

## OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED STEM CELLS (hASC)

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Human mesenchymal stem cells (hMSCs) have the potential to differentiate into cells of connective tissue lineages, including bone, cartilage, fat, muscle and also neurons. In our study we examined either the phenotypic profile of human adipose tissue-derived stem cells (hASC) and their osteogenic potential, comparing different osteogenic-inductive media. Cells were enzymatically isolated from adipose tissues derived by liposuction from several adult human donors, purified and then expanded. We obtained a cell yield of on average  $5 \times 10^5$  cells/ml of tissue, with a constant proliferative trend and a variable clonogenic capacity. hASC expressed MSCs-related cell surface antigens, like CD13, CD29, CD49d, CD 90 and CD105, determined by FACS analysis. At passage 4, hASC were induced to differentiate towards osteoblasts by culturing them in different inductive media, differing in ascorbic acid and dexamethasone concentrations. Osteogenic differentiation was determined by evaluating changes in cell-surface markers' expression, in osteogenic protein expression, like osteopontin and alkaline phosphatase, and in calcium deposition. Two weeks of osteogenic differentiation did not modify CD13 and CD90 expression, whereas CD14, CD29, CD49d and CD105 expressions were modulated. An evident osteopontin increase was detectable by immunofluorescence experiments and by FACS analysis in permeabilized differentiated hASC. A significant upregulation of ALP activity was observed in cells cultured in the presence of variable concentrations of dexamethasone and ascorbic acid, already after one week. This positive modulation was maintained for at least three weeks just in the presence of 10nM of dexamethasone and 150 $\mu$ M of ascorbic acid, whereas no significant upregulation was retained in hASC grown in medium with higher dexamethasone (100nM) and lower ascorbic acid concentration (50  $\mu$ M). Furthermore, 21 days differentiated cells showed a significant calcium deposition increase (more than 100%) which was determined by Alizarin Red S staining quantification. Extracellular matrix production was also confirmed in cells differentiated on different scaffolds, like hydroxyapatite and deproteinized bone, where no cytotoxic effects were observed. We conclude that hASC maintain their undifferentiated MSC-phenotype for more than two months in culture, and that the best osteogenic differentiation condition was observed with cells cultured in medium containing low dexamethasone and high ascorbic acid concentration, either in the absence or presence of scaffolds.