolo *et al*[60] have shown a greater tendency for senescence in cMSCs than in human MSCs when grown in monolayer culture. These findings suggest that the optimal culture conditions for cMSCs should be reconsidered; for instance, the inclusion of supplemental growth factors, such as fibroblast growth factor-2 (FGF-2), could increase the proliferation ability of cMSCs[61].

Table 1 shows the surface marker expression of cMSCs derived from various sources. The expression of cell surface markers is one of the identification criteria for MSCs[45]; however, the criteria used for human MSCs may not be completely compatible with those required for cMSCs because of interspecies differences and the lack of validated antibodies and controls for cMSCs. The definitive expression of surface antigens by cMSCs has not been demonstrated, and results for cMSC surface antigen expression have been contradictory among various studies. For example, high expression (> 90%) of CD90 was reported in cMSCs derived from adipose tissue[53,56,57,62] and bone marrow[47,53,56,63] in some studies, whereas other studies have shown low expression (< 40%) of CD90 for cMSCs derived from adipose tissue[49,64,65] and bone marrow[49,64]. Surface epitope expression has a known correlation with differentiation potential and proliferation ability as well as with stemness[66,67]; therefore, further studies are needed to identify appropriate antibody sets for cMSC characterization.

Differentiation potential of cMSCs

Multipotent differentiation potential is one of the defined criteria proposed by ISCT and is a crucial characteristic of MSCs used in regenerative therapy. Adipogenesis, osteogenesis, and chondrogenesis in cMSC studies are validated by specific staining

Table 1 Surface marker expression in canine mesenchymal stem cells

Source	Positive marker	Negative marker	Ref.
Adipose tissue	CD90, CD44, CD73, CD105	CD45, CD34, CD14	Kang *et al*[50], 2012
Bone marrow	CD90, CD44, CD73, CD105	CD45, CD34, CD14	
Umbilical cord	CD90, CD44, CD73, CD105	CD45, CD34, CD14	
Wharton's jelly	CD90, CD44, CD73, CD105	CD45, CD34, CD14	
Adipose tissue	CD90, CD44	CD45, CD34, CD146	Kisiel *et al*[52], 2012
Bone marrow	CD90, CD44	CD45, CD34, CD146	
Muscle	CD90, CD44	CD45, CD34, CD146	
Periosteum	CD90, CD44	CD45, CD34, CD146	
Adipose tissue	CD90, CD44	CD45, CD11b	Sasaki *et al*[40], 2018
Bone marrow	CD90, CD44	CD45, CD11b	
Synovium	CD90, CD44	CD45, CD11b	
Infrapatellar fat pad	CD90, CD44	CD45, CD11b	
Adipose tissue	CD90, CD44, CD105, CD9	CD45, CD34, Stro-1	Bearden *et al*[53], 2017
Bone marrow	CD90, CD44, CD105, CD9	CD45, CD34, Stro-1	
Synovium	CD90, CD44, CD105, CD9	CD45, CD34, Stro-1	
Adipose tissue	CD90	-	Reich *et al*[56], 2012
Bone marrow	CD90	-	
Adipose tissue	CD90, CD44, CD29	CD45, CD34	Takemitsu *et al*[64], 2012
Bone marrow	CD90, CD44, CD29	CD45, CD34	
Adipose tissue	CD90, CD44, MHCI	CD34, CD29, CD14, MHCII	Screven *et al*[49], 2014
Bone marrow	CD90, CD44, MHCI	CD34, CD29, CD14, MHCII	
Adipose tissue	CD90, CD44	CD45, CD34, CD14	Sullivan *et al*[118], 2016
Bone marrow	CD90, CD44	CD45, CD34, CD14	
Adipose tissue	CD90, CD44, CD29, CD73, CD4, CD8, MHCI	CD45, CD34, CD14, MHCII	Russell *et al*[57], 2016
Bone marrow	CD90, CD44, CD29, CD73, CD4, CD8, MHCI	CD45, CD34, CD14, MHCII	
Adipose tissue	CD90, CD44, MHCI	CD45, CD34, CD14, CD3, CD4, CD8, CD172a, CD11c, HLA-DR, sIgM	Kang *et al*[62], 2008
Adipose tissue	CD90, CD44, CD29	CD45, CD34, CD14, CD117, CD13 CD105, CD73	Vieira *et al*[48], 2010
Adipose tissue	CD90, CD44, CD140, CD117	CD45, CD34	Martinello *et al*[65], 2011
Bone marrow	CD90, MHCI	CD45, CD34, MHCII	Kamishina *et al*[119], 2006
Bone marrow	CD90, CD105	CD45, CD34	Csaki *et al*[46], 2007
Bone marrow	CD44, Stro-1	CD45, CD34	Hodgkiss-Geere *et al*[120], 2012
Bone marrow	CD90, CD44	CD14	Bertolo *et al*[73], 2015
Bone marrow	CD90, CD44, CD105, CD73, CD166, vimentin	CD31	Zhang *et al*[47], 2018
Bone marrow	CD90, CD44, CD29, CD105, CD166	CD45, CD34	Li *et al*[63], 2018
Synovium	CD90, CD44	-	Wijekoon *et al*[54], 2017
Synovial fluid	CD90	CD45, CD34	Krawetz *et al*[55], 2012

or analysis of mRNA expression (Figure 3 and Table 2).

In vitro adipogenesis of cMSCs has been confirmed for AD-cMSCs, BM-cMSCs, Sy-cMSCs, and IF-cMSCs (Table 2). The formation of oil lipid droplets is verified with oil red-O staining; however, cMSCs sometimes cannot sufficiently differentiate into adipocytes in the induction medium used for adipogenic differentiation of human MSCs, so the induction media need to be optimized for cMSCs. Neupane *et al*[68] utilized rabbit serum, rosiglitazone, and a higher glucose concentration in their medium to promote the adipogenic differentiation of AD-cMSCs. The optimized induction medium increased the numbers and sizes of fat globules and the expression of adipogenic transcription factors, such as PPARγ2, when compared to the conventional adipogenic differentiation medium used for human MSCs[68]. A higher adipogenic differentiation ability has been reported for AD-cMSCs and IF-cMSCs than for BM-cMSCs and Sy-cMSCs[40,49,53].

Table 2 Surface marker expression in canine mesenchymal stem cells

Source	Differentiation potential	Ref.
Adipose tissue	Adipogenesis, osteogenesis, chondrogenesis	Csaki *et al*[46], 2007
Adipose tissue	Adipogenesis, osteogenesis, neurogenesis	Kang *et al*[62], 2008
Adipose tissue	Adipogenesis, osteogenesis, chondrogenesis	Neupane *et al*[68], 2008
Adipose tissue	Adipogenesis, osteogenesis, chondrogenesis, myogenesis	Vieira *et al*[48], 2010
Adipose tissue	Adipogenesis, osteogenesis, myogenesis	Martinello *et al*[65], 2011
Adipose tissue	Chondrogenesis	Reich *et al*[56], 2012
Bone marrow		
Adipose tissue	Osteogenesis	Kang *et al*[50], 2012
Bone marrow		
Umbilical cord		
Wharton's jelly		
Adipose tissue	Adipogenesis, osteogenesis	Kisiel *et al*[52], 2012
Bone marrow		
Muscle		
Periosteum		
Adipose tissue	Adipogenesis, osteogenesis	Takemitsu *et al*[64], 2012
Bone marrow		
Bone marrow	Chondrogenesis, cardiogenesis	Hodgkiss-Geere *et al*[120], 2012
Synovial fluid	Chondrogenesis	Krawetz *et al*[55], 2012
Bone marrow	Adipogenesis, osteogenesis, chondrogenesis	Volk *et al*[59], 2012
Adipose tissue	Adipogenesis, osteogenesis, chondrogenesis	Guercio *et al*[58], 2013
Adipose tissue	Adipogenesis, osteogenesis, chondrogenesis	Screven *et al*[49], 2014
Bone marrow		
Bone marrow	Adipogenesis, osteogenesis, chondrogenesis	Bertolo *et al*[60], 2015
Adipose tissue	Osteogenesis, chondrogenesis	Sullivan *et al*[118], 2016
Bone marrow		
Adipose tissue	Adipogenesis, osteogenesis	Russell *et al*[57], 2016
Bone marrow		
Synovium	Adipogenesis, osteogenesis, chondrogenesis, osteoclast differentiation	Wijekoon *et al*[54], 2017
Adipose tissue	Adipogenesis, osteogenesis, chondrogenesis	Bearden *et al*[53], 2017
Bone marrow		
Synovium		
Bone marrow	Adipogenesis, osteogenesis, chondrogenesis Hepatocyte and functional insulin-secreting cell differentiation	Zhang *et al*[47], 2018
Adipose tissue	Adipogenesis, osteogenesis, chondrogenesis	Sasaki *et al*[40], 2018
Bone marrow		
Synovium		
Infrapatellar fat pad		
Bone marrow	Chondrogenesis	Endo *et al*[61], 2018

During osteogenic differentiation, the morphology of cMSCs progresses from a fibroblast-like shape to a cuboidal-like appearance, together with the formation of aggregates that stain with von Kossa or alizarin red stains[46,52,58,68]. Unlike human MSCs, cMSCs have been known to roll up into a sheet and become easily detached from culture dishes during mineralization assays[40,53,58,68]. BM-cMSCs have shown superiority in osteogenic differentiation in some studies, when compared to AD-cMSCs, Sy-cMSCs, and IF-cMSCs[40,49,53]. However, Kang *et al*[50] showed a greater osteogenic ability of AD-cMSCs when compared with BM-cMSCs and Wharton's jelly-derived cMSCs.

The chondrogenic differentiation potency is a crucial characteristic of cMSCs intended for use in cartilage regenerative therapy. *In vivo* cartilage formation during embryogenesis is initiated by condensation of cells, and the cell-to-cell contacts

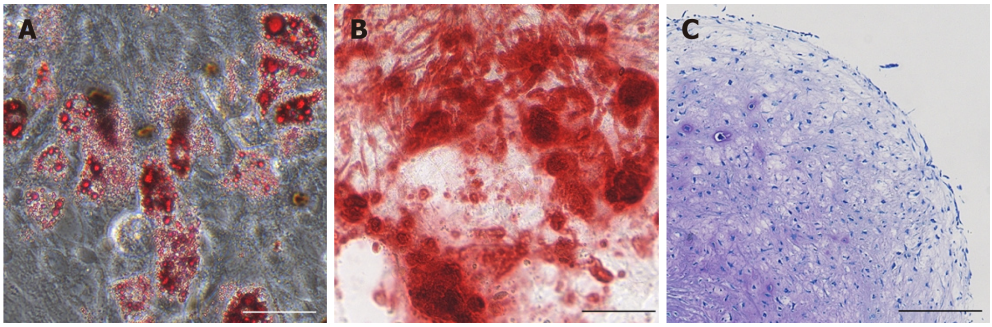

Figure 3 Trilineage differentiation of canine mesenchymal stem cells. A: Adipogenic differentiation confirmed by oil red-o staining. Bar = 50 μm; B: Osteogenic differentiation confirmed by alizarin red. Bar = 100 μm; C: Chondrogenic differentiation confirmed by toluidine blue staining. Bar = 250 μm.

induced by condensation are crucial for the onset of chondrogenesis[69]. The growth factor transforming growth factor-β (TGF-β) is crucial for the formation of three-dimensional aggregates during chondrogenesis in human MSCs[70], whereas cMSCs intrinsically aggregate in micromass culture even in the absence of TGF-β[52,68]. Nevertheless, some studies have reported difficulties in attaining substantial chondrogenic differentiation of cMSCs[52,56,57,60].

Since no definitive methods have been established for chondrogenic differentiation of cMSCs, the various studies have all used different tissue source origins, growth factors, and culture methods. The chondrogenic differentiation potential of cMSCs varies with the tissue source origin, as indicated by the larger cartilage pellets obtained from differentiating Sy-cMSCs and IF-cMSCs than from differentiating BM-cMSCs and AD-cMSCs[40,53]. The cartilage pellets that differentiate from BM-cMSCs are small, but they show intense staining with toluidine blue, alcian blue, or collagen type II antibody when compared to cartilage pellets differentiated from Sy-cMSCs, IF-cMSCs, or AD-cMSCs[53,56]. Conversely, AD-cMSCs, despite their abundance and ready availability, form only small cartilage pellets that show low stainability with toluidine blue, alcian blue, or collagen type II antibody and that sometimes show signs of necrosis[40,56]. Human Sy-MSCs and BM-MSCs are also known to have higher chondrogenic differentiation capacity when compared with AD-MSCs[44,71,72]. Interestingly, Bertolo *et al*[73] showed that cMSCs from different dog breeds can also vary in their differentiation potential.

Chondrogenic differentiation can be enhanced *in vitro* by supplementing the culture medium with growth factors, such as TGF-β and bone morphogenic protein (BMP). TGF-β, which is crucial for chondrogenic differentiation of MSCs, exists as three isoforms: TGF-β1, TGF-β2, and TGF-β3. Of these, TGF-β1[48,52,56,61] or TGF-β3[47,49,57] have been the most commonly used in studies of chondrogenesis in cMSCs. TGF-β3 is reported to result in higher and more rapid chondrogenic differentiation in human MSCs[74], but the optimal TGF-β isoform for chondrogenesis in cMSCs remains to be elucidated.

BMPs have been reported to promote chondrogenesis[75], and a greater stimulation of chondrogenesis in human MSCs has been reported for BMP-2 than for BMP-4 or BMP-6[76]. In addition to promoting chondrogenesis, BMPs can also stimulate endochondral ossification[77]. Many studies of cMSCs have shown the efficacy of BMPs for osteogenesis[53,78], although one study reported no improvement in chondrogenesis by BM-cMSCs or AD-cMSCs[56] in response to BMP-2. Therefore, the effectiveness of BMPs at inducing chondrogenesis in cMSCs remains unclear.

Chondrogenic differentiation ability decreases with the loss of CD90 in human MSCs[79,80] and cMSCs[55], indicating the importance of surface epitope expressions. Therefore, the initial cell selection may improve the chondrogenic potential of MSCs. The *in vitro* expansion of human MSCs, rabbit MSCs, and cMSCs in culture also impairs chondrogenic differentiation potential[44,81-83]. Moreover, senescence is reportedly more rapid in cMSCs than in human MSCs, particularly after passage 3[60]. Sekiya *et al*[84] reported that cartilage pellets were larger, stained more extensively for proteoglycans, and expressed higher levels of mRNA for type II procollagen when prepared from cultures enriched for small and rapidly self-renewing cells than from cultures containing more mature and slowly replicating cells. The smaller and more rapidly proliferating cells can be produced using FGF-2 during monolayer culture of cMSCs[61]. FGF-2 preconditioning of cMSCs also improved their chondrogenic differentiation potential[61] in a similar fashion to that observed for human MSCs[85]. Therefore, it is worthwhile to consider the use of FGF-2 preconditioning for cMSC expansion, as well as the use of cMSCs at early passages, for cartilage regenerative

therapy.

IN VIVO STUDY OF CARTILAGE REGENERATION WITH cMSCs

While *in vitro* studies provide a substantial amount of information about the potential of MSCs for cartilage repair, evaluation of the *in vivo* behavior and function of these cells is also required. *In vivo* studies of cMSCs for cartilage regeneration have involved the use of surgically induced cartilage defect models, including partial-thickness cartilage defect models[62,85-87] (Figure 4A), full-thickness cartilage defect models[88,89] (Figure 4B) and cranial cruciate ligament transection models[90]. (Table 3) In every model, intraarticular autologous[86-88] or allogeneic[51,63,89] cMSC injection demonstrated excellent regenerative effects, including hyaline-like cartilage regeneration, when compared with control groups.

El-Tookhy *et al*[86] reported that earlier injections of BM-cMSCs (*i.e.*, one day after creating the chondral defect) resulted in hyaline cartilage regeneration in the defect area, whereas the therapeutic effect of a later injection (*i.e.*, one month after creating the chondral defect) was limited to fibro-hyaline cartilage regeneration[86]. Similar results were reported for AD-cMSCs[87]. These findings suggest that injured tissue may express specific receptors or ligands that promote the adhesion or infiltration of MSCs to the defect site in the early phase of injury and that these receptors or ligands may be down-regulated as time progresses after the injury.

Mokbel *et al*[87] showed the homing of cMSCs in dog stifle joints with a partial thickness chondral defect after intraarticular injection of autologous AD-cMSCs labelled with green fluorescent protein. They detected labelled cells in the neocartilage at 8 wk after the cMSC injection[87]. Koga *et al*[91] showed that human synovial MSCs attached to the chondral defect in knee joints of humans and rabbits after placement of an MSC suspension on the defect for 10 min, and the MSCs that attached to the chondral defect expressed several adhesion molecules[91]. Injected MSCs could also be detected in the neocartilage tissue at 8 wk after injection in a rabbit osteochondral defect model, however, no labeled MSCs could be detected at 12 wk[92]. Most of the MSCs injected into synovial joints migrate into the synovial membrane, in addition to the articular cartilage, in rat OA models[93] and in normal or OA horses[94]. After migration to the synovium, the MSCs maintain their MSC properties, without differentiating into another lineage, for at least 28 d. Meanwhile, they secrete several trophic factors, such as PRG-4, BMPs, and TSG-6, which are the key trophic factors for chondroprotection and immunosuppression[93]. Thus, in early phase of cartilage injury, intraarticularly injected MSCs may migrate into the chondral defect and regenerate neocartilage tissue, while most of the residual MSCs migrate into the synovium in the joints and produce trophic factors that provide chondroprotective and immunosuppressive effects. The immunomodulatory effects of cMSCs in dog joints have also been confirmed in several studies[51,90,95].

Some studies have demonstrated greater repair effects, determined by histological evaluation of chondral defects, for intraarticular injections of cMSCs in combination with either hyaluronic acid[63,88] or platelet-rich plasma[90] when compared with injection of cMSCs alone. Therefore, some synergistic effects of cMSCs are expected when used in combination with chondroprotective materials.

CLINICAL APPLICATIONS OF cMSCs FOR CARTILAGE INJURY AND OA

Conventional OA therapies in dogs include drug therapy, weight loss regimens, exercise programs, nutraceuticals, and supplements[96]. The objectives of these treatment methods are to minimize joint pain, reduce inflammation, and restrain the progression of the cartilage damage; however, they often do not provide complete pain relief[27,97,98]. MSCs are a promising therapeutic tool for OA as they can secrete trophic factors[93,99], recruit endogenous cells to the damaged lesion[100,101], and differentiate into chondrocytes[102], thereby ameliorating the cartilage injury. Several clinical studies have utilized cMSCs for OA in dogs, with most treating OA with AD-cMSCs (Table 4).

Dogs in actual conditions of OA can serve as vulnerable translational animal models for human medicine in terms of the use of MSCs. Each cMSC study has demonstrated improvement in OA symptoms after single intraarticular, intravenous, or acupuncture point injections of cMSCs. Some studies have injected cMSCs in

Table 3 *In vivo* study of canine mesenchymal stem cells for cartilage repair

Source	Injection / trans-plantation	Combination use	Cell number (million)	Model	Evaluation method	Ref.
Bone marrow	Single intraarticular, autologous	-	7-8	Partial thickness articular cartilage defect	Histology	El-Tookhy *et al*[86], 2008
Adipose tissue	Single intraarticular, autologous	-	7-8	Partial thickness articular cartilage defect	Morphology, histology, fluorescence analysis	Mokbel *et al*[87], 2011
Adipose tissue	Four times intraarticular, allogeneic	-	5 (three times), 66 (once)	Intact	Pain and lameness scoring, immunohistochemistry	Park *et al*[95], 2013
Bone marrow	Transplantation with scaffold, allogeneic	Scaffold	0.01	Full-thickness cartilage defect	Histology, immunohistochemistry, micro CT	Duan *et al*[89], 2013
Synovium	Single intraarticular, autologous	Hyaluronic acid	0.05, 5 or 50	Partial thickness articular cartilage defect	Histology	Miki *et al*[88], 2015
Adipose tissue	Single intraarticular, allogeneic	Platelet-rich plasma	10	Cranial cruciate ligament transection	Lameness scoring, focal compression strength, extracellular matrix composition, histopathology, real-time PCR	Yun *et al*[90], 2016
Bone marrow	Single intraarticular, allogeneic	Hyaluronic acid	10	Partial thickness articular cartilage defect	Gross appearance, magnetic resonance imaging, histology, immunohistochemistry	Li *et al*[63], 2018
Umbilical cord	Single intraarticular, allogeneic	-	1	Surgical manipulation of articular cartilage	Magnetic resonance imaging, radiography, ultrasonography, blood test, scanning electron microscope	Zhang *et al*[51], 2018

combination with platelet-rich plasma[103,104] or hyaluronic acid[105]. Improvements in pain, lameness, and range of motion were observed from 1 week[103,106] after the cMSC injection up to 180 d after cMSC injection alone[107] or up to 12 mo after cMSC injection in combination with hyaluronic acid[105]. However, one report showed a decrease in the effects of cMSCs between 30 and 90 d after the cMSC intraarticular injection[108]. Since the number of cells is known to decrease rapidly after administration of MSCs into the stifle joint[93], longer lasting effects of cMSCs might be obtained by periodic injections.

Most of the clinical studies have used subjective evaluation methods, including pain, lameness, range of motion, and functional disability scoring or owner questionnaires, while two studies provided objective evaluations with gait analysis using a force platform[104,108]. Assessing pain in an objective manner is important, especially in nonverbal patients, and gait analysis with a force platform is a valuable method that offers repeatable and quantitative measurements of ground reaction force from the feet of the dog[109]. However, the objective measures cannot provide information on some features, such as activity level, reluctance to run in dogs, and regeneration of damaged articular cartilage. Therefore, multi-aspect evaluation is needed for the measurement of cMSC effects. One study by Kriston-Pál *et al*[105] utilized arthroscopic and histological evaluation in addition to owners' questionnaires and demonstrated that the repaired articular cartilage in the cMSC-treated joint consisted of hyaline-like cartilage.

The immunomodulatory effect of BM-cMSCs was demonstrated by Muir *et al*[110] for dog stifles with cranial cruciate ligament rupture. Intraarticular and intravenous injection of autologous BM-cMSCs in dogs with partial rupture of the cranial cruciate ligament suppressed systemic and stifle joint inflammation, including C-reactive protein concentrations and circulating $CD8^+$ T lymphocyte number.

No serious adverse events have been reported in the clinical studies using intraarticular injection, acupuncture point injection, and intravenous injection of cMSCs. Nevertheless, Kang *et al*[111] indicated that the development of pulmonary

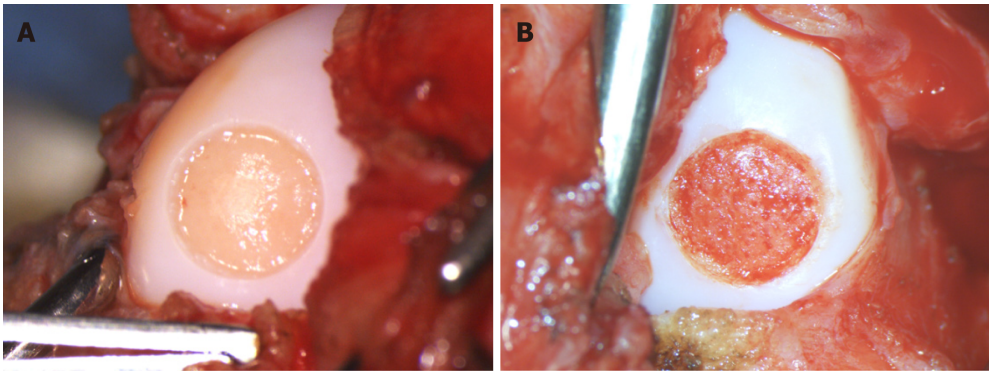

Figure 4 Cartilage defect models in dog stifle joints. A: A macroscopic image of a partial-thickness cartilage defect model in a dog stifle joint; B: A macroscopic image of a full-thickness cartilage defect model in a dog stifle joint.

edema and hemorrhage are possible adverse reactions following intravenous injections of cMSCs. Therefore, dogs treated with cMSC transplantation should be observed cautiously after injection.

DISCUSSION

This is the first review of cMSC isolation, characterization, differentiation, and use in *in vivo* models for cartilage repair and clinical trial for cartilage diseases. The cMSCs can be isolated from various tissues in the body, and the cMSCs derived from each tissue have specific characteristics. Although the cMSCs used in most studies at least partially meet the criteria for human MSCs declared by ISCT, suitable surface epitopes for cMSCs have not yet been established. cMSCs can differentiate into adipocytes, osteocytes, and chondrocytes *in vitro*; however, the differentiation methods differ among studies, and some studies have reported difficulties in inducing chondrogenic differentiation. Thus, suitable differentiation protocols for cMSCs should be identified to ensure sufficient differentiation in *in vitro* studies.

Dogs can serve as an experimental animal model for studies on cartilage repair, and cMSCs can lead to hyaline cartilage repair of experimentally created cartilage defects *in vivo*. Although dogs have a variety of unique characteristics compared to other experimental animals, they also share some problems, including body size and ethical concerns. For these reasons, farm animals, including pigs, sheep, and goats, can supersede dogs as experimental animal models for human medicine.

One distinctive feature of dogs is that many dogs naturally develop OA during their lives, which means that clinical trials can be performed. The results of clinical studies have proved the great cartilage regenerative and immunomodulatory effects of cMSCs in veterinary patient dogs with OA. Since the current therapeutic objective for OA treatment in both human and veterinary patients is management of pain and functional recovery of joints[112], the results of clinical studies in dogs can provide significant information for the treatment of cartilage lesions in both human and veterinary medicine. However, it should be noted that cell therapies in veterinary patients are not strictly supervised by regulatory agencies in most countries[113]. Regulations and guidelines for the use of MSCs in veterinary medicine are still urgently required. In addition, dogs cannot communicate the presence or degree of pain in diagnostic or therapeutic effect measurement phases, so caution is required in interpreting the results of studies using veterinary MSCs.

In stifle joints, meniscus damage is also known to induce and progress cartilaginous damage in dogs and humans[114-116], and the meniscal tear is one of the most prevalent injuries in the stifle joint[115,117]. Therefore, the effectiveness of cMSC treatments for meniscal injury as well as chondral lesions is expected to be elucidated in the future.

CONCLUSION

In recent years, expectations have increased regarding the use of MSCs in both human and veterinary medicine, and many *in vitro* or *in vivo* studies support the therapeutic effects of cMSCs for treatment of cartilage lesions and OA in dogs. Dogs can serve as clinical models as well as experimental animal models for human medicine, while

Table 4 Clinical study of canine mesenchymal stem cells for osteoarthritis in dogs

Source	Injection	Cell number (million)	Target joint	Observation period	Evaluation points	Ref.
Adipose tissue	Single intraarticular, autologous	4.2-5	Hip	30, 60, 90 d	Lameness, pain, range of motion, functional disability, owner questionnaire	Black *et al*[121], 2007
Adipose tissue	Single intraarticular, autologous	3-5	Elbow	30, 60, 90, 180 d	Lameness, pain, range of motion, functional disability, owner questionnaire	Black *et al*[107], 2008
Adipose tissue	Single intraarticular with platelet-rich plasma or hyaluronic acid, autologous	3-5	Elbow	1 wk, 1 mo	Not described	Guercio *et al*[103], 2012
Adipose tissue	Single intraarticular with platelet-rich plasma, autologous	Over 30	Hip	0, 30, 90, 180 d	Gait analysis	Vilar *et al*[104], 2013
Adipose tissue	Single intraarticular, autologous	30	Hip	1, 3, 6 mo	Pain, functional disability, range of motion, owner questionnaire	Cuervo *et al*[122], 2014
Adipose tissue	Single intraarticular, autologous	15	Hip	0, 30, 90, 180 d	Gait analysis	Vilar *et al*[108], 2014
Adipose tissue	Acupoint injection, allogeneic	0.2-0.8	Hip	7, 15, 30 d	Pain, functional disability, range of motion	Marx *et al*[106], 2014
Adipose tissue	Single intraarticular, allogeneic	12	Hip, elbow, stifle, shoulder	60 d	Pain, owner questionnaire	Harman *et al*[123], 2016
Bone marrow	Single intraarticular and single intravenous, autologous	5 for intraarticular, 2 for intravenous	Stifle	4, 8 wk	Circulating T lymphocyte, C reactive protein and cytokine concentration, total cell count in synovial fluid	Muir *et al*[110], 2016
Adipose tissue	Single intraarticular with hyaluronic acid, allogeneic	12	Elbow	6, 9, 12 mo	Owner questionnaire, arthroscopic image, histology	Kriston-Pál *et al*[105], 2017
Adipose tissue	Single intraarticular and/or single intravenous, allogeneic	Not described	Various joints	10 wk	Lameness, pain, range of motion, functional disability	Shah *et al*[124], 2018

they themselves, as veterinary patients, require effective treatment methods for cartilage lesions and OA. Thus, despite several limitations and problems, including ethical issues and difficulty of effect measurement, studies of MSCs in dogs have great significance from various points of view. Regulations and guidelines for cMSCs should be established in the future, and standardized methods for cMSC usage would provide more unified and reliable results from the studies. More data on cMSC characteristics and their use as an OA treatment in dogs will be needed, and they must be meaningful for the improvement of cartilage repair treatment in both human and veterinary medicine.

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