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Influence of Seeding Concentration of Cells on Efficiency of Occupation of Ca-P 3D Scaffolds with Bone Marrow Stromal Stem Cells and Differentiation in Bone-like Tissue .

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INTRODUCTION: Bone marrow-derived stromal stem cells (BM SSCs) have the capacity for renewal and the potential to differentiate into multiple lineages of mesenchymal tissues (bone, cartilage, fat and muscle). These cells are very attractive cell source in tissue engineering. BM SSCs are capable of forming bone when implanted ectopically in an appropriate scaffold. Two important parameters in scaffold-based tissue engineering include the cell seeding efficiency and the uniform distribution of cells in the matrix. The practical application of BM SSCs in 3D scaffolds is hampered by its low cell seeding efficiency. The aim of this study was to assess the efficiency of seeding concentration of cells on bone formation with BM SSCs in CaP scaffolds.

METHODS: Cells were isolated from BM obtained by puncturing iliac crest under the intravenous anesthesia of patient, expanded and detected by expression of surface antigens by flow cytometer FACS (Epics Elite Coulter). For derivation of human 3D transplants of bone tissue we used CaP scaffolds (BD Biosciences). Cell suspension containing 2.5 x 10^5, 1 x 10^6, 2 x 10^6 cells in 200 ml of media loaded onto scaffolds, respectively, and incubated the tube with gentle agitation (~50 rpm) on an orbital shaker at 37^{o}C for 2-hours. The osteogenic culture media was DMEM–LG supplemented with 10% FBS, 0.2 M ascorbic acid, 1x10^{-7}dexamethasone, 10mM β-glycerophoshate. Cell-scaffold constructions fixed in 4% paraformaldehyde for 2 days, then decalcified for further 10 days in 10% EDTA prior to embedding in paraffin. For histological analysis, 5 mm sections of the implants were prepared and stained with hematoxylin and eosin. Immunohistochemical analysis of samples was detected using Abs for osteopontin, produced by R&D Systems.

RESULTS: BM SSCs were positively stained with Abs for the Ags: CD10, CD13, CD29, CD44, CD49a,b, CD54, CD71, CD73, CD90, CD105, CD166, HLA ABC. The cells were negative for CD34, CD45, CD133, CD31, Flk, CD117, STRO-1, HLA DR, DP and DQ. Comparative analysis of results has shown that at loading cells in concentration 2.5 x 10^5, 1 x 10^6, 2 x 10^6 in scaffolds, 2 x 10^5, 8.5 x 10^5 and 1.26 x 10^6 cells occupied porous sponges, accordingly (Fig.1 a, b, c). Furthermore, only seeding cell concentration 2 x 10^6 allowed cells to occupy scaffolds densely and to differentiate in osteogenic pathway. Culturing of cells in this concentration in scaffolds with osteogenic medium for 21 days led to formation bits of bone-like tissue in vitro. Histochemical analysis showed that cells had morphology of osteoblasts and osteocytes. Immunohistological analysis detected expression of osteopontin (Fig1.d). The cells supported an uniform area of bone formation in scaffolds.

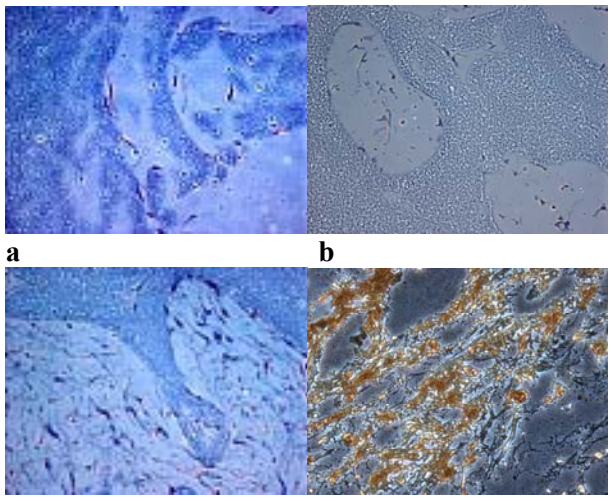

Fig.1:Effect of seeding cell concentration on occupation of Ca-P scaffolds and differentiation BM SSCs in cells of bone tissue after 3 week of culture: a) 2.5 x10^5, b) 1 x 10^6, c) 2 x 10^6 d) expression of osteopontin.

DISCUSSION& CONCLUSIONS: A typical Ca-P scaffold for tissue engineering should consist of inter-connected macroporous structures 200-400 μm to facilitate cell distribution and enhance diffusion rates to and from the center of the scaffold. Therefore, low cell seeding efficiency is a prevalent problem for such 3D scaffolds with similar architecture. Results of our research have shown that seeding cell concentration influences not only settling scaffolds with cells, but also on a differentiation of BM SSCs in osteogenic pathway. So, seeding cell concentration 2 x 10^6 is optimal for engineering of bone tissue in vitro in CaP scaffolds.