Encapsulated Allogeneic Synovial Membrane Mesenchymal Stem Cells Provide Better Outcomes of Chondral Lesions in Horses

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Research Article

Keywords: alginate, bioengineering, horses, immunomodulation, mesenchymal cells

Posted Date: December 22nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-587036/v2>

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Abstract

Osteoarthritis is the main cause of equine lameness, and its treatment remains ineffective. Synovial membrane mesenchymal stem cells ($_{SM}MSCs$) provide satisfactory outcomes in joint injuries, mainly due to their immunomodulatory and reparative properties. This study aimed to evaluate the effect of $_{SM}MSCs$, either encapsulated in alginate hydrogel or free, in chondral lesions of horses.

Methods: Chondral lesions were surgically induced in the medial trochlea of the talus of fifteen horses. Animals were treated with PBS free $_{SM}MSCs$ or encapsulated $_{SM}MSCs$. Physical evaluations, assignment of lameness scores and synovial fluid analysis were performed (cytological analysis and dosage of IL-1, IL-10, IL-6, INF- γ , TNF α , P substance, serum amyloid A, TGF- β , IGF and PGE2) for two weeks. Cartilage biopsies were performed 150 days after induction for histological analysis and immunohistochemistry staining.

Results: All groups initially presented inflammation. Although free $_{SM}MSCs$ showed moderate tissue repair, encapsulated $_{SM}MSCs$ had a lower grade of inflammation with superior tissue macro- and microscopic aspects at the end, while the control group showed fibrosis and poor cartilage aspect. This study suggests better effectiveness of stem cells in chondral defects when encapsulated MSCs are used.

Conclusion: While the absence of treatment perpetuates cartilage degradation, encapsulated $_{SM}MSCs$ respond better to initial inflammation, interacting and modulating the environment through the release of anti-inflammatory cytokines. Better outcomes observed in encapsulated MSCs were related to the immuno- and physical barriers provided by the alginate hydrogel, allowing a longer period of permanence and interaction between MSCs and the environment.

Introduction

Osteoarthritis (OA) is the main cause of lameness in horses, leading to poor performance and important economic impacts (1). Even though its etiology is multifactorial, mechanical forces are the major causes of chondral lesions, generating chondrocyte injury and the release of proteases which result in chondral fibrillation (2, 3). Cell-based therapies and tissue engineering can help to promote better chondral healing, reducing articular damage and pain (4).

Strategies combining mesenchymal stem cells (MSCs), biocompatible scaffolds and bioactive components provide mechanical support and a cell source, contributing to joint repair (4, 5). Although several cell sources have been used with positive results, synovial membrane-derived mesenchymal stem cells ($_{SM}MSCs$) present high proliferation capacity and chondrogenic differentiation (6). They can also be obtained through minimally invasive techniques (6–9). Treatment of OA using MSCs relies on its immunomodulatory and paracrine effects, decreasing lymphocyte activation and releasing several molecules (IL-10, IL 1ra, TGF- β and PGE2) (5, 10, 11) and growth factors (12–17) involved in the articular repair process. Despite the noticeable benefit tissue engineering have brought to orthopedics, some

undesired events remain, especially systemic dispersion (18) and decreased viability after MSC intra-articular injection (19). Thus, encapsulated stem cells can be used in joint injuries for protection and to maintain cell viability and stimulate chondrogenic differentiation (19).

The aim of this study was to evaluate the effect of $_{SM}$ MSCs encapsulated in alginate hydrogel on immunomodulation and healing of induced tibiotarsal lesions of horses.

Methods

Fifteen healthy geldings were used in this study, with ages between 3 and 8 years and a mean weight of 330 kg. The animals were divided into 3 groups of 5 animals each: $_{SM}$ MSCs alone (free SMMSC), encapsulated $_{SM}$ MSCs (encapsulated SMMSC) and the control (PBS control).

Cell culture and encapsulation

All $_{SM}$ MSCs were previously characterized and stored in a biobank. Cells were cultured until reaching 1×10^7 $_{SM}$ MSCs in the third passage (P3) according to the technique described by (5,20) and divided into free and encapsulated groups. Cells from the encapsulated $_{SM}$ MSC group were resuspended in 1.5% (w/v) sodium alginate. The mixture was dripped in a gelling solution of 102 mM $CaCl_2$ using a 10-ml syringe and a 21-G needle in an infusion pump. The drops were maintained within the solution for 10 minutes for the crosslinking reaction and capsule formation. Capsules were washed three times in 0.15 M NaCl before the injection.

Cell viability was assessed by the trypan blue exclusion method in a Neubauer chamber after dissolving alginate hydrogel with sodium citrate³.

Arthroscopic procedure and treatments

Tibiotarsal joints were subjected to arthroscopy to induce chondral lesions, and treatment was performed at the moment of the surgical procedure. Thus, arthroscopy was set as the initial moment (0 h). Measurement of clinical parameters and synovial fluid analyses were performed at 0, 24, 48, 96, 168 and 336 h.

Chondral lesions were made according to the technique described by McIlwraith (21). In brief, a shaver was used to create lesions 15 mm in diameter (Figure 1). Ferris-Smith forceps were used to remove the hyaline and calcified cartilage without reaching subchondral bone (mean depth of 1.5-3.2 mm).

All treatments were performed at the initial time immediately after the induction of chondral lesions. The capsules were injected through the arthroscopic portal using a no. 20 Levine catheter. Free $_{SM}$ MSCs were diluted in 10 ml of PBS and injected. The control group received only 10 ml of PBS.

Clinical and laboratorial evaluations

Clinical parameters (physical examination and lameness score) were assessed before arthrocentesis by two evaluators in a blinded manner. Tibiotarsal arthrocentesis was performed at all time points. The synovial fluid analysis included fibrinogen concentration and cytological evaluation (total nucleated cells and differential counting). Stored synovial fluid was used for quantification of IL-1, IL-10, IL-6, INF- γ , TNF α^4 , P substance⁵, serum amyloid A⁶, TGF- β^7 , IGF⁸ and PGE2⁹ by ELISA following the manufacturer's instructions. Absorbance was read at a wavelength of 450 nm.

A new arthroscopy was performed after 150 days to observe the macroscopic appearance of the chondral surface *in situ* and to harvest cartilage for histological analysis and immunohistochemistry for type II collagen. The cartilage aspect was graded blindly by 6 evaluators following the International Cartilage Repair Society (ICRS) score, which evaluates cartilage repair, integration of lesion edges, macroscopic appearance and general aspect of the repairing tissue.

Chondral tissue was harvested and cryopreserved using Tissue-Tek® O.C. T^{TM10} for histological evaluation (hematoxylin-eosin and toluidine blue staining) according to the O'Driscoll histological score. The evaluators were also uninformed of the experimental groups at the time of evaluation.

Immunohistochemistry (IHC) was performed according to the technique described by (22) using the peroxidase and 3,3'-diaminobenzidine tetrahydrochloride (DAB) method. Antigen retrieval was performed with citrate buffer (pH 6) in a pressure cooker,¹¹ and the glass slides were put into the *Dako Cytomation Autostainer*¹² platform. Anti-type II collagen antibody was detected using a mouse secondary monoclonal antibody¹³ at a 1/200 dilution. Immunologic staining was performed using the Histofine¹⁴ method, and the slides were counterstained with hematoxylin. The positive control was pulmonary tissue, and the negative control was made by not using primary antibody.

All samples were evaluated in bright field microscopy and graded in a semiquantitative scoring system, where the absence of expression was graded as 0; from 1% to 25% of positive staining was graded as 1; from 26% to 50% of positive staining was graded as 2 (weak); from 51% to 75% of positive staining was graded as 3 (moderate); and a score of 4 was graded when more than 75% cells were stained (strong).

Statistical analysis

Normality tests were performed using the Kolmogorov–Smirnov test. In the absence of normality, the Kruskal–Wallis test was applied for comparisons between groups at the same time points, and the Friedman test was applied for comparisons between time points of each group. Tukey's test was used for comparisons between medians using Sigmastat 3.5 software when there was significance.

Correlations between treatments and cytokines are shown in a heatmap (pheatmap package) grouping data hierarchically by Euclidean distance and the Ward method. "R" software was used for Pearson's correlation coefficient.

Data regarding treatment response were analyzed by general linear model ANOVA (GLM) considering the evaluator and the experimental group as constants. Mean values of significant results were compared by Tukey's test.

Descriptive analysis is presented as the mean $\pm$ SEM or proportion. Significant difference was considered when $P \leq 0.05$, and tendency toward statistical significance was considered when $P < 0.10$.

³Anticoagulant Acid Citrate Dextrose Solution (ACD-A) - Haemonetics Corporation - Braintree, MA

⁴Milliplex Map Kit, Equine cytokine (EQCYTMAG-93K) - EMD Millipore Corporation – Germany.

⁵Horse Substance P (SP) Elisa Kit – MYBIOSOURCE – USA.

⁶Horse Serum Amyloid A (SAA) Elisa Kit – MYBIOSOURCE – USA.

⁷Milliplex Map Kit, TGF beta single plex magnetic bead kit – EMD Millipore Corporation – Germany.

⁸Horse IGF-1 (Insulin-like growth Factor 1) Elisa Kit- MYBIOSOURCE – USA.

⁹PGE2 (Prostaglandin E2) Elisa Kit – Elabscience Blotechnology Inc. – USA.

¹⁰Sakura Finetek Europe B.V. Germany.

¹¹Pascal®; Dako, Carpinteria, CA, USA.

¹²Dako, Carpinteria, CA, USA.

¹³Sigma Aldrich, Saint Louis, Missouri, USA.

¹⁴414154F, Nichirei Biosciences, Tokyo, JP.

Results

The mean count and viability of $_{SM}MSCs$ were 1.4×10^7 and 98.20% in the free $_{SM}MSC$ group and 1.5×10^7 and 97.40% in the encapsulated $_{SM}MSC$ group, respectively. Each capsule contained approximately 1×10^5 cells (Figure 2).

The physical parameters remained within physiological ranges for the species. Lameness scores reached grade 2/5 at 24 h and 48 h, accompanied by synovial effusion. Both parameters decreased to 1/5 after 168 h.

The synovial fluid analysis (Figure 3) revealed significant differences in total nucleated cells (TNCs) between the encapsulated $_{SM}MSC$ group and the other groups at 24 h ($P=0.0473$). The comparison of the moments within the same group showed a significant increase in TNC at 24 h in the encapsulated $_{SM}MSC$ group, whereas in the free $_{SM}MSC$ group the significant increase remained until 48 h ($P=0.002$).

The neutrophil count showed a significant increase at 24 h in the encapsulated $_{SM}MSC$ group compared with the other groups ($P=0.006$). The comparison of the moments of each group revealed a significant increase in neutrophil count at 24 and 48 h ($P<0.001$) in the free $_{SM}MSC$ and encapsulated $_{SM}MSC$ groups.

The lymphocyte count in the encapsulated $_{SM}MSC$ group presented a significant difference at 24 h ($P=0.004$), while the increase remained for 48 h in the free $_{SM}MSC$ group ($P=0.009$) and until 168 h in the PBS control group ($P=0.015$).

Macrophages increased significantly in all groups initially. However, the treated groups presented an earlier decrease illustrated by a significant difference between the control and treated groups at 96 h ($P=0.025$).

The total protein (TP) of both treated groups increased significantly at 24 h compared with the initial moment ($P<0.05$).

Values of interleukin-6 (IL-6) only showed significant differences at 168 h ($P=0.030$). The encapsulated $_{SM}MSC$ group presented higher values compared with the control group but did not differ from the free $_{SM}MSC$ group.

Individually, there was a peak of IL-6 in the free $_{SM}MSC$ group at 48 and 96 h ($P=0.003$) and only at 96 h in the encapsulated $_{SM}MSC$ group ($P=0.004$). The PBS control group did not present significant differences throughout the time points ($P=0.218$).

No differences were observed between groups or within the same group in SAA, P substance, PGE2, IL-1 α , TNF α , IGF 1 or TGF β ($P>0.05$), even though the TGF- β levels remained higher in the control group.

Higher levels of IL-10 at 24 h, 48 h and 96 h were found in the encapsulated $_{SM}MSC$ group compared with the initial moment ($P=0.012$), but not at 336 h.

The hierarchical grouping divided treatments into two groups: the control group and the treated group (which included both free and encapsulated cells). The variables were divided into four groups: 1: SAA and IGF; 2: TNCC, TP, IFN, TGF, TNF, IL-10, NC and IL-6; 3: MC, LC and IL-1; and 4: SP and PGE2.

Encapsulated MSCs were related to higher SAA, IGF, TNCC, TP, IFN- γ , TGF β , TNF α , IL-10, neutrophil and IL-6 levels, whereas free MSCs led to higher IL-1, macrophage and lymphocyte counts, and the control group was associated with higher SP and PGE2 levels (Figure 5).

There was a positive correlation between PGE2 and TGF β ($r=0.47$), IL-1 and IL-10 ($r=0.96$), IFN- γ and TNF α ($r=0.87$), IL-10 and IL-6 ($r=0.37$), SP and TNF α ($r=0.49$) and IL-6 and NC ($r=0.23$) in the free $_{SM}MSC$ group. The encapsulated $_{SM}MSC$ group revealed positive correlations between NC and IL-10 ($r=0.43$), IL-10 and IL-6 ($r=0.32$), IFN- γ and TNF α ($r=0.75$), IFN and IL-6 ($r=0.23$), TNF α and IL-10 ($r=0.38$), and MC and TNF α ($r=0.19$). The PBS control group showed a positive correlation between IL-6 and IL-10 ($r=0.77$) and between IGF and TP ($r=0.44$) (Figure 6).

The encapsulated $_{SM}MSC$ group presented a better macroscopic appearance similar to naive cartilage. Conversely, chondral erosions and peripheral detachments were observed in the free $_{SM}MSC$ group.

However, no signs of synovitis, fibrillation or erosion were observed in either group (Figure 7). The PBS control group presented fibrosis with unfilled, friable and eroded areas.

The Global Repair Evaluation (GRE) showed higher scores in the encapsulated $_{SM}MSC$ group than in the PBS control group ($P=0.007$). The results of all groups are presented in Table 1.

Table 1

ANOVA for comparing the scores of the groups at T150 in the variable “Global Repair Evaluation” (GRE) (* there was a statistical difference between the groups).

	Group	Average	Standard error	Standard deviation	Median	P
Global Repair Evaluation (GRE)	Free $_{SM}MSC$	8.600	0.400	0.894	8.000	0.007*
	Encapsulated $_{SM}MSC$	10.000	0.707	1.581	10.000	
	PBS control	6.600	0.245	0.548	7.00	

The histological analysis (HE and toluidine blue) revealed different degrees of fibrocartilage deposition. Overall, while the control group presented only fibrosis and fibrocartilage with no chondrocytes or extracellular glycosaminoglycan matrix, the encapsulated $_{SM}MSC$ and free $_{SM}MSC$ groups led to better tissue organization, visible chondrocytes and extracellular matrix deposition (Figure 8).

Immunohistochemistry for type II collagen did not reveal significant differences, even though encapsulated $_{SM}MSC$ and free $_{SM}MSC$ groups led to higher labeling scores (4 and 3, respectively) compared with the PBS control group (score of 2)(Figure 8).

O’Driscoll histological scoring of revealed significant differences between groups in structural integrity ($P=0.028$), chondrocyte grouping ($P=0.013$) and lateral integration of the tissue ($P=0.031$) (Table 2).

Table 2

Values resulting from histological scores of O'DRISCOLL (average, standard error, standard deviation, median and p-value), ANOVA and Tukey test for comparison between groups in 150 days (* there was statistical difference between groups).

	Group	Average	Standard error	Standard deviation	Median	P
Tissue morphology	_{SM} MSC free	2.833	0.167	0.408	3.0	0.071
	_{SM} MSC encapsulated	2.167	0.307	0.753	2.0	
	PBS control	1.667	0.333	0.816	1.5	
Coloring matrix	_{SM} MSC free	2.500	0.224	0.548	2.5	0.590
	_{SM} MSC encapsulated	2.333	0.211	0.516	2.0	
	PBS control	2.167	0.167	0.408	2.0	
Structural integrity	_{SM} MSC free	3.333	0.333	0.816	3.5	0.028*
	_{SM} MSC encapsulated	2.333	0.211	0.516	2.0	
	PBS control	2.167	0.167	0.408	2.0	
Chondrocyte clustering at the implant site	_{SM} MSC free	2.667	0.211	0.516	3.0	0.013*
	_{SM} MSC encapsulated	3.0	0.0	0.0	3.0	
	PBS control	3.0	0.0	0.0	3.0	
Histological surface evaluation	_{SM} MSC free	2.833	0.167	0.408	3.0	0.149
	_{SM} MSC encapsulated	2.333	0.333	0.816	2.5	
	PBS control	2.000	0.258	0.632	2.0	
Lateral integration of the implanted material	_{SM} MSC free	2.667	0.211	0.516	3.0	0.031*
	_{SM} MSC encapsulated	2.500	0.224	0.548	2.5	
	PBS control	1.833	0.307	0.753	2.0	
Inflammation	_{SM} MSC free	1.33	0.211	0.516	1.0	0.237
	_{SM} MSC encapsulated	1.167	0.167	0.408	1.0	

	PBS control	1.0	0.0	0.0	1.0
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A tendency toward statistical significance occurred between the encapsulated _{SM}MSC and PBS control groups in tissue morphology (P=0.07). The median chondrocyte grouping was lower in the free _{SM}MSC group.

Discussion

Several strategies have been developed to avoid chondral loss and OA development. Stem cells are an important treatment option due to their immunomodulatory properties (23, 24). Synovial membrane MSCs have demonstrated better chondrogenic differentiation (8, 25) and were previously proven to be compatible with alginate capsules (20).

The recommended therapeutic cell concentration fluctuates at approximately 10 to 30 million (26). However, free MSC injection can lead to cell dispersion in 7 days (17, 27–29). To reduce cell dispersion, previous studies used activated PRP as a cell scaffold, achieving significant results (30). Other studies using MSCs in scaffolds also demonstrated good therapeutic and differentiation potential in bone defects (31). However, to the authors’ knowledge, the use of MSCs encapsulated in sodium alginate to treat chondral lesions in horses has not been previously described.

The maintenance of physical parameters after MSC injection reinforces the biosafety of this treatment and corroborates previous studies (32, 33). Transitory lameness was described by some authors after MSC injection (32, 34). Similar findings were reported in LPS-induced synovitis treated with MSCs (35, 36). In contrast, the absence of lameness has also been described after MSC injection (37).

Intraarticular injection of autologous, allogeneic or xenogeneic bone marrow MSCs alone can increase TNCC in horses (32, 37, 38). In our study, in addition to MSCs, alginate and the experimental lesion could have contributed to the inflammation process. Although alginate itself is inert, the calcium used in the crosslinking process can exert an immunogenic effect (39). Additionally, alginate capsules injected in the peritoneal cavity of mice caused a significant increase in inflammatory cells after 48, 96 and 168 h (40). In this sense, the absence of a group with alginate alone (without MSCs) to evaluate the isolated effect of alginate capsules is a limiting factor of this study.

The insertion of MSCs in an alginate scaffold could have reduced their effect initially, leading to a higher inflammatory process. However, the inflammatory process decreased over time, such that at the end of the study, the encapsulated SMMSC group revealed the lowest cell count, which can indicate an immunomodulatory paracrine effect of MSCs despite the initial inflammation. This effect is achieved by the release of cytokines and growth factors (41–43) that can be added to the porous structure of alginate, allowing oxygen, metabolite and nutrient diffusion (44) and stimulating cell proliferation and survival (45). A similar immunomodulatory effect was observed in a previous study that used MSCs in

alginate hydrogels to treat neuroinflammation, demonstrating that alginate can not only act as a delivery scaffold but also enhance the therapeutic effect of MSCs (46).

Although MSC injection can contribute to the increase in neutrophil count (47), we state that the experimental lesion was the main cause of the initial inflammatory process as all groups, including the control group, initially presented inflammation. While some studies showed a decrease in neutrophil count after 7 to 9 days (33, 48), it remained high for 14 days in our study, corroborating other previous data (49).

Lymphocytes are cells from the adaptive immune system that are attracted chemotactically by several cytokines (50). The increase in lymphocyte count (LC) at 168 h only in the control group might occurred due to the ability of MSCs to reduce lymphocyte activation (12, 51).

Macrophages have different subtypes with different functions. They can polarize into either a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype depending on the environment (52, 53). M2 macrophages release chondrogenic factors, including IL-10, IL-1Ra and TGF β (52, 53). Although specific labeling for M1 and M2 was not performed, the positive correlation between macrophage count (MC) and IL-10 and the statistically higher IL-10 in the encapsulated SMMSC group indicate a tendency toward M2 polarization, as IL-10 is required for macrophage M2 polarization (54). Interestingly, the PBS Control group also presented a correlation between MCs and IL-10, which indicates an anti-inflammatory response against inflammation. However, as further analyses of cartilage scores revealed bad cartilage aspects and histologic architecture, the role of MSCs in interacting, organizing and orchestrating the reparative process was corroborated. The final result found in the PBS control group indicates that most of the synovial macrophages did not polarize into M2, as a better outcome would be expected in a pro-resolutive (M2) scenario.

An increase in total protein (TP) up to 5 g/dL has been demonstrated after allogeneic and xenogeneic MSC injections, indicating inflammation (32), similar to the 24 h time point in the encapsulated $_{SM}$ MSC group. The positive correlation between TP and macrophage count in the encapsulated SMMSC group, with a simultaneous decrease in both variables, suggests that MSCs modulated the inflammatory process.

Interferon γ increased only at 24 h in the encapsulated $_{SM}$ MSC group and at 24, 168 and 336 h in the free SMMSC group. As IFN γ is related to M1 polarization of macrophages and an increase in neutrophil and monocyte activity (55), the increase in its levels reflects the initial inflammatory process, where most macrophages generally adopt M1 polarization (52, 53). Even in the presence of a strong positive correlation between IFN γ and TNF- α , a better outcome was noticed in both treated groups, which shows that despite the proinflammatory commitment of these cytokines, the initial inflammatory process elicited an MSC anti-inflammatory response, corroborating previous findings (56).

The role of IL-6 in horses is not completely understood. It can be released in LPS-induced arthritis (57), acting as a proinflammatory cytokine through the delay in lymphocyte and neutrophil apoptosis and

decrease in T-cell stimulation (58, 59). However, immunoregulatory properties have also been attributed to IL-6, demonstrating a dual role of this cytokine (60). As the peak of IL-6 occurred at 96 h in the encapsulated s_M MSC group along with a decrease in neutrophil and total nucleated cell counts and an increase in IL-10, we assumed that IL-6 exerted an immunoregulatory effect in this case. The absence of simultaneous increases in IL-6, IL-1 and TNF α corroborates this affirmation because in a pro-inflammatory scenario, IL-6 is involved in chondral matrix degradation along with IL-1 and TNF α (61–63).

In contrast, IL-10 was increased in the encapsulated s_M MSC group. When properly stimulated, MSCs can release IL-10 and other anti-inflammatory molecules, such as IL-1ra, indoleamine 2,3-dioxygenase (IDO), TGF- β and PGE2 (10, 29, 30). Thus, the release of IL-10 can have occurred in response to the initial inflammatory process.

The presence of well-attached, white and firm cartilage-like tissue has been previously reported after PRP injection in both experimental and natural chondral lesions in horses (64–66). When PRP was associated with MSCs, superior type II collagen deposition and macroscopic and histological appearance were reported (67). Better macro- and microscopic aspects were observed in the encapsulated s_M MSC group, with higher glycosaminoglycan deposition and better O'Driscoll scores, showing great contrast with the free s_M MSC and PBS control groups, which revealed the prevalence of fibrous tissue.

Taken together, the data of this study indicate a better outcome in cartilage condition after injecting MSCs encapsulated in alginate. In addition to providing a scaffold for MSCs tridimensional organization, alginate encapsulation is a cell delivery mechanism capable of improving therapeutic cellular effects due to the maintenance of MSCs for a longer time at the site of injection (68–71), which can facilitate cell-to-cell interaction and consequent secretion of specific cytokines (72) and cell stimulation (29, 42, 73–75) that direct the articular environment toward a pro-resolutive scenario. It is important to note that the large synovial cavity allowed the injection of a high number of capsules. However, each case needs to be evaluated individually because the number of capsules to be injected is directly related to the cell concentration. A longer follow-up period for the animals would have provided considerable data regarding type II collagen deposition and morphological and histological scores. As each MSC source has its own particularities that may lead to different behaviors, even when facing the same conditions, the comparison of the synovial membrane to other MSC sources would also contribute to a better understanding of the events associated with chondral healing in horses.

Conclusion

Alginate capsules with MSCs elicited a marked initial inflammatory process. However, inflammation was modulated over time, and encapsulated MSCs produced better outcomes in terms of chondral aspect and composition. Some grade of inflammation is necessary and even desirable, which can be beneficial to the MSC response, releasing anti-inflammatory cytokines that guide the reparative process toward resolution. In addition to safety in application, the positive results observed after administration of encapsulated

MSCs in chondral defects substantiate further studies to clarify the immunomodulatory effect of encapsulated MSCs under different conditions.

Abbreviations

SMMSC: Synovial membrane mesenchymal stem cells; PBS: Phosphate Buffered Saline; IL- interleukin; INF: interferon; TNF: tumor necrosis factor; TGF: Transforming growth factor; IGF: insulin growth factor; PGE: prostaglandin E; OA: osteoarthritis; MSC: Mesenchymal stem cell; CaCl: calcium chloride; NaCl: Sodium chloride; ICRS: International Cartilage Repair Society; IHC: Immunohistochemistry; DAB: 3,3'-diaminobenzidine tetrahydrochloride; TNC: total nucleated cells; TP: total protein; SAA: serum amyloid A; SP: substance P; NC: nucleated cells; MC: macrophages cells; LC: lymphocytes cells; GRE: Global Repair Evaluation; HE: hematoxylin eosin; COL II: collagen type II; PRP: plasma rich platelets; LPS: lipopolysaccharide.

Declarations

Ethics Committee approval

All experimental procedures were performed in accordance with the approved guidelines and regulations of the Ethics Committee of São Paulo State University (protocol n. 032/2020).

Acknowledgments

We thank the São Paulo Research Foundation (FAPESP grant numbers 2017/12815-0 and 2017/14460-4), the Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Council for Scientific and Technological Development (CNPq) for funding this study.

Authors' contributions

VH conceived the study and participated in its design, execution, and coordination, in addition to writing the manuscript. JP, FC, MC and EV conceived the study and participated in its design, execution, data collection and manuscript review. GS conceived the study and participated in its design, execution, data collection and manuscript review. LV and TR participated in the design of the study and performed the statistical analysis. CE participated in the design of the study and performed histological and immunohistochemical analysis. AL conceived the study, coordinated the research, and carried out the writing and revision of the manuscript. All authors read and approved the final manuscript.

Funding

São Paulo Research Foundation (FAPESP grant number 2017/12815-0 and 2017/14460-4), financial assistance for carrying out the entire research project. The Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Council for Scientific and Technological Development (CNPq), contributed to the assistance of authors with scholarships.

Availability of data and materials

The authors do not share the data due to the pioneering work in the equine species.

Ethics Committee approval

All experimental procedures were carried out in accordance with the approved guidelines and regulations of the Ethics Committee of São Paulo State University (protocol n. 032/2020).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Initial tibiotarsal arthroscopy (talus medial trochlea). (A) Chondral lesion at time point 0 h made using the shaver, removing hyaline cartilage. (B) Chondral defect 15 mm in diameter.

Figure 2

Equine synovial membrane-derived mesenchymal stem cells encapsulated in 1.5% sodium alginate. (A) Capsules after gelification; (B) bright field microscopy (20x objective lens) showing cell density, viewed as small spherical structures.

Figure 3

Total nucleated cell, neutrophil, lymphocyte and macrophage counts (cells/μL) and total protein (g/dL) of synovial fluid of the three groups. * indicates P<0.05.

Figure 4

Median interleukin (μ L), serum amyloid A (SAA), IGF, TNF α , P substance, IL-1 α , IL-10, IL-6, IFN γ , PGE2 and TGF- β levels in the synovial fluid of the three groups.

* indicates $P < 0.05$ within the group; ** indicates $P < 0.05$ between groups.

Figure 5

Heatmap of the correlation between synovial fluid SAA, IGF, TNCC, TP, IFN- γ , TNF α , TGF β , IL-10, neutrophil count (NC), IL-6, macrophage count (MC), lymphocyte count (LC), IL-1 α , SP, and PGE2 and the treatments: free MSCs (free $_{SM}$ MSC), encapsulated MSCs (encapsulated $_{SM}$ MSC) and the control group (PBS control).

Figure 6

Pearson's correlation coefficient test between synovial fluid SAA, IGF, TNCC, TP, IFN - γ , TNF α , TGF β , IL-10, NC, IL-6, MC, LC, IL-1 α , SP, and PGE2 and treatments: free MSCs (free $_{SM}$ MSC), encapsulated MSCs (encapsulated $_{SM}$ MSC) and the control group (PBS control).

Figure 7

Macroscopic evaluation after 150 days. (A) Encapsulated MSC group (encapsulated s_M MSC) showing total filling of the lesion by the newly formed tissue. (B) Free MSC group (free s_M MSC) revealing chondral erosions and peripheral detachments. (C) Control group (PBS control) exhibiting fibrosis, erosion, and friable and poorly attached tissue.

Figure 8

Bright field microscopy at 150 days. Tissue was stained in HE and toluidine blue and immunohistochemistry for type II collagen (COL II). Encapsulated and free MSCs revealed better structural organization and the presence of chondrocytes and extracellular matrix.