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The secretome of equine bone marrow-derived mesenchymal stem cells enhanced regenerative phenotype of equine articular chondrocytes

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► To cite this version:

Manon Jammes, Romain Contentin, Frédéric Cassé, Bastien Bourdon, Arnaud Bianchi, et al.. The secretome of equine bone marrow-derived mesenchymal stem cells enhanced regenerative phenotype of equine articular chondrocytes. International Society of Extracellular Vesicles Congress 2022 (ISEV2022), May 2022, Lyon, France. hal-03710365

HAL Id: hal-03710365

<https://hal.science/hal-03710365>

Submitted on 30 Jun 2022

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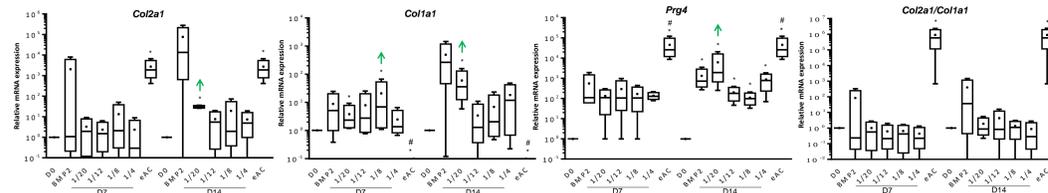
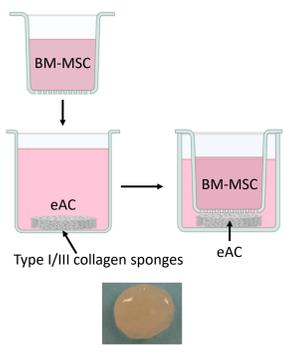
Introduction

Equine **osteoarthritis** (OA) is a sequential disease which leads to cartilage degradation and painful bone frictions. It induces impaired animal well-being, premature cessation of sport activity, and financial losses. Fibrocartilage synthesis occurring during cartilage destruction is a physiological response, allowing bone protection but reducing tissue mechanical resistance. To date, there is a **lack of curative therapies**. Mesenchymal stem cell (MSC)-based therapies are promising for cartilage repair, but face limitations inherent to cell itself which can be overcome using their **secretome** through acellular therapy approaches.

To understand the effects of equine bone marrow (**BM**)-MSC secretome on equine articular chondrocytes (eAC) phenotype, indirect co-culture experiments were first performed. Then, we wanted to recapitulate the effects we observed in co-culture on eAC using the MSC-conditioned medium (CM) to make acellular therapy conceivable. We assessed hyaline cartilage and fibrocartilage markers at the transcription and protein levels, and evaluated eAC migratory capacities, which are of interest during OA therapy to favor the filling of the cartilage defects. To optimize **immunomodulation properties of MSC secretome** for future experiments, MSC priming relevance with interleukin (**IL**)1-β was evaluated. Suspected to be the principal vectors of the effects observed, exosomes were isolated through chemical precipitation and then characterized to confirm their nature.

Results

Co-culture experiments

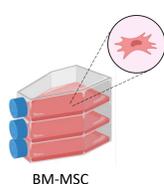


Cartilage markers
Col2a1 : Hyaline cartilage
Col1a1 : Fibrocartilage, less resistant than type II collagen
Prg4 : Lubricin, joint lubricant

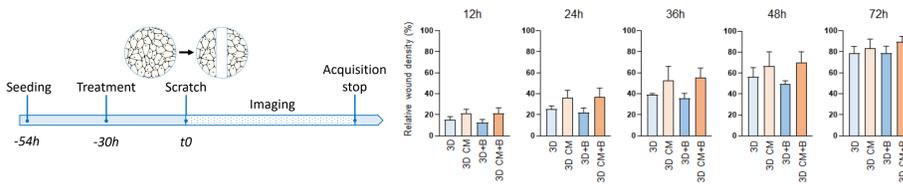
mRNA levels of hyaline cartilage and fibrocartilage matrix markers were assessed by RT-qPCR after 7 (D7) and 14 days (D14) of culture. Normalization was performed according to D0 (eAC cultured in monolayer until P3). The significance of the values was tested using a Mann-Whitney test (* p<0,05). A chondrogenic control was performed using bone morphogenetic protein 2 (BMP2), and eAC condition refers to mRNA extracted from eAC at P0.

eAC co-culture with BM-MSC improved eAC phenotype by enhancing *Col2a1*, *Col1a1*, *Prg4* markers expressions, but *Col2a1/Col1a1* remained unchanged.
→ **BM-MSC secretome modulates eAC phenotype**

Conditioned media experiments

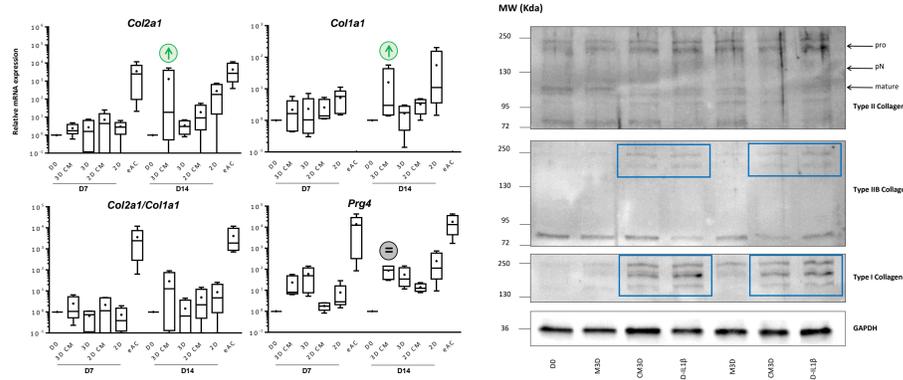


1. MSC-CM enhanced eAC migratory capacities
3D medium corresponds to 2D eAC culture medium supplemented with ascorbic acid. Media were tested with or without the BMP2 chondrogenic molecule.



2. CM improved mRNA and protein expression of healthy and pathologic cartilage markers by eAC
After 7 and 14 days of 2D and 3D culture of eAC with MSC-CM, mRNA expression of hyaline cartilage and fibrocartilage markers was assessed by RT-qPCR. Normalization was realized towards the D0 condition.

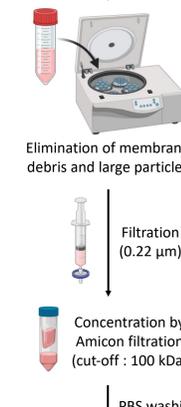
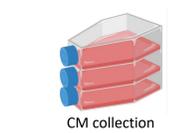
CM3D-IL1β condition refers to CM from MSC primed with interleukine(IL)-1β (10 ng/mL) during 24h prior to conditioning. After 14 days, eAC cultured with CM supplemented or not with BMP2, in organoid-like collagen sponges were harvested and protein extraction was performed.



MSC-CM enhanced hyaline cartilage and fibrocartilage markers expression of eAC after 14 days and increased their migratory capacities
→ **BM-MSC-CM recapitulates the effect of co-culture on eAC**

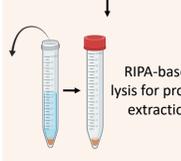
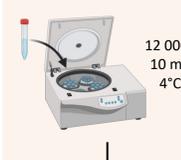
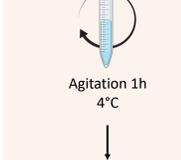
MSC secretome was able to increase collagen protein accumulation especially when primed with the **OA-related inflammatory cytokine IL-1β**, suggesting that the therapeutic potential of MSC-CM could be enhanced by an optimized cytokine priming.

Exosomes characterization



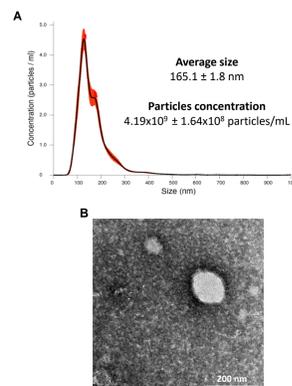
Exosomes precipitation

PEG 50%
Protamine sulfate 2%



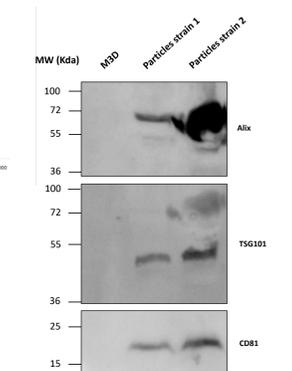
1. Physical and morphological characterization

MSC-CM nanoparticle size distribution and concentration were determined by NTA (A). Particle morphology was observed by TEM to confirm their EV nature and assess EV integrity (B).

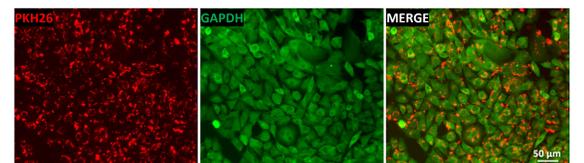
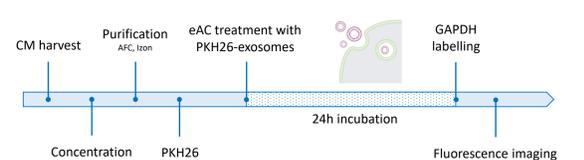


2. Expression of exosomal specific markers

Protein extraction was performed after purification of exosomes according to the detailed protocol. Then, a western-blot was performed with antibodies against exosome-specific proteins.

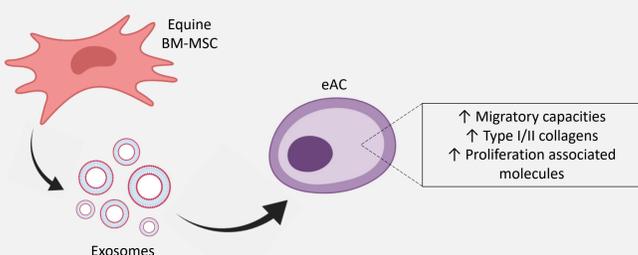


3. Uptake of exosomes by eAC



CM from BM-MSC contains undamaged exosomes expressing Alix/TSG101/CD81. These extracellular vesicles interact with eAC and can be uptaken by the cells.

Conclusions



MSC-CM were able to favor eAC migratory capacities and cartilage matrix synthesis *in vitro* by increasing types II and I collagens protein amounts. These effects could be mediated by exosomes since we have demonstrated that MSC-CM contain these nanovesicles that can be uptaken by eAC. However further investigations are needed to better define the contribution of exosomes to the therapeutic potential of MSC-CM in OA.

These results demonstrate that **MSC secretome influences eAC phenotype** and could relieve OA symptoms or delay OA outcomes in equine medicine. Consequently, an acellular therapy avoiding disadvantages of direct cell use must be considered, especially because this process could be potentially transposed to human medicine according to the One Health concept.

The European projects "EQUISTEM" and "OA-ACTIVE" are funded by the European Union within the framework of the Operational Program ERDF/ESF 2014-2020