Amniotic fluid mesenchymal stem cells have differentiation potential

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Objective

Amniotic fluid (AF) cells have emerged recently as a possible source of mesenchymal stem cells (MSCs) , with potential applications in regenerative medicine. The aim of this study was to isolate MSCs from human amniotic fluid, and to study their differentiation potential into adipocytes, chondrocytes, and osteocytes.

Methods

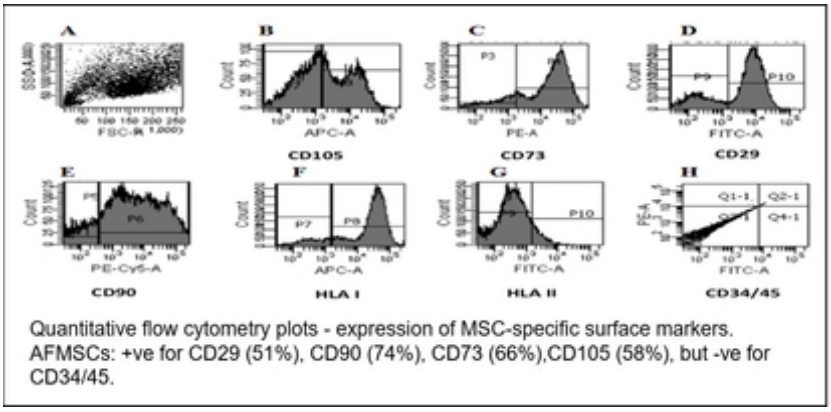
In 23 women undergoing ultrasound guided amniocentesis for genetic study after 16 weeks of gestation, after informed consent, an additional 5 ml of AF was taken for our study.The AF was centrifuged and cell pellet was mixed with media and seeded. They were isolated expanded and characterised. Phenotypic characterization of AFMSCs was done by flow cytometry. In 18 samples, the isolated mesenchymal stem cells (AFMSCs) were studied for adipocyte, chondrocyte and osteocyte differentiation using adipocyte differentiation media (Dexamethasone, Indomethacin, IBMX & Insulin), Chondrocyte Differentiation Kit (Stempro, Life Technologies)and osteocyte differentiation media (Dexamethasone, β-glycerophosphate & Ascorbic Acid 2 Phosphate) respectively.

Results

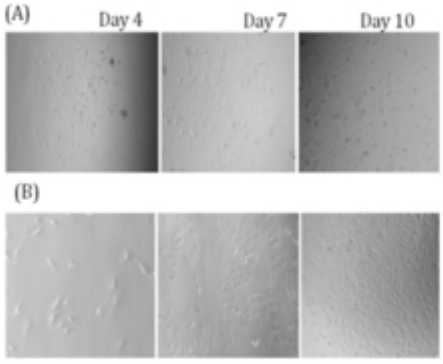
The mean period of gestation was 18.8 weeks (range 16+6- 23 weeks); 18/23 samples showed cell growth; 5 samples showed bacterial contamination and no cell growth was found. Primary culture cells from amniotic fluid showed mesenchymal cells with spindle-shaped morphology under phase contrast microscope. Average 5 million cells were obtained at the 3rd passage. AFMSCs +ve for CD29 (51%), CD90 (74%), CD73 (66%) and CD105 (58%), but -ve for CD34/45 : characteristic of mesenchymal stem cells. Cells from all 18 samples showed tri-lineage differentiation into osteocyte, chondrocytes, and adipocytes that were confirmed by Alizarin Red, Alcian blue, and Oil red O staining respectively.

Conclusion

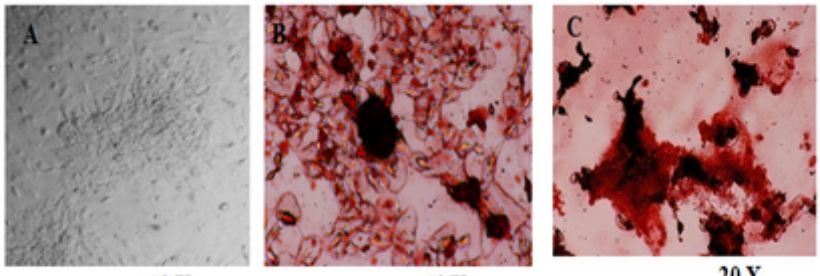
Successfull differentiation of AFMSCs into osteocyte, chondrocytes and adipocytes has been done. Thus, AF may provide an excellent source of MSCs both for basic research and for potential therapeutic applications.



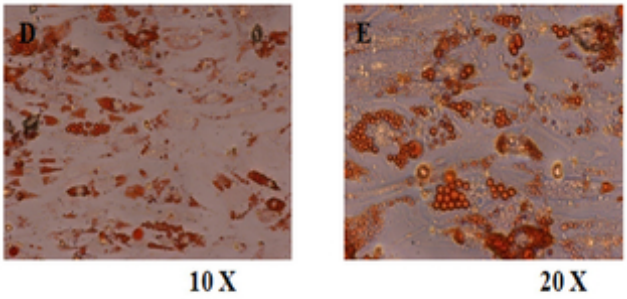
Quantitative flow cytometry plots - expression of MSC-specific surface markers. AFMSCs: +ve for CD29 (51%), CD90 (74%), CD73 (66%),CD105 (58%), but -ve for CD34/45.



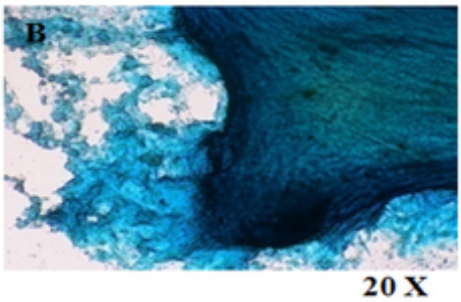
Phase contrast images of AFMSCs



Cells in osteoinductive culture exhibiting Osteogenic differentiation of AFMSCs, deposition of matrix mineral, as confirmed by Alizarin red S staining.



Adipogenic differentiation of AFMSCs from adipogenic induction media showing oil droplets and positive staining with Oil red O stain



Chondrogenic differentiation of AFMSCs from chondrogenic induction media showing positive staining with Alcian blue stain