CANINE MESENCHYMAL STEM CELLS FROM VISCERAL AND SUBCUTANEOUS ADIPOSE TISSUE FOR CELL-BASED THERAPY

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INTRODUCTION AND OBJECTIVES

Mesenchymal stem cells (MSCs) are nonhematopoietic multipotent stem cells shown for the first time in bone marrow, but they have also been isolated from other sources. Particularly, MSCs derived from bone marrow (BM-MSCs) and adipose tissue (AD-MSCs) are the most highly characterized and are considered comparable. Both have demonstrated broad multipotency with differentiation in adipocytic, osteocytic and chondrocytic cell lineages. However, the easy and repeatable access to adipose tissue, the simple isolation procedure and the greater numbers of fresh MSCs derived from equivalent amounts of fat versus bone marrow provide a clear advantage in using AD-MSCs over BM-MSCs.
This study compared some characteristics of canine AD-MSCs from subcutaneous and visceral fat. These findings were directed to obtain high quantity and quality AD-MSCs for clinical cell-based therapy.

MATERIAL AND METHODS

Subcutaneous and visceral fat samples were collected (Figure 1) from 2 different groups of 10 healthy donor dogs. The AD-MSCs were isolated from each sample and were cultured in Dulbecco's modified Eagle's medium-Low Glucose with 20% Fetal Bovine Serum.
Cell yield (number of cells/g of fat) was evaluated. AD-MSCs were sub-cultured up to passage (P) 6 and their proliferation potential was evaluated. The identity of AD-MSCs of each passage was verified by their ability to attach to flasks surface, to produce colony-forming units (CFU) (Figure 2) and to differentiate in chondrocytes, adipocytes and osteocytes.
The presence of transcription factors indicating self-renewal and undifferentiation (Oct4, Nanog and Sox2) were also investigated by RT-PCR analysis.

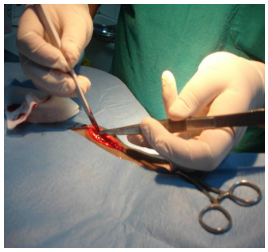

Fig. 1. Sample collection

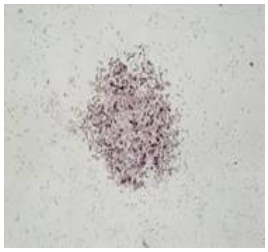

Fig. 2. CFU 50X

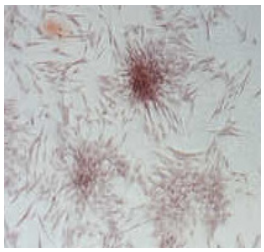

Fig. 3. Alizarina Red Staining (Ca^{2+})

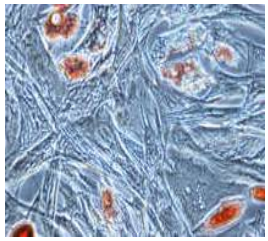

Fig.4. Oil Red O Staining (lipids)

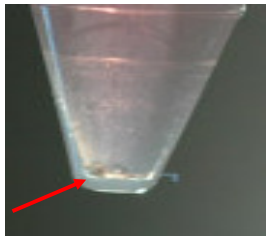

Fig. 5. Chondrocyte nodule

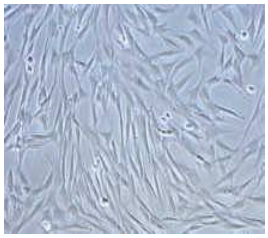

Fig.6. Fibroblast-like AD-MSCs

RESULTS AND CONCLUSION

The isolated AD-MSCs adopted a fibroblast-like shape (Figure 6). Statistical analysis demonstrated that subcutaneous and visceral fat yielded the same number of cells/g of fat. We also demonstrated that in a non-inductive medium the AD-MSCs reached the highest proliferative capacity, especially when derived from subcutaneous fat (Figure 7). Data shown that the obtained CFU number grew up to P2 and decreased in subsequent passages.
Cells derived from subcutaneous fat gave higher mean values of CFU than those of cells deriving from visceral fat in all of six passages (Figure 8). AD-MSCs differentiated in chondrocytes, adipocytes and osteocytes (Figures 3, 4, 5) up to P4. RT-PCR analysis revealed that cells expressed pluripotency-associated transcription factors Oct4, Nanog and Sox2 up to P6, but the mRNA expression level was higher at P2.
Finally, the results of our study showed that the AD-MSC from subcutaneous fat, grown up to P2 in our culture condition, were most suitable to use in regenerative therapy.

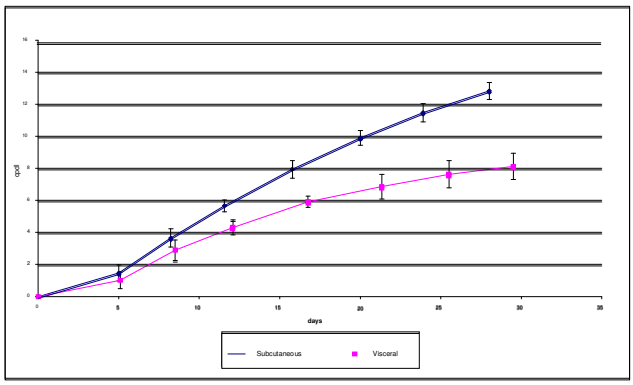

Fig.6. Cumulative pd of AD-MSC derived from visceral and subcutaneous fat

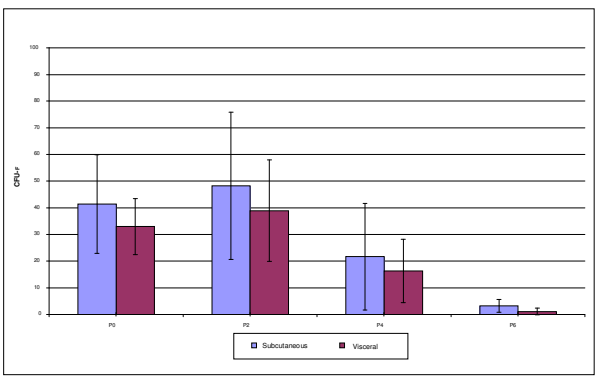

Fig. 7. CFU-F potential of AD-MSCs from canine visceral and subcutaneous fat

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