# Comparison of the chondrogenic potential of mesenchymal stem cells derived from bone marrow and umbilical cord blood intended for cartilage tissue engineering

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## Introduction

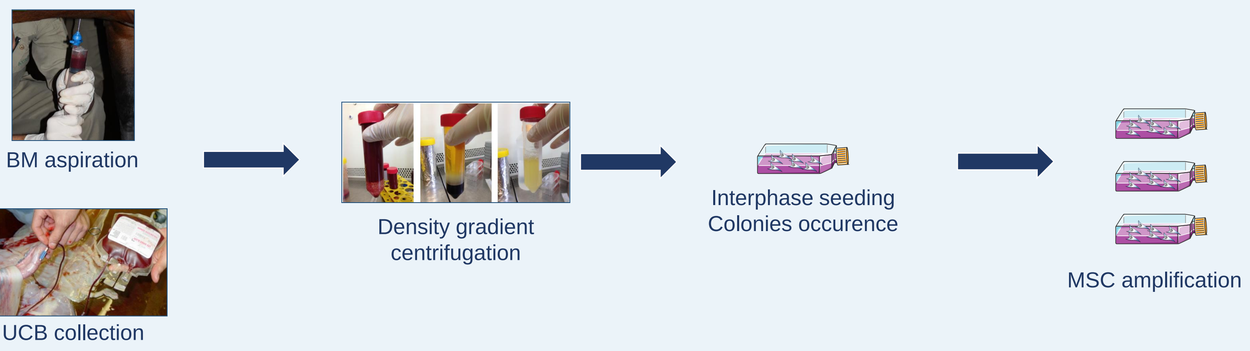
Articular cartilage is frequently altered upon trauma or in osteoarthritis (OA), a degenerative disease that is currently incurable. Cartilage tissue engineering/cell therapy offer new insights to cure these articular disorders. Since the first generation of cartilage tissue engineering led to a poor quality cartilage rich in the hyaline cartilage atypical type I collagen, it has been followed with improved generations. One way to improve these strategies was to diversify the cell type used. Mesenchymal stem cells (MSC) are considered as an interesting cell type in order to produce a hyaline cartilage substitute. This study aimed to compare umbilical cord blood (UCB) and bone marrow (BM) derived MSC.

BM and UCB MSC were isolated and then amplified in monolayer culture. We characterized MSC by assessing their proliferative and multipotence capacities, and the presence of cluster of differentiation (CD) characteristic of MSC. Then, we compared MSC at their basal state and after a chondrogenic differentiation, which consisted of culture in hypoxia or normoxia in a biomaterial, with chondrogenic factors (BMP-2, TGF-ß1). Criteria used to compare MSC were the mRNA level and protein amount of several osteogenic (osteocalcin/Runx2), hypertrophic (type X collagen) and chondrogenic markers (type II collagen). Furthermore, we analysed the extracellular matrix(ECM) composition/structure by immunochemistry.

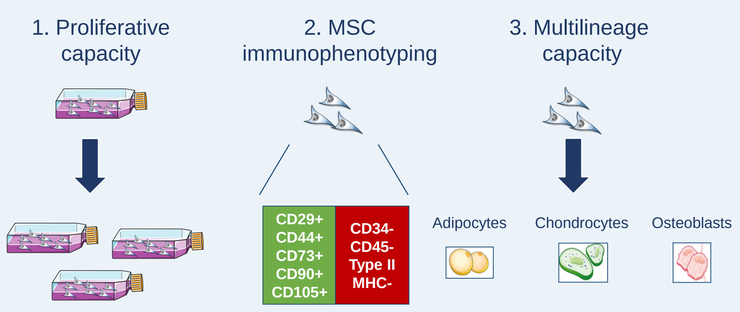
## Results

### MSC isolation

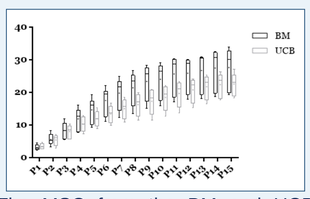
Equine UCB was collected by venipuncture of the umbilical veins and equine BM was collected from sternal puncture in a cohort of horses with ages ranging from 2 to 4 years. Then, MSC were isolated by density gradient centrifugation with Ficoll followed with a seed of the interphase in a plastic flask filled with an isolation medium to select cells which were able to adhere and form colonies, the MSC. After the appearance of several colonies, cells were detached using trypsin/EDTA and then reseeded at 5000 cells/cm$^2$.




### MSC characterization



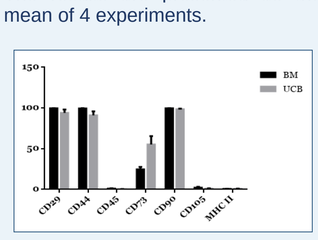

#### 1. Proliferative capacity



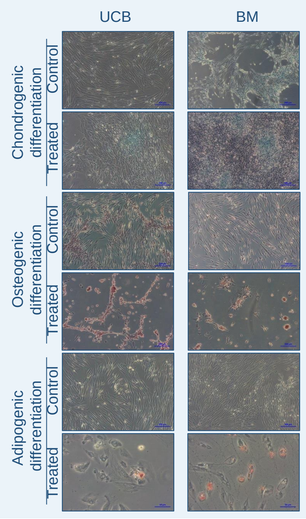

The MSC from the BM and UCB were isolated and seeded at 5000 cells/cm$^2$. A passage with trypsin/EDTA was performed every 7 days, followed by counting and re-seeding at the same cell density (n = 4). The significance of the results was evaluated using a Mann Whitney test (*p < 0.05, **p < 0.01, ***p < 0.001).

#### 2. MSC immunophenotyping

At P4, the cells were detached and suspended at 10 million cells per ml in PBS. They were incubated with the indicated specific monoclonal antibodies, or relevant anti-IgG isotype controls for 30 min at 4 °C in a dark room. The cells were then washed and resuspended in PBS. The results are presented as the mean of 4 experiments.




#### 3. Multilineage capacity



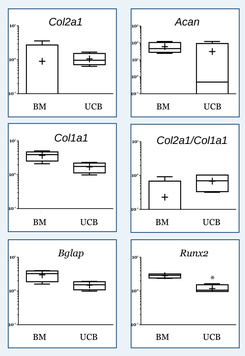

The cells were amplified at P4 and cultured at 50% of confluence with media specific for osteoblastic (n = 4), adipocyte (n = 4) or chondrogenic (n = 4) differentiation (treated panels) or amplification medium (control panels) for 14 days. After fixing with paraformaldehyde, cells were stained with Alizarin red, Oil Red O and Alcian blue to respectively reveal the presence of calcium deposits, lipid droplets and sulfated proteoglycans.

### MSC at their basal state

Because MSC could exhibit differences involved in their chondrogenic abilities, we investigated a comparison of both MSC at their basal state. Thus, during the amplification of MSC we performed mRNA (1) and protein (2) level analyses.

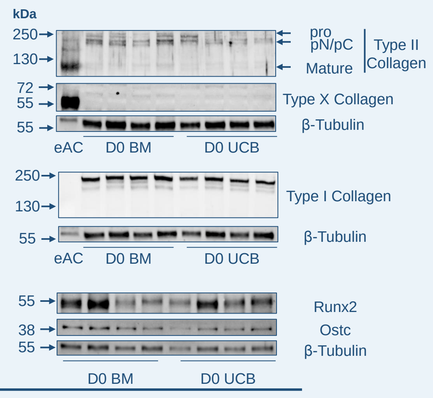
#### 1. Comparison of mRNA expression in BM and UCB MSC at their basal level

MSC were cultured in monolayer until P4 with the amplification medium and their mRNA were extracted. The mRNA were estimated by RT-qPCR after normalization relative to the *β-actin* reference gene. The *Col2a1 : Col1a1 ratio* is shown. The results are represented as box plots (median, quartiles, extreme values) and the significance of the values was tested using a Mann-Whitney test (* p < 0.05).

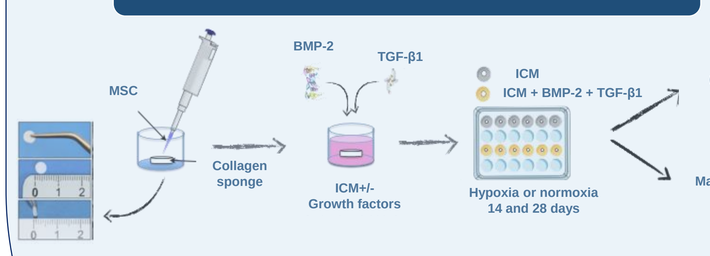



#### 2. Comparison of protein expression in BM and UCB MSC at their basal level

MSC were cultured in monolayer until P4 with the amplification medium and proteins were extracted. The total protein extracts were separated by electrophoresis on a 7.5% acrylamide gel under denaturing conditions. The proteins were then transferred to a PVDF membrane which was incubated with the antibodies indicated on the right.

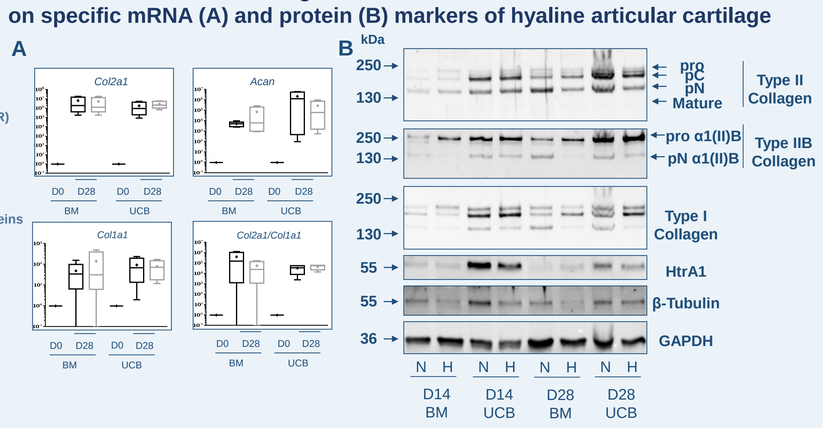



### MSC after chondrogenic differentiation



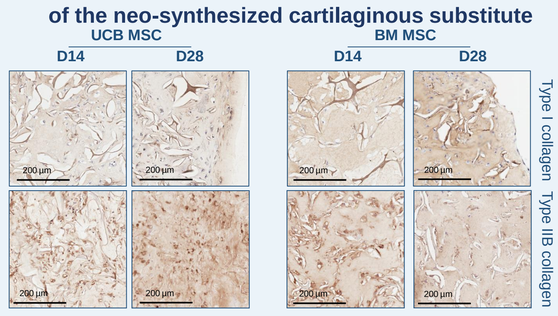

Equine MSC derived from BM or UCB were seeded in collagen sponges at P4 (n = 5). They were then grown in hypoxia (3% $O_2$) or normoxia (21% $O_2$) for 14 and 28 days (D14 and D28) in the presence of incomplete chondrogenic medium enriched with BMP-2 (50 ng/ml) and TGF-β1 (10 ng/ml). The D0 condition corresponds to MSC cultured in monolayer at P4 with the amplification medium and the equine articular cartilage (eAC) condition corresponds to the protein extracted from healthy cartilage.

#### 1. Effect of the chondrogenic differentiation of BM and UCB MSC on specific mRNA (A) and protein (B) markers of hyaline articular cartilage




(A) mRNA levels were estimated by RT-qPCR after normalization relative to the *β-actin* reference gene. The results are represented as box plots (median, quartiles, extreme values) and the significance of the values was tested using a Mann-Whitney test (* p < 0.05). (B) The total protein extracts were separated by electrophoresis on a 7.5% acrylamide gel under denaturing conditions. The proteins were then transferred to a PVDF membrane which was incubated with the antibodies indicated on the right. N : Normoxia, H : Hypoxia

#### 2. Assessment of the ECM of the neo-synthesized cartilaginous substitute




At D14 and at the end of the differentiation protocol, sponges were embedded in paraffin and sectioned in 4 µm-thick slices. Then immunostaining was performed in order to detect type I collagen or type IIB collagen.

**BM:**

**-High isolation efficiency**
**-Harvested throughout the year**
**-Minimally invasive**

**UCB:**

**-High isolation efficiency**
**-Harvested during the spring**
**-Not invasive**

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**BM and UCB MSC:**

**- Multipotent**
**- High proliferative capacities**
**- Express characteristic CD**

**BM MSC:**

**- Highest proliferative capacities**
**- Strongest abilities to differentiate into adipocytes**

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**BM and UCB MSC:**

**- Inter strain variabilities**

**BM MSC:**

**- Highest amount of type I collagen**
**- Highest protein level of type II collagen**
**- Largest level of osteoblatic markers**

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**BM and UCB MSC:**

**- Hypoxia limits the expression of hypertrophic markers**
**- Biomaterial is colonized with ECM**
**- No type I collagen at the vicinity of the nuclei**

**UCB :**

**- Largest amount of ECM**
**- Type I collagen decreases at 28 days**

**BM :**

**- Lower expression of HtrA1**
**- Lower protein amount of type I collagen**

## Conclusions

Although samples of BM and UCB allow the isolation of cells possessing MSC characteristic, differences can be observed between the two sources without predicting a better chondrogenic potential for a MSC source rather the other. mRNA and protein analyses of undifferentiated MSC suggest a stronger possibility for BM MSC to synthesize collagens compared to UCB MSC, which is attractive considering their use in cartilage tissue engineering. Nevertheless, BM MSC express also a higher amount of hyaline cartilage atypical molecules such as osteocalcin and type I collagen, to a greater extent than UCB MSC. Both MSC were able to produce a hyaline like cartilage matrix upon a chondrogenic differentiation protocol. However, considering that type I collagen is the major unwanted component in the *in vitro* cartilage synthesis, BM MSC seem to be the best MSC candidate for cartilage tissue engineering with the *in vitro* chondrogenic differentiation protocol used here. However, the decrease of the type I collagen protein amount only observed in UCB MSC at D28, even if it remains higher than BM MSC, could only be the beginning of the decrease. Thus, by lenghtening the time of the chondrogenic differentiation protocol, UCB could also be an attractive MSC source for cartilage tissue engineering.

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