Journal of Biosciences and Medicines, 2023, 11, 181-189
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Mesenchymal Stem Cell Allograft Improved Pain Management in Dogs with Osteoarthritis

Jonathan R. T. Lakey[1,2*], Wenyi Guo[3], Michael Alexander[1], Mike K. S. Chan[4,5], Michelle B. F. Wong[4,5], Todd Scott[6]

[1]Department of Surgery, University of California Irvine, Irvine, USA
[2]Department of Biomedical Engineering, University of California Irvine, Irvine, USA
[3]Department of Pancreatic Surgery, General Surgery, Qilu Hospital of Shandong University, Jinan, China
[4]European Wellness Biomedical Group, Edenkoben, Germany
[5]Baden R&D Laboratories GmbH, Sabine Conrad Ferdinand-Laselle-Strasse, Edenkoben, Germany
[6]Crestwood Veterinary Clinic, Edmonton, Canada
Email: *jlakey@uci.edu

How to cite this paper: Lakey, J.R.T., Guo, W.Y., Alexander, M., Chan, M.K.S., Wong, M.B.F. and Scott, T. (2023) Mesenchymal Stem Cell Allograft Improved Pain Management in Dogs with Osteoarthritis. *Journal of Biosciences and Medicines*, **11**, 181-189.
https://doi.org/10.4236/jbm.2023.1111016

Received: October 18, 2023
Accepted: November 17, 2023
Published: November 20, 2023

Abstract

Background: Osteoarthritis is one of the most common bone diseases, triggered by bone destruction stemming from the inflammatory response of chondrocytes. The disease progresses slowly, but halting its progression or finding a cure remains elusive. The treatment of pain associated with osteoarthritis has yielded unsatisfactory results. In recent years, mesenchymal stem cells (MSCs) have emerged as a potential avenue for addressing the condition. In this study, we used MSCs to treat companion dogs with osteoarthritis. **Methods:** For this study, 26 animals were included in this study to assess the pain and mobility one month after treatment. The pain scores were obtained from owners using a questionnaire based on the Helsinki Chronic Pain Index, and the Liverpool Osteoarthritis in Dogs (LOAD) Owner questionnaire to assess the mobility of the dogs from stem cell infusion. **Results:** Questionnaires were administered to dog owners before and one month after treatment, and we found that dogs treated with MSCS experienced an 81.2% ± 6.8% reduction in pain and a 77.9% ± 10.1% increase in mobility, whereas most of the dogs in the untreated control group experienced disease progression. **Conclusions:** The transplantation of stem cells into companion pets is a promising and expanding opportunity for pet owners with aging and arthritic dogs. MSCs may play an important role in the treatment of OA without complications in companion pets.

Keywords

Stem Cell Therapy, Canine, Pain, Osteoarthritis, Mesenchymal Stem Cell

1. Background

Osteoarthritis is the most common degenerative joint disease characterized by articular cartilage damage, particularly affecting the hands and knees [1]. Its occurrence is age-related and is the main cause of disability in the elderly [2]. Obesity and joint overuse are important factors leading to the degeneration of articular cartilage [3]. Its incidence will continue to increase with the aging of the population and the increase in the proportion of the obese population [4]. The pathogenesis of osteoarthritis is complicated. During the pathogenesis, it is accompanied by the degeneration of articular cartilage and the formation of osteophytes. The resulting discomfort and pain severely affect the quality of life for patients. The current treatment of this disease includes early and mid-term treatments such as exercise, weight loss, anti-inflammatory and analgesic drugs, and finally joint replacement. These treatments have limitations, and there is no effective method to prevent the occurrence and progression of osteoarthritis [5].

In recent years, stem cell therapy has been applied to the treatment of osteoarthritis. Due to the characteristics of easy acquisition and rapid proliferation of stem cells, the method of using stem cells to replace diseased tissues has been widely studied, such as degenerative diseases, tumors, trauma, etc., which is called regenerative therapy [6]. Mesenchymal stem cells (MSCs) are an important part of stem cell regeneration therapy, which have been used in clinical trials of various diseases such as ulcerative colitis, diabetes and its complications, and liver cirrhosis. In addition, MSCs have been clinically used in diseases such as Crohn's disease [7]. In recent years, studies have shown that MSCs can be used for the treatment of osteoarthritis. The role of MSC in the treatment of osteoarthritis has been discovered for more than ten years. As early as 2003, after Murphy *et al.* induced goat knee OA, they injected MSCs into the knee joints and found that articular cartilage degeneration, osteophyte remodeling and subchondral sclerosis were reduced in the cell-treated joints [8]. Numerous studies and clinical trials since then have shown the great potential of MSC in the treatment of osteoarthritis. This study aims to evaluate whether such benefits can be reproduced in dogs, who as companion pets are suffering from similar diseases. In this study reported clinical data of using allograft MSCs to treat dogs with osteoarthritis.

2. Methods

2.1. Animals

Companion pet dogs (including females and males) with osteoarthritis, aged seven years and older, were enrolled in the study after the owners signed a consent form. Prior to the study, consulting veterinarians performed a standard physical exam. The diagnosis of osteoarthritis was based on veterinarians' examination. Standard blood work was collected and added to the study file at the veterinarian offices. The dogs were offered the treatment of MSC cells: For this particular analysis, the

treatment group (n = 10) that received the intra-articular injections of the stem cell product. A group of pet owners who declined the MSC infusion, untreated control group (n = 6) that received standard non-steroidal anti-inflammatory drugs (NSAIDs) treatment. The type and dosage of NSAIDs given to the control animals were determined individually by licensed veterinarians as part of their regular practice. Dogs with active infections were excluded from this study as well as those pet owners who choose not to be involved in this study and subsequent follow up. The study protocol was reviewed and approved by the institutional animal care and use committee (IACUC).

2.2. Preparation and Culture of Stem Cells

For this study, mesenchymal stem cells (MSCs) were derived from commercially available canine bone marrow and cultured in humidified 37˚C tissue culture incubators in canine MSC growth medium containing 10% fetal bovine serum (Corning 35-010-CV) and 1% antibiotic-antimycotic (Corning 30-004-CI). After a period of tissue culture, tissue culture flasks containing the expanding MSC were divided and subdivided into new tissue culture flasks. For subculturing, MSCs were removed from the flask by trypsin-EDTA (Gibco 15-400-C4) and neutralized by complete media. MSC passages 3-7 were used for subsequent experiment. For cell freezing, MSCs were removed from the flask and the media was replaced by DMSO based BamBanker freezing media (Bulldog Bio BB-01), aliquoted at 5×10^5 cells/vial, and frozen in liquid nitrogen. Vials were labelled with specific lot number, date and identification as Canine MSC cells. Lots were collected and quarantined and an aliquot was tested and maintained until the results were collected, analyzed and reviewed by our quality control individual. Approved lots were released from quarantine and place in the release. One vial from each lot was retained as a long term archived sample and placed in low temperature storage. Frozen MSC aliquots were shipped on dry ice to the vet office where they were stored in a low temperature liquid nitrogen dewar. The storage dewar was monitored daily and filled with liquid nitrogen weekly to ensure the samples remained frozen during the storage interval.

2.3. Treatment Protocol

Vials of MSC cells were collected from the storage dewar, Lot # recorded and the vial was rapidly thawed in a 37˚C sterile water bath. The vial was sprayed with 70% ethanol and the vial opened with the contents drawn into a 3 cc sterile syringe with a 18 gauge needle. The companion dogs were treated with 1 ml suspension product (5×10^5 cells/mL) via intra articular infusion after the site was shaved and cleaned with aseptic spray. Animals were monitored post implantation for a period of 2 - 3 hours post infusion before being released back to their owners. Pets were followed up at 1 month and 3 months after injection. Below parameters were measured at baseline and each follow-up visit: 1) Physical Exam by veterinarian with routine blood work. 2) Owner-reported pain and activity

from questionnaire. 3) Imaging evaluations by MRI and/or X-ray. 4) Specific blood levels of serum inflammatory markers, such as C-reactive protein and erythrocyte sedimentation rate.

2.4. Assessment of Pain and Mobility

The dogs returned to their homes after their participation in the study. At one month post stem cell injection, the owners were asked to evaluate the degree of pain and mobility of their dogs through well-established questionnaire. Out of the dogs treated with MSCs, 10 were assessed using the Helsinki Chronic Pain Index (**Figure 1**) and using the Liverpool Osteoarthritis in Dogs (LOAD) Owner questionnaire (**Figure 2**). Control groups were also evaluated by Helsinki Chronic Pain Index and LOAD index after one month of initial vet visit. The dogs continued to live with their owners and received ongoing care in their familiar environment as part of regular pet visits as per their owners.

2.5. Statistical Analysis

Quantitative values were presented as mean ± standard deviation. Statistical analysis was conducted using SPSS Statistics software. A t-test was used to compare differences between the treatment and the control groups at specific time points. For categorical data, the Chi-Square test was employed to assess differences between groups. A value of $p < 0.05$ was considered statistically significant.

Dog's Name: Date:

Helsinki Chronic Pain Index

Hielm-Bjorkman HK, Rita H, Tulamo R-M. Psychometric testing of the Helsinki chronic pain index by completion of a questionnaire in Finnish by owners of dogs with chronic signs of pain caused by osteoarthritis. Am J Vet Res. 70: 727 – 734, 2009.

(As translated from Finnish to English)

Circle the pain and function description that best represents your dog's behaviour:

Rate your dog's attitude and/or mood:

0	1	2	3	4
Very alert	Alert	Neither alert nor disinterested	Disinterested	Very disinterested/ lethargic

Rate your dog's willingness to participate in play or interact:

0	1	2	3	4
Very willing	Willing	Reluctant	Very reluctant	Does not participate or interact at all

Rate your dog's frequency in vocalization or discomfort behaviour (audible whining, grunting, yelping, or unusual licking):

0	1	2	3	4
Never	Hardly ever	Sometimes	Often	Very often

Rate your dog's eagerness to walk:

0	1	2	3	4
Very eager	Eager	Reluctant	Very reluctant	Does not want to walk at all

Rate your dog's ability and/or willingness to walk up and/or down stairs:

0	1	2	3	4
Very willing/able	Willing/able	Reluctant	Very reluctant	Does not do stairs at all

Dog's Name: Date:

Helsinki Chronic Pain Index con't

Rate your dog's ability and/or willingness to run:

0	1	2	3	4
Very willing/able	Willing/able	Reluctant	Very reluctant	Does not run at all

Rate your dog's ability and/or willingness to jump (onto bed, couch, vehicle, etc):

0	1	2	3	4
Very willing/able	Willing/able	Reluctant	Very reluctant	Does not jump at all

Rate your dog's ease in lying down:

0	1	2	3	4
Very easy	Easy	Neither easy nor difficult	Difficult	Very difficult

Rate your dog's rising from a down position:

0	1	2	3	4
Very easy	Easy	Neither easy nor difficult	Difficult	Very difficult

Rate your dog's ease of movement after a long rest:

0	1	2	3	4
Very easy	Easy	Neither easy nor difficult	Difficult	Very difficult

Rate your dog's ease of movement during and/or after exercise/walks (tired, dragging feet, scuffing nails, lying down):

0	1	2	3	4
Very easy	Easy	Neither easy nor difficult	Difficult	Very difficult

Figure 1. Helsinki chronic pain index.

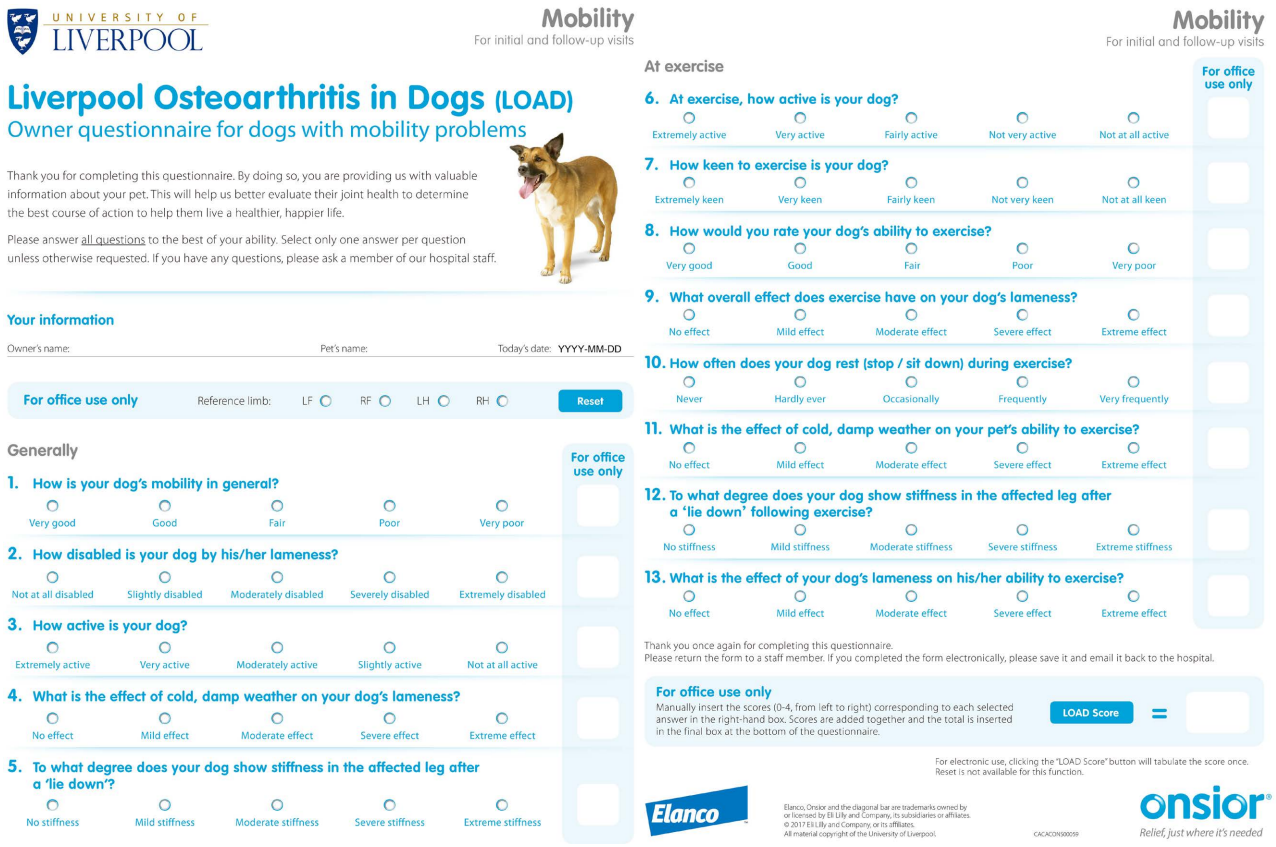

Figure 2. Liverpool osteoarthritis in dogs (LOAD).

3. Results

The MSCs products were manufactured in bulk and labelled with lot # and manufacturing date. Samples from each lot was assessed for quality control parameters (Table 1) and shipped in dry ice (−140˚C) under strict quality assurance regulations and procedures; no aerobic, anaerobic, or fungal contamination was detected.

After MSC treatment, dogs exhibited an 81.2% ± 6.8% decrease in Helsinki Chronic Pain Index questionnaire scores. LOAD Owner questionnaire showed that their mobility increased by 77.9% ± 10.1%. However, the mobility of 6 untreated control dogs without MSCs treatment decreased by 6.2% ± 7.8% (Table 2). There were significant differences between the MSCs-treated group and control group ($p < 0.001$).

All dogs tolerate the MSCs injection well. There were no adverse reactions found in the MSCs treated dogs during the treatment or during follow-up. There were no acute or chronic complications or issues following the injections.

4. Discussion

Chondrocytes are considered difficult to regenerate, and none of the existing treatments can alter the outcome of osteoarthritis [9]. Clinical investigators have encountered numerous challenges in treating osteoarthritis, prompting the

search for potential cures. Stem cell regeneration therapy has been extensively explored in various diseases and is thus regarded as a promising avenue for addressing osteoarthritis. This method holds the potential to facilitate the repair of degenerated chondrocytes and promote the generation of new chondrocytes [10]. Previous studies have demonstrated that MSCs and their derived exosomes may expedite meniscus regeneration, while also accelerating cartilage formation. Furthermore, their presence can hinder cartilage degradation and suppress osteoclast activity through paracrine signaling rather than direct differentiation [11] [12] [13] [14]. Similarly, our study showed positive results in safety and efficacy of MSCs therapy in pet dogs with osteoarthritis.

Table 1. Sample quality control reports generated per shipment of MSCs.

Canine MSC Lot #	M062017W4
Release Date	1/30/2023
MSC origin and lot #	M062017W4
Antibiotic/Antifungal	0.5% Pen/Strep, 0.5% Amphotericin B
Passage #	3
Culture condition	37˚C, 5% CO_2
Date frozen (−80C and LN2)	12/2022
Cell Concentration	5×10^5 cells/mL
Release amount	20 vials, 1 mL/vial
Shipping Temp	Shipped on Dry ice

	48 hr	**5 days**
Aerobic	No growth	No growth
Anaerobic	No growth	No growth
Fungal	No growth	No growth

Endotoxin	<5 EU/mL	0.8 EU/mL
Gram Stain	Negative	Pass

Table 2. Chronic pain and osteoarthritis scores. MSC-treated dogs with osteoarthritis were evaluated before and after 1 month of treatment. Dogs with osteoarthritis receiving no MSC treatment were used as control, and evaluated for osteoarthritis score 1 month apart.

MSC-Treated Dogs					
Helsinki Chronic Pain Index			**Liverpool Osteoarthritis in Dog (LOAD)**		
Pre	Post	% Change	Pre	Post	% Change
40.1 ± 5.7	7.7 ± 3.3	81.2% ± 6.8%	52.8 ± 5.2	11.6 ± 5.2	77.9% ± 10.2%
Untreated Dogs					
Helsinki Chronic Pain Index			**Liverpool Osteoarthritis in Dog (LOAD)**		
Pre	Post	% Change	Pre	Post	% Change
40.1 ± 13.0	42.1 ± 13.9	−4.9% ± 13.5%	35.4 ± 15.2	31.3 ± 16.7	−6.3% ± 32.0%

Pain and immobility constitute the primary factors underlying the adverse emotional experiences of individuals afflicted with osteoarthritis, severely impinging on their quality of life. In the absence of intervention, the likelihood of disability becomes significantly elevated. Khatab *et al.* demonstrated that the MSC secretome could effectively diminish joint pain and mitigate cartilage damage in an osteoarthritis mouse mode [15]. In a study by He *et al.*, MSC-derived exosomes were found to downregulate the expression of IL-1β within cartilage tissue of osteoarthritic rats. This process attenuated inflammation within chondrocytes and alleviated neuropathic and inflammatory pain [16]. Stanley *et al.*, in a canine osteoarthritis model, confirmed that umbilical cord-derived MSCs notably alleviated pain in comparison to the control group [17].

Intravenous administration of MSCs has also exhibited beneficial effects in osteoarthritis. Carlien *et al.* revealed that equine peripheral blood-derived MSCs ameliorated pain and lameness in dogs afflicted with osteoarthritis [18]. In a prospective study, the application of adipose-derived MSCs via intra-articular injection to 329 osteoarthritis patients yielded symptomatic improvement in 87.9% of cases, with a remarkable 10.8% of patients achieving complete recovery [19]. In summary, both the intra-articular injection of MSCs and the utilization of their cellular constituents have demonstrated the potential to attenuate or even reverse osteoarthritis symptoms in animal models.

In 2022, $35.9 billion was spent on Veterinary services in the United States. Osteoarthritis in pet dogs brings not only mental but also financial stress to owners. In this study, we employed the questionnaire based on the Helsinki Chronic Pain Index and the LOAD Owner questionnaire to assess pain scores and the mobility of the pet dogs. We then measured changes in these two sets of data before and after MSCs treatment in dogs with osteoarthritis. The Helsinki Chronic Pain Index comprises an 11-question survey aimed at assessing the degree of chronic pain in dogs and monitoring its changes [20]. On the other hand, the LOAD questionnaire was utilized to investigate clinical symptoms such as joint pain and the range of motion reduction in dogs with osteoarthritis during various activities [21]. Our results demonstrated that, in comparison to the control group, dogs treated with MSCs exhibited reduced pain and significantly improved mobility, with their overall mobility being significantly enhanced ($p < 0.001$) without any adverse reactions or other complications. This safe and effective treatment may greatly relieve the mental and financial stress of pet dog owners.

While this study highlights the significant potential of mesenchymal stem cell therapy in alleviating osteoarthritis symptoms, our findings come with certain limitations. Firstly, widespread adoption of this therapy may require additional time. Secondly, the data collected through questionnaires is subject to a degree of subjectivity. Finally, further exploration is needed to fully understand the underlying mechanisms of this treatment. To gain deeper insights, future studies will involve a larger cohort of dogs.

5. Conclusion

Transplantation of stem cells is a promising technology that has garnered considerable scientific interest. This study in companion dogs has demonstrated both short-term safety and efficacy of MSC therapy in treating osteoarthritis in pet dogs. It holds significant promise, and further research in this area is anticipated. Future studies are planned to further examine and validate the technology, including the long-term safety especially potential for tumorigenicity, as well as long-term efficacy including whether the disease recurs and if repeated MSC injection will be necessary.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Hunter, D.J. and Bierma-Zeinstra, S. (2019) Osteoarthritis. *The Lancet*, **393**, 1745-1759. https://doi.org/10.1016/S0140-6736(19)30417-9

[2] Martel-Pelletier, J., Barr, A.J., Cicuttini, F.M., Conaghan, P.G., Cooper, C., Goldring, M.B., *et al.* (2016) Osteoarthritis. *Nature Reviews Disease Primers*, **2**, Article No. 16072. https://doi.org/10.1038/nrdp.2016.72

[3] Guilak, F. (2011) Biomechanical Factors in Osteoarthritis. *Best Practice & Research: Clinical Rheumatology*, **25**, 815-823. https://doi.org/10.1016/j.berh.2011.11.013

[4] Ng, J., Little, C.B., Woods, S., Whittle, S., Lee, F.Y., Gronthos, S., *et al.* (2020) Stem Cell-Directed Therapies for Osteoarthritis: The Promise and the Practice. *Stem Cells*, **38**, 477-486. https://doi.org/10.1002/stem.3139

[5] Zhu, J., Yang, S., Qi, Y., Gong, Z., Zhang, H., Liang, K., *et al.* (2022) Stem Cell-Homing Hydrogel-Based miR-29b-5p Delivery Promotes Cartilage Regeneration by Suppressing Senescence in an Osteoarthritis Rat Model. *Science Advances*, **8**, eabk0011. https://doi.org/10.1126/sciadv.abk0011

[6] Bacakova, L., Zarubova, J., Travnickova, M., Musilkova, J., Pajorova, J., Slepicka, P., *et al.* (2018) Stem Cells: Their Source, Potency and Use in Regenerative Therapies with Focus on Adipose-Derived Stem Cells—A Review. *Biotechnology Advances*, **36**, 1111-1126. https://doi.org/10.1016/j.biotechadv.2018.03.011

[7] Wang, R., Yao, Q., Chen, W., Gao, F., Li, P., Wu, J., *et al.* (2021) Stem Cell Therapy for Crohn's Disease: Systematic Review and Meta-Analysis of Preclinical and Clinical Studies. *Stem Cell Research & Therapy*, **12**, Article No. 463. https://doi.org/10.1186/s13287-021-02533-0

[8] Murphy, J.M., Fink, D.J., Hunziker, E.B. and Barry, F.P. (2003) Stem Cell Therapy in a Caprine Model of Osteoarthritis. *Arthritis & Rheumatology*, **48**, 3464-3474. https://doi.org/10.1002/art.11365

[9] Im, G.I. (2018) Tissue Engineering in Osteoarthritis: Current Status and Prospect of Mesenchymal Stem Cell Therapy. *BioDrugs*, **32**, 183-192. https://doi.org/10.1007/s40259-018-0276-3

[10] Nejadnik, H., Hui, J.H., Feng Choong, E.P., Tai, B.C. and Lee, E.H. (2010) Autologous Bone Marrow-Derived Mesenchymal Stem Cells versus Autologous Chondrocyte Implantation: An Observational Cohort Study. *The American Journal of*

Sports Medicine, **38**, 1110-1116. https://doi.org/10.1177/0363546509359067

[11] Mao, G., Zhang, Z., Hu, S., Zhang, Z., Chang, Z., Huang, Z., *et al.* (2018) Exosomes Derived from miR-92a-3p-Overexpressing Human Mesenchymal Stem Cells Enhance Chondrogenesis and Suppress Cartilage Degradation via Targeting WNT5A. *Stem Cell Research & Therapy*, **9**, Article No. 247. https://doi.org/10.1186/s13287-018-1004-0

[12] Liu, Y., Zeng, Y., Si, H.B., Tang, L., Xie, H.Q. and Shen, B. (2022) Exosomes Derived from Human Urine-Derived Stem Cells Overexpressing miR-140-5p Alleviate Knee Osteoarthritis through Downregulation of VEGFA in a Rat Model. *The American Journal of Sports Medicine*, **50**, 1088-1105. https://doi.org/10.1177/03635465221073991

[13] Bjorge, I.M., Kim, S.Y., Mano, J.F., Kalionis, B. and Chrzanowski, W. (2017) Extracellular Vesicles, Exosomes and Shedding Vesicles in Regenerative Medicine—A New Paradigm for Tissue Repair. *Biomaterials Science*, **6**, 60-78. https://doi.org/10.1039/C7BM00479F

[14] Ibanez, L., Guillem-Llobat, P., Marin, M. and Guillen, M.I. (2022) Connection between Mesenchymal Stem Cells Therapy and Osteoclasts in Osteoarthritis. *International Journal of Molecular Sciences*, **23**, Article No. 4693. https://doi.org/10.3390/ijms23094693

[15] Khatab, S., van Osch, G.J., Kops, N., Bastiaansen-Jenniskens, Y.M., Bos, P.K., Verhaar, J.A., *et al.* (2018) Mesenchymal Stem Cell Secretome Reduces Pain and Prevents Cartilage Damage in a Murine Osteoarthritis Model. *European Cells & Materials*, **36**, 218-230. https://doi.org/10.22203/eCM.v036a16

[16] He, L., He, T., Xing, J., Zhou, Q., Fan, L., Liu, C., *et al.* (2020) Bone Marrow Mesenchymal Stem Cell-Derived Exosomes Protect Cartilage Damage and Relieve Knee Osteoarthritis Pain in a Rat Model of Osteoarthritis. *Stem Cell Research & Therapy*, **11**, Article No. 276. https://doi.org/10.1186/s13287-020-01781-w

[17] Kim, S.E., Pozzi, A., Yeh, J.C., Lopez-Velazquez, M., Au Yong, J.A., Townsend, S., *et al.* (2019) Intra-Articular Umbilical Cord Derived Mesenchymal Stem Cell Therapy for Chronic Elbow Osteoarthritis in Dogs: A Double-Blinded, Placebo-Controlled Clinical Trial. *Frontiers in Veterinary Science*, **6**, Article No. 474. https://doi.org/10.3389/fvets.2019.00474

[18] Brondeel, C., Weekers, F., van Hecke, L., Depuydt, E., Pauwelyn, G., Verhoeven, G., *et al.* (2023) Intravenous Injection of Equine Mesenchymal Stem Cells in Dogs with Articular Pain and Lameness: A Feasibility Study. *Stem Cells and Development*, **32**, 292-300. https://doi.org/10.1089/scd.2022.0296

[19] Freitag, J., Wickham, J., Shah, K. and Tenen, A. (2022) Real-World Evidence of Mesenchymal Stem Cell Therapy in Knee Osteoarthritis: A Large Prospective Two-Year Case Series. *Regenerative Medicine*, **17**, 355-373. https://doi.org/10.2217/rme-2022-0002

[20] Hielm-Bjorkman, A.K., Rita, H. and Tulamo, R.M. (2009) Psychometric Testing of the Helsinki Chronic Pain Index by Completion of a Questionnaire in Finnish by Owners of Dogs with Chronic Signs of Pain Caused by Osteoarthritis. *American Journal of Veterinary Research*, **70**, 727-734. https://doi.org/10.2460/ajvr.70.6.727

[21] Hercock, C.A., Pinchbeck, G., Giejda, A., Clegg, P.D. and Innes, J.F. (2009) Validation of a Client-Based Clinical Metrology Instrument for the Evaluation of Canine Elbow Osteoarthritis. *Journal of Small Animal Practice*, **50**, 266-271. https://doi.org/10.1111/j.1748-5827.2009.00765.x