

CHARACTERIZATION OF FETAL TRABECULAR BONE CELLS FOR TISSUE ENGINEERING

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INTRODUCTION: Each year, 450,000 bone grafts are performed in the USA alone. Tissue engineering to replace diseased tissues with living substitutes has been attracting significant interest in the area of medical technology. Three elements are central in bone tissue engineering: 1) scaffold, b) cells, and c) proteins. In our laboratory we have been interested in fetal cell tissue engineering as bone cells originating from fetal tissue have the advantage that they are less differentiated, possess a vast capacity for multiplication and due to their immaturity can more easily pass the immunological incompatibility seen with adult cells. By associating fetal bone cells with a biodegradable scaffold, it is possible to obtain a cellular tissue where the cells are protected/resistant and that could easily be transplanted for tissue repair.

METHODS: Bone biopsies: Bone samples were obtained from a 30 year old patient during hip surgery and from fetal tissue following voluntary abortion (16 weeks) and tissues were obtained with accordance with the University Hospital Ethics Committee. Osteoblast culture: primary osteoblast cultures were established by rinsing tissue in PBS (containing penicillin and streptomycin 3 times). Trabecular bone was mechanically dissociated with a scalpel blade and medium was added (DMEM + 10% FCS). Medium was changed every two days. Growth curves: Cells were seeded at 1×10^4 cells. At each time point, cells were detached with trypsin/EDTA, centrifuged and counted.(triplicate). Alkaline Phosphatase activity: The alkaline phosphatase activity was measured using a kit (Sigma chemicals Co.). Treatment: Cells were treated with vitamin D3 10^{-8} M and dexamethasone 10^{-8} M. The activity was normalized to total cellular protein, which was determined by a Bio-Rad protein assay. 3-D culture on scaffold: Biodegradable scaffolds (Baxter Tissue Fleece, native equine collagen) were used as a support for fetal bone cells. Cells were seeded into scaffolds and following

5-15 days, scaffolds were mounted in O.C.T. and frozen in isopentane cooled in liquid nitrogen. Frozen sections were cut and colored with H&E

RESULTS and DISCUSSION: We have seen that fetal bone cells can be isolated and have alkaline phosphatase activity after differentiation treatment (Fig.1). They have very high dividing potential when compared to cells of the 30 year old patient. In addition, fetal bone cells grow extremely well in association with a biodegradable collagen scaffold (unlike adult cells) which results in the creation of a 3-D tissue, which is easily manipulated.

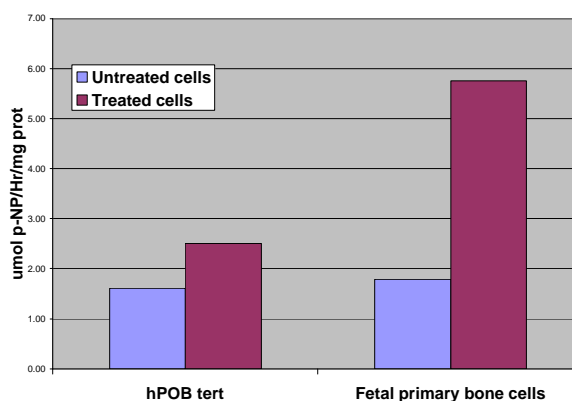


Fig. 1: Alkaline phosphatase expression after 1 week culture treatment with vitamin D3 and dexamethasone in comparison with an immortalized cell line.

REFERENCES: ¹ Oreffo RO, Triffitt JT. *Bone*. 1999 Aug;25(2 Suppl):5S-9S.

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