Supplemental data to:

Chondrogenic potential of mesenchymal stromal cells derived from equine bone marrow and umbilical cord blood

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Comparative chondrogenic potential of cells derived from umbilical cord matrix, adipose tissue, and bone marrow

Putative mesenchymal stromal cells (MSC) derived from umbilical cord matrix (CM-MSC), adipose tissue (AT-MSC), and bone marrow (BM-MSC) were assessed histologically for signs of chondrogenic phenotype after 28-days induction in pellet culture, as described in the Materials and methods section of the main article. Three AT-MSC, three BM-MSC, and two CM-MSC cell populations were included in the study. Cell populations represent individual horses. All cells were primary, polyclonal in origin, and had been cryopreserved prior to the study. The differentiation studies were performed simultaneously with the cord blood and bone marrow-derived cells of the main article.

Materials and methods

Cell culture techniques and chondrogenic induction was performed as described in the main article. Outcome was assessed after 28-day induction by hematoxylin and eosin, Safranin O, and Alcian Blue stain of paraffin embedded sections. The isolation methods for putative CM- and AT-MSC are described in more detail.

Nucleated cell isolation from umbilical cord matrix

Plastic adherent cells with a fibroblast-like morphology were established from explant cultures of the umbilical cord matrix as described elsewhere (1). Sections of the umbilical cord (20-30 cm) were stored in plastic bags at 4°C until processing. Upon arrival to the laboratory, the cord was rinsed under running tap water to remove gross contamination with stall bedding material. The cord matrix (Wharton's Jelly) was evident to varying degrees as an amber coloured gelatinous matrix in the soft tissue between the blood vessels of the cord. Multiple pieces (2x2x2 mm) were sharply dissected using sterile scissors. The pieces were rinsed twice in phosphate buffered saline (PBS) and once in culture medium prior to seeding in 6-well plates. The culture medium was completely replaced 18-24 hours after initial seeding, and hereafter every two to three days. Subculturing and cryopreservation was done as described in the above general section.

Nucleated cell isolation from adipose tissue

Adipose tissue was collected from the site between the skin and the dorsal gluteal muscles of four mature horses immediately after euthanasia. One to 15 grams of fat was collected and transported in sterile PBS to the laboratory. The nucleated cells were released from the fat tissue using enzymatic digestion as described elsewhere (2, 3). The fat was rinsed three times in PBS to remove all visible blood, fibrous tissue and blood vessels. The fat was dissected into pieces less than 10-20 mg (2x2x2 mm) and rinsed once with PBS. In 50 ml conical tubes 4 ml of digestion solution was added per 1 gram of fat and incubated for one hour at 38.0 ºC in humidified atmosphere containing 5% CO_2 with shaking scheduled every 15 minutes. The digestion medium consisted of 1% collagenase type I (Sigma) in Hank's balanced salt solution supplemented with 1% bovine serum albumin (Sigma). The tissue solution was filtered through a 250-micron syringe filter to obtain the stromal-vascular fraction (SVF) containing the nucleated cells of the fat tissue. The SVF was centrifuged at 300 g for five minutes to remove floating adipocytes. The pellet was resuspended in culture medium and a manual cell count was performed. Cells were seeded in T75 culture flasks at approximately 3 x 10^4 cells/cm^2. The culture medium was replaced three days after seeding and subsequently every two to three days. Subculturing and cryopreservation was done as described in the above general section.

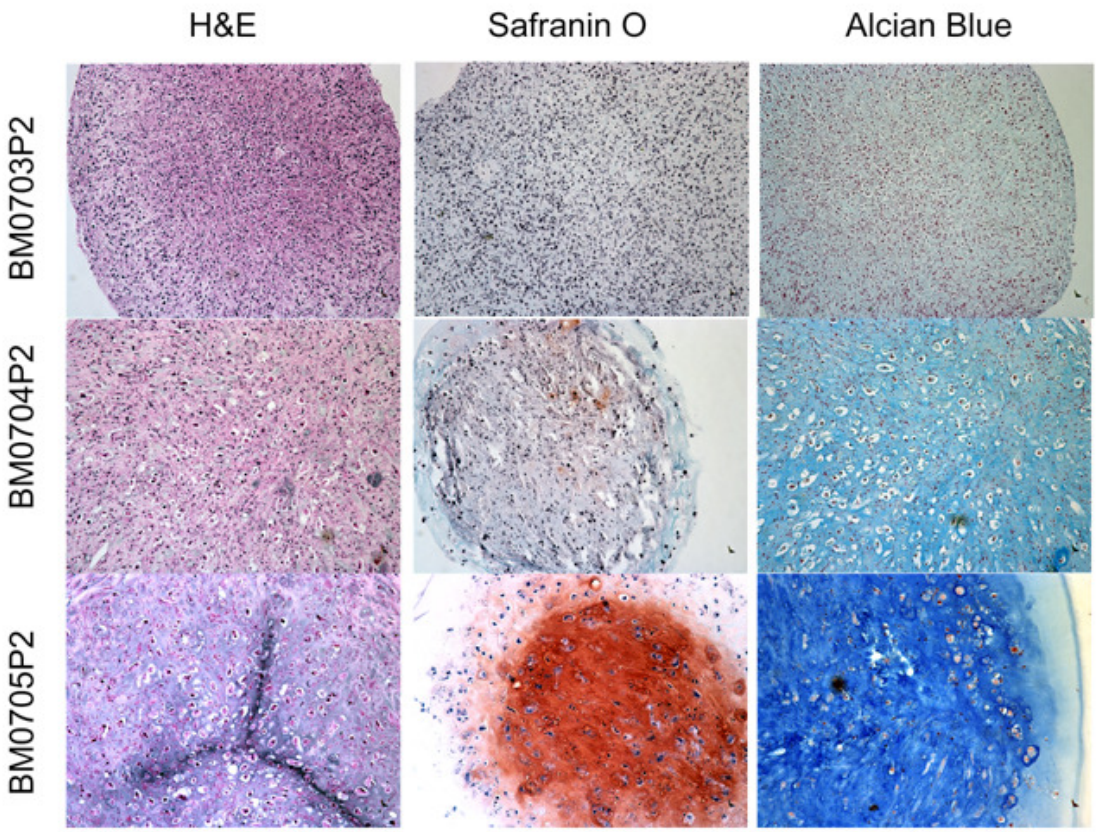
Results

Poor to moderate chondrogenesis was present in the three BM-MSC cell sources (Fig. 1 Suppl.). Chondrogenic induction was unsuccessful in AT-MSC and CM-MSC cell sources (Fig. 2 and 3 Suppl).

Discussion

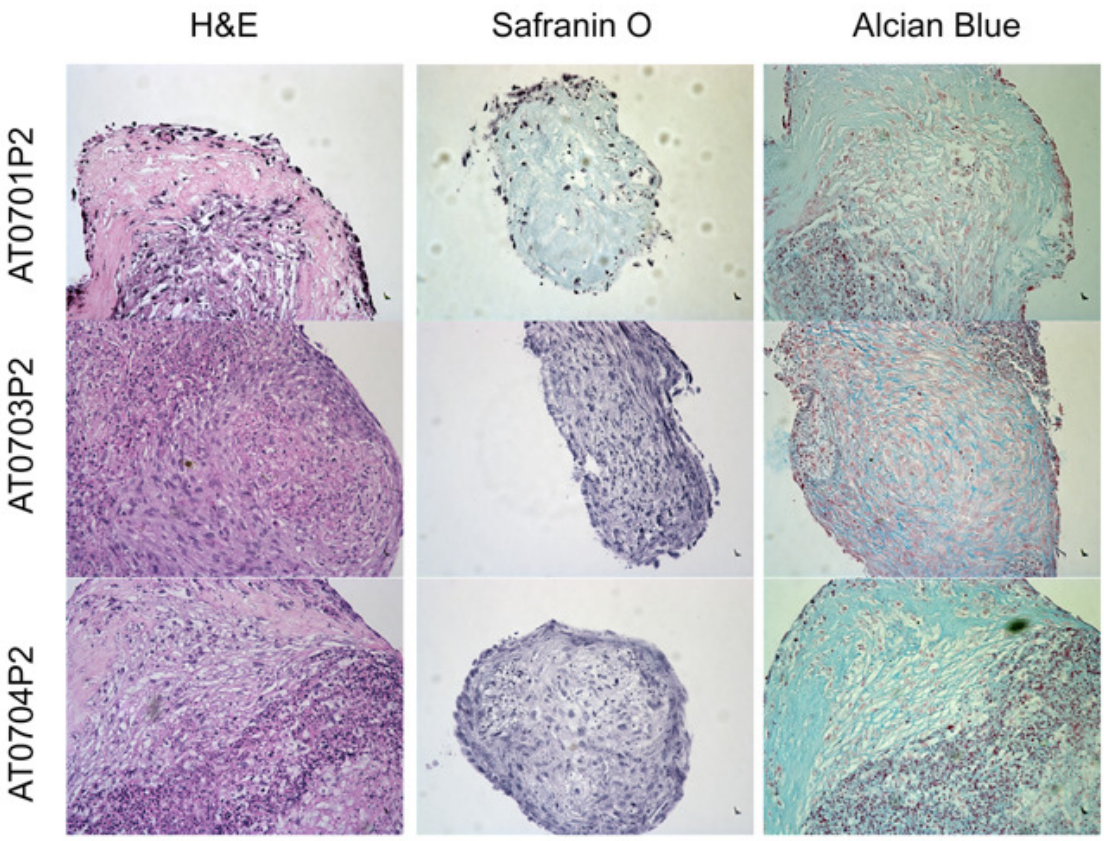
When compared to the robust chondrogenic differentiation present in the cord blood-derived cell line of the main article (Fig. 2D-F) this data further supports cord blood as a potential source of chondrogenic progenitor cells. As discussed in the main article the inclusion of dexamethasone in the isolation medium of cord blood cells may greatly influence the potential of the cells. More studies are needed to evaluate if AT-MSC and CM-MSC generally lack chondrogenic potential.

Fig. 1 suppl.: Bone marrow-derived putative MSCs chondrogenesis.



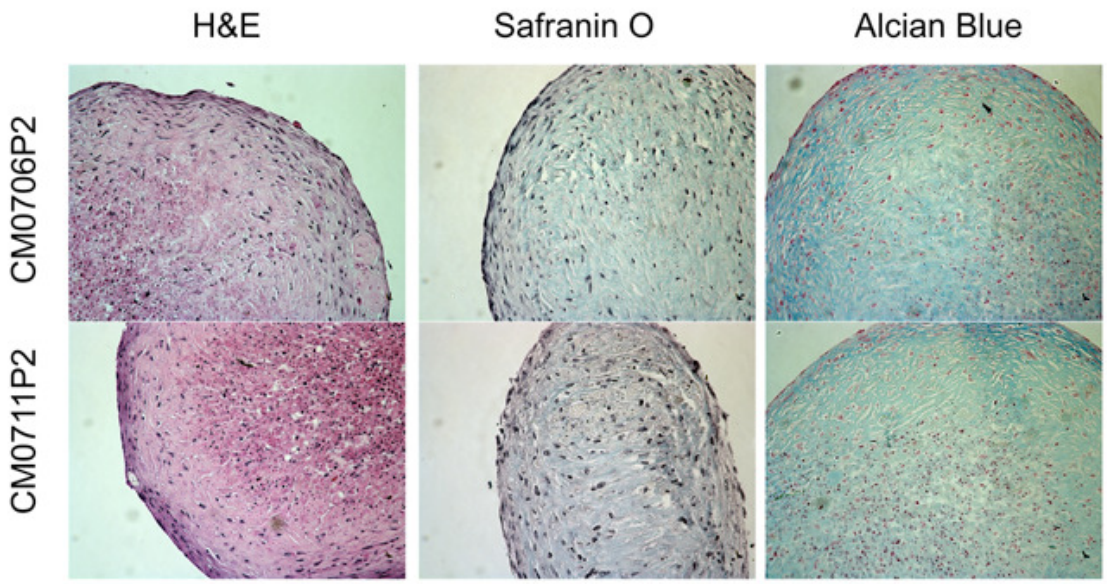
The chondrogenic potential of three independent bone marrow-derived cell populations is shown by histology after 28-day induction. Chondrogenic induction was unsuccessful in cell line BM0703P2 as evident by the chaotic morphology lacking resemblance to hyaline cartilage and the lack of extracellular glycosaminoglycans as illustrated by the negative Safranin O and Alcian Blue stains. Moderate chondrogenesis occurred in cell sources BM0704P2 and BM0705P2, which display varying degrees of hyaline-like cartilage morphology with the extracellular matrix being Alcian Blue positive and Safranin O positive as well for BM0705P2. H&E = hematoxylin and eosin stain.

Fig. 2 suppl.: Adipose tissue-derived putative MSCs chondrogenesis.



The chondrogenic potential of three independent adipose tissue-derived cell populations is shown by histology after 28-day induction. Chondrogenic induction was unsuccessful as evident by the chaotic morphology lacking resemblance to hyaline cartilage and the lack of extracellular glycosaminoglycans as illustrated by the negative Safranin O and Alcian Blue stains. H&E = hematoxylin and eosin stain.

Fig. 3 suppl.: Cord matrix-derived putative MSCs chondrogenesis.



Chondrogenic potential of two independent cord matrix-derived cell populations is shown by histology after 28-day induction. Chondrogenic induction was unsuccessful as evident by the chaotic morphology lacking resemblance to hyaline cartilage and the lack of extracellular glycosaminoglycans as illustrated by the negative Safranin O and Alcian Blue stains.

References

1. Mitchell KE, Weiss ML, Mitchell BM et al. Matrix cells from Wharton's jelly form neurons and glia. Stem Cells 2003; 21: 50-60.
2. Zuk PA, Zhu M, Ashjian P et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002; 13: 4279-4295.
3. Zuk PA, Zhu M, Mizuno H et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001; 7: 211-228.