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Surface protein characterization of human osteoblasts derived from iliac crest and adipose tissue

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Introduction

Mesenchymal stem cells (MSCs) are multipotent stromal cells that have extensive proliferative potential and the ability to undergo multilineage differentiation. Traditionally, osteogenic differentiation of mesenchymal stem cells has been studied in cells isolated from bone marrow and iliac crest. However, these harvest techniques are associated with several problems, including donor morbidity, pain, and limited amount of cells. Only a few years ago, adipose tissue has been identified as another source of mulitpotent MSCs, which are referred to as Adipose Derived Stem Cells (ADSCs).

Aim of our study

The aim of our study was to provide a comparative analysis of primary osteoblasts from the iliac crest and osteogenic differentiated MSCs from adipose tissue, using osteoblast-specific protein expression.

Patients and methods

We harvested cells from 80 adult patients, undergoing elective surgery (mostly Hip and Knee replacement) with the patient's consent.

Table 1

Cell type	No. of patients	Age	First passage
ADSCs	37	53±14.7	10 days
BMSCs	17	51.4±16.6	12 days
OB	30	54±14.7	10days
ADOBs	17	61±9	7days

ADSCs: Adipose Derived Stromal or Stem Cells
BMSCs: Mesenchymal stromal or stem cells (MSCs) derived from Bone Marrow
OB: Osteoblasts from Iliac Crest
ADOBs Adipose Derived Osteoblasts (Osteoblasts cultured from adipose tissue)


Fig.1. Fat Tissue


Fig. 2. After digestion in collaginase type1

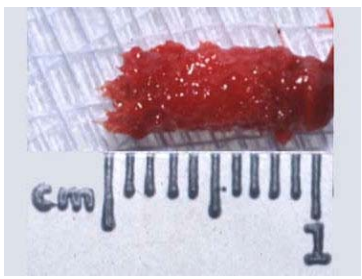


Fig.3. Osteoblasts culture tissue from iliac crest

Osteoblasts from cancellous bone were harvested with α MEM medium, BMSCs and ADSCs with DMEM medium and ADOB with osteogenic medium, (α MEM medium supplemented with 0.1μM-dexamethasone and 10mM β-glycerophosphate) and 10%FBS, ascorbic phosphate, and antibiotics/antimycotic as described by Zuk et al. Characterization of osteoblasts from cancellous bone (OB) and ADOB are confirmed by the expression of phenotypic markers of protein (osteocalcin, ALP, Collagen type1 and CBFA1) using RT-PCR, western blot, and immunocytochemistry. We also analyzed marker genes of ADSCs and BMSCs: nucleostemin, CD34, CD105, CD 10, CD 13, CD 59, and CD 166.

Results

RT-PCR analysis revealed that the non-differentiated ADSCs contained different types of stromal cells with a large variety of CD marker expression.
The osteoblasts cultured from adipose tissue (ADOBs) were 100% confluent on day 7, while primary osteoblasts reached 100% confluence at day 10. Bone specific protein expression, osteocalcin and collagen type1, was significantly weaker in osteoblasts derived from adipose tissue (ADOB) than in osteoblasts cultured from iliac crest Surface protein expression (CD) did not differ significantly in cells isolated from either fat tissue or bone, except CD13.

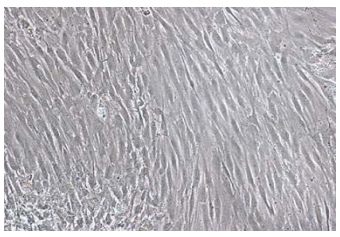


Fig. 4a. OB Passage 0 Day 10

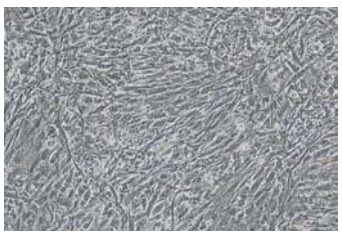


Fig. 4b. ADOB Passage 0 Day 7

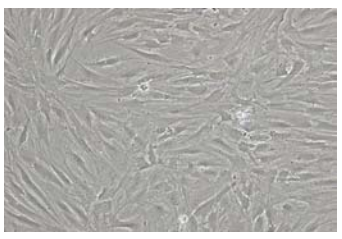


Fig. 5a. OB Passage 1 Day 14

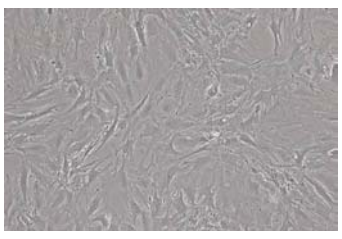


Fig. 5b. ADOB Passage 1 Day 11

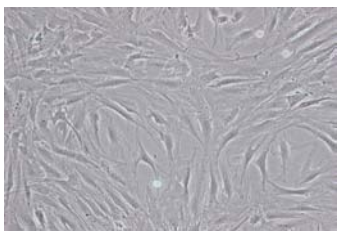


Fig. 6a. OB Passage 4 Day 26

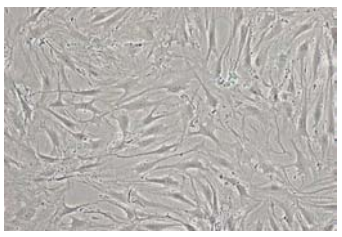


Fig.6b. ADOB Passage 4 Day 23

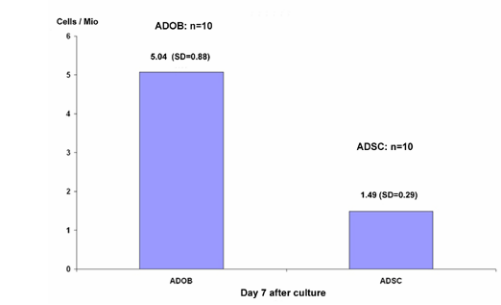


Fig. 7. Growth of cells

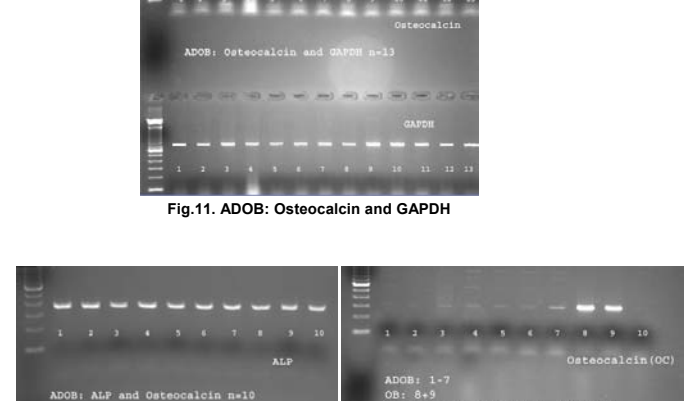
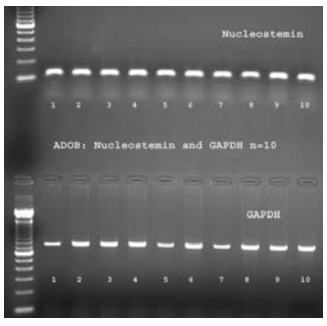


Fig. 9. ADOB: CD10, CD59, CD44, CD105

Fig.10a.OB: CD13 and GAPDH

Fig.10b. ADOB: CD13,CD166

Fig.11. ADOB: Osteocalcin and GAPDH



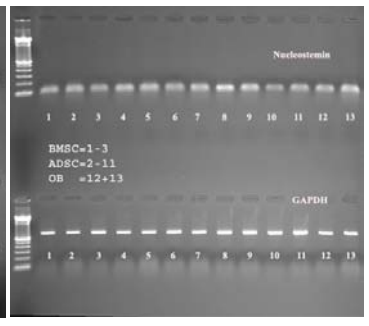


Fig. 8. ADOB: Nucleostemin und GAPDH

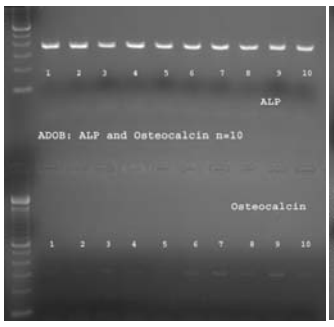


Fig.12a. ADOB: ALP and OC

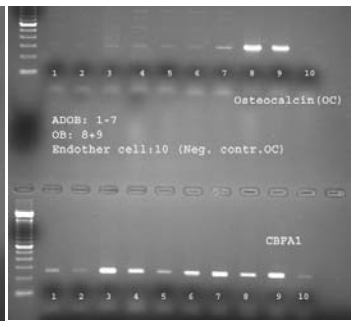


Fig.12b. OC and CBFA1

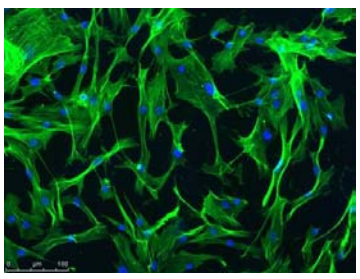


Fig.13a. ADOB: OC

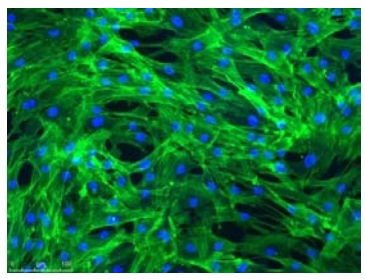


Fig.13b.OB: OC.

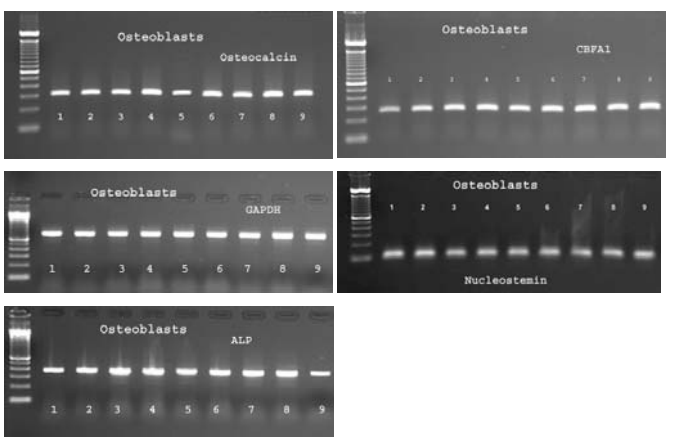


Fig.14. OB from iliac crest: Marker expression

Conclusion

The use of adipose tissue as a potential source for multipotent cells is a promising approach for future tissue engineering applications, due to its general availability and low donor morbidity.
However, future studies are necessary to establish the role of permanent differentiation of these multilineage potential cells.