



## Current research and application of stem cells in the dog and cat

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**SUMMARY:** Stem cells (SCs) are multipotent cells with differentiation and proliferation capacities in many cell lineages. The majority of literature applications are about bone marrow derived stem cells (BMSCs) and adipose derived stem cells (ADSCs). Most clinical trials have been done for the treatment of musculoskeletal and neurological problems in canine patients. Hematopoietic SCs (HSCs), synovium (SDSCs) and cartilage (CSPCs) - derived SCs, umbilical cord blood-derived SCs (UCSCs), muscle, dental, cardiac and hepatic SCs have been used with promising results. Despite the overall progress crucial questions about SCs remain unanswered. It is still unclear if the regeneration mechanism of SCs owed to their differentiation into specific progenitor cells or due to their immunomodulatory and anti-inflammatory secretions. Also, there are questions about the best origin of stem cells, whether they should be delivered in situ or systemically, if they should be embedded into scaffolds or not and which is the suitable transplantation stem cells number. Many of the published studies have limitation regarding to sample size, blind randomization, control groups and homogeneity of population. In addition, the long-term efficacy and safety of MSCs need further evaluation. This review is an update in usage of SCs, mainly focused on BMSCs and ADSCs in small animals and its purpose is to present the late developments in this field. Also, the advantages, the disadvantages and the limitations of the current literature review are discussed.

**Keywords:** ADSCs, cats, dogs, MSCs, stem cells, transplantation.

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## INTRODUCTION

Stem cells (SCs) are multipotent cells with differentiation and proliferation capacities in many cell lineages. They produce factors with immunomodulatory and angiogenesis properties (Takemitsu et al., 2012). They divided into embryonic SCs (ESCs) and adult mesenchymal SCs (MSCs). The latter can be harvested by mesodermal, endodermal or ectodermal tissues (de Bakker et al., 2013). Depending on their origin they have advantages and limitations. For example, bone marrow - derived mesenchymal SCs (BMSCs) have the higher differentiation capacity in musculoskeletal cells, but their culture is time consuming (Fortier and Travis, 2011). Their osteogenic potential is better than adipose tissue - derived SCs (ADSCs) (Park et al., 2012), while the latter exhibit higher proliferation rate (Webb et al., 2011). Also, allogeneic versus autologous transplantation is still debatable (Zhang et al., 2015). In vitro studies proved that allogeneic transplantation is safe (Ryan et al., 2005), due to a protective mechanism against T and B cells that forms a safe micro-environment (Ryan et al., 2005). Moreover, xenogeneic SCs transplantation was also presented. Human SCs promoted healing in dogs with bone defects (Cruz et al., 2015, Zang et al., 2016), dermatological problems (Ferrer et al., 2015) and neurological diseases (Lee et al., 2009, Chung et al., 2013).

SCs usage in tissue engineering is a progressive technique that continues to expand increasingly in the veterinary world. Many scientific papers have been published over the last 10 years (Arinze et al., 2003, Yan et al. 2007, Cui et al. 2007, Wang et al. 2014, Nantavisai et al., 2019, Voga et al. 2020).

## ADVANCES IN STEM CELLS

### Adipose tissue - derived stem cells (ADSCs)

#### Dogs

##### *Musculoskeletal applications*

In 2007, Cui et al. (2007) showed that autologous ADSCs enhanced bone regeneration in critical sized bone defects in canine models. Same conclusions retrieved from trials on mandibular (Haghighat et al., 2011) and long bone defects (Bigham-Sadegh et al., 2012). In 2013, Lui et al. repeated the same model with allogeneic ADSCs and presented similar results without adverse reactions (Liu et al., 2013).

The application of autologous ADSCs in coxofemoral (Black et al., 2007) and humeroradial joints with osteoarthritis (Black et al., 2008, Guercio et al., 2012) resulted in clinical improvement. Another study reinforced these results with kinetic force assessments (Vilar et al., 2014). Equally effective was the application of allogeneic ADSCs (Harman et al., 2016). Moreover, the combination of autologous ADSCs with platelet-rich plasma (PRP) (Vilar et al., 2013, Cuervo et al., 2016, Yun et al., 2016) reduced pain in cases of osteoarthritis, but the studies lacked of control groups.

In 2014, an innovative administration of allogeneic ADSCs for hip dysplasia, in acupuncture points, showed clinical improvement (Marx et al., 2014). Also, promising was the administration of allogeneic ADSCs in elbow dysplasia (Kriston-Pal et al., 2017). Nevertheless, these studies did not present arthroscopic findings. Recently, another research demonstrated that the local injection of ADSCs was superior compared to intravenous for degenerative arthritis (Shah et al., 2018). Unfortunately, the outcomes were based only on clinical evaluation.

ADSCs combined with PRP accelerated the reconstruction of partial ruptured anterior cruciate ligament (Canapp et al., 2016a). In contrast, the use of ADSCs during tibial tuberosity advancement procedure did not diminish the healing time (Dos Santos et al., 2018). The results about ADSCs advantages were controversial, but both studies had heterogeneous populations and different rehabilitation protocols. Furthermore, preliminary results proposed the combination of ADSCs with PRP for refractory supraspinatus tendinopathy (Canapp et al. 2016b), semitendinosus myopathy (Gibson et al., 2017) and tracheal cartilage defects (Hashemibeni et al., 2012). Lastly, the combination of ADSCs with growth factor showed promising results in a canine tendon injury model (Sheh et al., 2018).

##### *Neurological applications*

ADSCs were used for spinal cord injury (SCI) (Roszek et al., 2016). Canine models investigated ADSCs capacities in situ (Ryu et al., 2009). Interestingly, ADSCs promoted functional improvement after SCI (Park et al., 2012) and accelerated disk regeneration after disk degeneration disease (DDD) (Ganey et al., 2009). However, the results were based on experimental conditions. In clinical field, two studies described the benefits of ADSCs transplantation in dogs with thoracolumbar DDD (Kim et al., 2016)

and peripheral (facial) nerve trauma (Ghoreishian et al., 2013). Both confirmed clinical improvement, but lacked of histopathological confirmation.

#### *Dentistry and ophthalmology applications*

ADSCs were used successfully in periodontal trauma (Tobita et al., 2013), atonic oral ulcers (Alamoudi et al., 2014) and as an osseointegration factor for dental implants (Bressan et al., 2015). To the authors' knowledge, no clinical studies are currently available. After the proof of ADSCs safety inperiocular (Wood et al., 2012) and intra-lacrimal (Park et al., 2013) injections, interest was concentrated in ADSCs usage against keratoconjunctivitis sicca (KCS) (Villatoro et al., 2015). Peri- and intra-lacrimal ADSCs transplantation led to KCS remission (Bittencourt et al., 2016). However, possible adverse reactions were not studied.

#### *Other applications*

The *in vitro* differentiation of ADSCs into cells similar to hepatocytes (Banas et al. 2007) triggered further studies. In one preclinical study, ADSCs decreased hepatic parameters after liver trauma (Teshima et al., 2017), and increased survival time in one dog with hepatocutaneous syndrome (Nam et al., 2017). Also, ADSCs were employed for dogs with inflammatory bowel disease (Perez-Merino et al., 2015). The conclusions are suggestive, as these studies lacked of long term efficacy and safety data. Despite the fact that ADSCs improve the cardiac function after myocardial infarction, clinical trials failed to prove their benefits in dilated cardiomyopathy (Pogue et al., 2013). A current comparison of ADSCs and BMSCs intrarenal injection in a canine renal injury model revealed better level of protection for ADCSs (Osman et al. 2020). Lastly, ADSCs application was beneficial for one dog with an atonic ulcer (Han et al., 2015) and another with pemphigus (Zubin et al., 2014), but was not helpful for allergies (Hall et al., 2010).

#### Cats

One pilot study showed improvement in renal function in cats with chronic kidney failure after intrarenal administration of autologous ADSCs (Quimby et al., 2011). However, the intravenous administration of allogeneic ADSCs was associated with adverse effects (Quimby et al., 2013). Further research in order to attempt to minimize these reactions failed (Quimby et al., 2016). The intravenous injection of ADSCs in

cats with inflammatory bowel disease led to (Webb and Webb, 2015) signs remission. Also, the intraperitoneal injection of ADSCs was shown to be safe and possibly effective for various feline diseases (Parys et al., 2016). ADSCs were employed successfully in autoimmune diseases like gingivostomatitis (Arzi et al., 2016), asthma (Trzil et al., 2015) and eosinophilic keratitis (Villatoro et al., 2018). Nevertheless, larger sample sizes are needed to obtain more reliable results.

### **Bone marrow-derived mesenchymal stem cells (BMSCs)**

#### Dogs

##### *Musculoskeletal applications*

The implantation of autologous BMSCs embedded into various scaffolds, encouraged bone formation in alveolar (Kim et al., 2009), mandible (Hu et al., 2014), orbital (Yang et al., 2014) and long bone defects (Ozdal-Kurt et al., 2014). Also, the combination of cell sheet and scaffolds resulted in healing in mandible injuries (Shan and Hu, 2017). All the macroscopic results were co-evaluated with imaging and histological findings. Despite the fact that these were control studies and their results were significant different between groups, their sample sizes were limited. Few experimental works studied the importance of scaffolds and the migration of systemically administered BMSCs. The intra-osseous injection of allogeneic BMSCs into femurs promoted bone regeneration in mandible defects (Liu et al., 2014). Similar were the results of intra-arterial infusion of autologous BMSCs in dogs with femoral head necrosis (Jin et al., 2016). Both studies concluded that the technique was not only promising for bone density problems, but it was also safe. Moreover, genetic modification of BMSCs enhanced specific capacities. The induced expression of vascular endothelial growth factor 165 (VEGF<sub>165</sub>) (Hang et al., 2012) or bone morphogenetic protein -2 (BMP-2) (Peng and Wang, 2017) stimulated the quantity and quality of new formed bone in femoral head osteonecrosis. These suggestions resulted from control-based studies with big sample sizes. Additionally, smaller studies concluded that microRNA-31 and dentin matrix protein-1 (DMP-1) manipulated BMSCs enhanced bone formation with superior features compared to simple BMSCs (Deng et al., 2014, Liu et al., 2016). BMSCs have also been

used for cartilage repair (Bornes et al., 2014). In 2005, Wayne et al. proposed that the injection of BMSCs in canine joints with articular defects promoted superior quality of new formed cartilage. Concurrently, the outcomes from intralesional injection of bone marrow into traumatized meniscus, implied healing properties of BMSCs (Abdel-Hamid et al., 2005). Equivalent findings concluded from the intravenous and intrarticular injection of BMSCs, in dogs with partial cruciate ligament rupture, despite the limited sample and volume of synovial fluid available for tests (Muir et al., 2016). Following studies focused on PRP combination with BMSCs, because it improved their proliferation rate (Chen et al., 2014) and chondrogenetic capacity (Kazemi et al., 2017). A large retrospective study suggested that their injection in dogs with supraspinatus tendinopathy resulted in clinical and ultrasonography improvement (McDougall et al., 2018). However, there are no immunohistochemical assessments of these findings. Lastly, BMSCs were used successfully in tendon repair not only in vitro (Ozasa et al., 2014) but also in vivo (Case et al. 2013) and esophagus reconstruction (Tan et al., 2013). Nevertheless, the data were preliminary.

#### *Neurological applications*

BMSCs were also helpful for neurological disorders (Kamishina et al., 2006). In 2008, Hiyama et al. (2008) investigated the intralesional autologous BMSCs injection in a canine model of DDD. The histological and imaging analysis showed alteration of the micro-environment and inflammation's inhibition (Hiyama et al., 2008). However, this was an experimental model. Therefore, scientists investigated the safety of the procedure in clinical cases of DDD. Their results were controversial. One study presented improvement of nociception and proprioception after autologous BMSCs transplantation (Besalti et al., 2015). Another study concluded that the procedure may be safe, but not useful (Steffen et al., 2017). Both reports failed to make a statement due to limited samples and heterogenous populations. Furthermore, clinical results highlighted BMSCs efficacy after decompression surgery, in SCI (Nishida et al., 2012, Besaltiet al., 2016) even in chronic cases (Nishida et al., 2011). The improvement was similar after autologous or allogeneic BMSCs implantation (Jung et al., 2009, Sarmiento et al., 2014). Concurrently investigation about the most suitable time for BMSCs application (Penha et al., 2014) and the number of implanted cells (Serigano et al., 2010) was made. These

parameters affected the outcomes, but no protocols were proposed. Recently, Wu et al. (2018) created a canine model with complete transection of spinal cord and presented that BMSCs could be beneficial even for devastating cases. Moreover, autologous BMSCs combined with scaffolds were used successfully in sciatic (Ding et al., 2010) and ulnar nerve defects (Kaizawa et al., 2016). Degeneration was accelerated functionally and histologically. Lastly, BMSCs were proposed for cases of autoimmune meningoencephalomyelitis. Although the diagnosis was made by clinical examination, there was noted signs remission (Zeira et al., 2015).

#### *Dentistry and ophthalmology applications*

One report studied autologous BMSCs implantation into alveolar clefts combined with scaffolds, PRP and bone grafts (Yuanzhneng et al., 2015). The combination of BMSCs with PRP showed the best results. The findings were equivalent to studies that support PRP's proliferation properties in BMSCs (Chen et al., 2014, Kazemi et al., 2017, McDougall et al. 2018). Another study proposed autologous, alveolar BMSCs as a different origin of BMSCs, due to their access and osteogenic potentials (Wang et al., 2018). Nevertheless, the conclusions were preliminary. Additionally, large oral (Aly et al., 2014), vocal fold (Kanemaru et al., 2003) and laryngeal ulcers (Irvani et al., 2017) were favoured by the intra-lesional administration of autologous BMSCs. These results were supported by histological findings, but more research is needed. In veterinary ophthalmology experimental models of corneal ulcer (Tognoli et al., 2008) and retinal degeneration (Tracy et al., 2016), showed that autologous BMSCs were safe and anti-inflammatory.

#### *Other applications*

Canine models of cirrhosis (Matsuda et al., 2017), renal trauma (Lim et al., 2016) and chronic enteritis (Xu et al., 2016) were used to investigate other effects. Autologous BMSCs improved function in liver failure, inhibited fibrosis in renal injuries and diminished clinical signs in post radiation enteritis (Xu et al., 2016). Also, the usage of allogeneic BMSCs promoted healing in cutaneous wounds without adverse effects (Kim et al., 2013). Furthermore, Memon et al. (2005) presented that the intra-lesional injection of BMSCs accelerated regeneration in ischemic myocardium. Also, their intracoronary injection reduced infarctions' areas, confirmed by histology and immunohistochemistry analysis (Hao et al., 2015). In the same

model, the adjunct of basic fibroblast growth factor augmented the engraftment and the differentiation of BMSCs (Wang et al., 2015). Undoubtedly, the previous studies had preliminary value and more research should be done in dosage, time points and administration routes.

### Cats

Feline BMSCs morphology and isolation are similar to human and rodents, (Martin et al., 2002, Zhang et al., 2011, Munoz et al., 2012) with higher neurogenic properties (Zhang et al., 2011). Also, c-kit<sup>+</sup> feline BMSCs differentiated into cardiac myocytes with cardiac action potentials (Kubo et al., 2009). However, feline BMSCs had culture limitations referred to the donor health status (Quimby et al., 2011). In vivo applications are few. A randomized clinical trial failed to demonstrate significant effect of BMSCs in acute kidney injury. Also, it left unanswered questions about the ideal routes, doses and frequency of BMSCs administration (Rosselli et al., 2016). Additionally, a case report of a cat with lumbar fracture that gained clinical rehabilitation after intraoperative BMSCs transplantation reinforced their beneficial effects in SCI (Penha et al., 2012).

## Different tissue derived mesenchymal stem cells

### *Synovium (SDSCs) and cartilage (CSPCs) - derived stem cells*

SDSCs showed osteogenic capacity similar to BMSCs and rapid proliferation like ADSCs (Bearden et al., 2017). As referred to chondrogenic differentiation, SDSCs were found to be superior to ADSCs and BMSCs (Sasaki et al., 2018). In fact, the intra-articular injection of autologous SDSCs combined with hyaluronic acid in a canine model for cartilage repair, resulted in better macroscopic and histological scores compared to the control group (Miki et al., 2015). However, the studies lacked of statistical analysis. Until now, only autologous CSPCs harvested from ear cartilage showed capacity to regenerate both elastic and hyaline chondral tissue (Mizuno et al., 2014).

### *Muscle stem cells*

Satellite cells become activated after trauma, as muscle precursor cells (MPCs). Satellite cells have high myogenic capacities, but they are no good candidates for transplantation, because of harvesting, survival and migration problems. However, their good

expanding and long term survival in vivo (Eberli et al., 2012) promoted their usage for functional regulation of urinary sphincter (Eberli et al., 2012) and re-innervated thyroarytenoid muscle (Paniello et al., 2018) in canine models. Histological analysis of both studies revealed the formation of new innervated muscle fibres. Muscle-derived stem cells (MDSCs) can be differentiated not only in myogenic, but also in other lineages, such as osteogenic, chondrogenic and tenocyte-like cells. In fact, MDSCs were superior compared to BMSCs for tendon repair (Ozasa et al., 2014). Nevertheless, these findings were concluded in vitro. The majority of studies investigated MDSCs efficacy in dystrophic models. In 2011, a study proposed the intra-arterial administration of allogeneic MDSCs in dystrophic dogs to promote muscle regeneration (Rouger et al., 2011). Despite the limitation of small sample size, clinical and histopathological outcomes were favourable. Additionally, MDSCs were studied in molecular lever (Robriquet et al., 2015, Lardenois et al., 2016). Furthermore, a trial to make their usage easier showed that transient immunosuppression of the hosts was sufficient (Lorant et al., 2018). Undoubtedly, more research is needed about the immunology features of MSDSs.

### *Cardiac derived stem cells (CSCs)*

Despite the fact that the majority of cardiac cells have not potentials to further differentiation, there is a population of CSCs with capacities to form myocytes and promote angiogenesis. In contrast to MSDSs, which could differentiate into muscle cells when injected into the myocardium (Yoon et al., 1995), CSCs could enhance both cardiac regeneration and function by producing functional cardiac cells (Welt et al., 2013, Taghavi et al., 2015). Autologous CSCs helped cardiac remodelling in infraction canine model (Welt et al., 2013) and improved cardiac function in feline cardiomyopathy model (Taghavi et al., 2015). However, in both studies the delivery method was invasive and new methods should be tried in order to make CSCs usage a possible clinical approach.

### *Hepatic stem cells*

Hepatic progenitor cells (HPCs) differentiated into hepatocytes and cholangiocytes (Kruitwagen et al., 2014). The activation, reaction and identification of HPCs were evaluated in normal dogs (Ijzer et al., 2010) and cats (Ijzer et al., 2009). Also, HPCs were assessed in dogs with various liver diseases, such as acute and chronic hepatitis, cooper toxicosis, cancer

or biliary problems (Ijzer et al., 2010, Kruitwagen et al. 2019) and in cats with feline cholangitis (Otte et al., 2018) and lipidosis (Valtolina et al., 2018). Despite the fact that HPCs could be an alternative to liver transplant, which is not feasible in veterinary medicine, no current trials of HPCs usage have been reported.

#### *Dental pulp (DSCs), skin and hair follicle (HFSCs) stem cells*

DSCs have high proliferation rate, easy accessibility and differentiation potentials into many cell lineage (Ashiry et al., 2018). Autologous DSCs were used successfully in canine models for pulp regeneration (Nakashima and Iohara, 2014, Chen et al., 2015, Ashiry et al., 2018) and periodontal healing (Khor-sand et al., 2013). Also, allogeneic DSCs application was efficacious for periodontitis management (Iohara et al., 2018). Moreover, allogeneic DSCs were used in degenerative heart disease (Petchdee and Sompeewong, 2016) and xenogeneic (human) DSCs in SCI in dogs (Feitosa et al., 2017). Despite the promising results, both studies lacked histological and imaging assessments. Also, periodontal stem cells (PDLSCs) were isolated as another tool for periodontitis therapy, but the studies were in vitro (Khoshhal et al., 2017). To the authors' knowledge skin and hair follicle stem cells were identified and characterized in dogs (Brachelente et al., 2013), but they have not been used in vivo.

#### *Umbilical cord blood-derived stem cells (UCSCs)*

UCSCs exhibit less immunogenicity, higher plasticity and production of cells per volume compared to MSCs (Jin et al., 2008). UCSCs were used mainly in SCI models (Lim et al., 2007, Park et al. 2011, Ryu et al. 2012) in order to promote clinical improvement and regulate inflammation (Park et al., 2011). However, histopathology and imaging evidence is needed (Lim et al., 2007, Ryu et al., 2012).

#### *Hematopoietic stem cells (HSCs)*

HSCs originate from bone marrow, umbilical cord and peripheral blood. HSCs form lymphoid and myeloid progenitor cells (Gomes et al., 2017). In 1962 and 1974, bone marrow transplantation in dogs implied its benefits (Thomas et al., 1962, Epstein et al., 1967, Dale and Graw, 1974). Later, studies used autologous hematopoietic stem cells transplantation (HSCT), against auto-immune diseases (Stolfi et al., 2016). Also, allogeneic HSCT was employed in the

modification of host-versus-graft reaction (Zorn et al., 2011, Mathes et al., 2014, Vrecenak et al., 2014, Rosinski et al., 2015). Interestingly, allogeneic HSCT improved hosts' tolerance towards skin allografts, by T-cells regulation (Mathes et al., 2014). The first clinical case of allogeneic HSCT was completed in a dog with lymphoma (Lupu et al., 2006). Then, two studies tried autologous HSCT for lymphoma (Escobar et al., 2012, Willcox et al., 2012) and acute leukemia (Suter et al., 2015). All agreed that HSCT increased the duration of signs remission. However, according to Schaefer et al. (2016) allogeneic HSCT had problems related to HSCs ability to reach bone marrow without being trapped in other tissues. Lange et al. (2017) tried to identify the best route of administration by comparing the intravenous and intraosseous HSCT. However, the study failed to prove superiority of one method over the other. Few are known about feline HSCs, which have similar proliferative capacities to canine (Abkowitz et al., 1993, Abkowitz et al., 1995). Feline HSCs were used after genetical manipulation for inhibition of coronavirus replication and development of feline infectious peritonitis (Anis et al., 2017).

## CONCLUSIONS

In conclusion, literature review cannot still answer questions regarding the best origin of SC, the most suitable route of administration, whether they should be embedded into scaffolds or not as well as which is the ideal number of transplanted cells. Also, it remains unanswered if the therapeutic potentials of SCs exist due to their differentiation into progenitor cells or due to their immunomodulatory secretions. Many experimental models are used for preliminary data, while the clinical use of SCs is in early stages. Most of these studies focused on the usage of BMSCs and ADSCs, because of their easy collection and culture, their sufficient differentiation capacity in musculoskeletal cells and high proliferation rate. However published data have limitations because of their small sample size, heterogeneity of population, lack of blind randomization and control groups. Therefore, a future research plan should involve methods and techniques capable to collect answers for all these questions.

## CONFLICT OF INTEREST

None declared.

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