

Effect of bone graft biomaterials at different chemical composition and geometry on human Bone Marrow Stromal Cells osteogenic differentiation

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Introduction: For the repair of bone defects, a tissue engineering approach would be to combine cells capable of osteogenic (i.e. bone-forming) activity with an appropriate scaffolding material to stimulate bone regeneration and repair. Bone Graft Materials (BGMs) constitute an essential adjuvant for bone tissue regeneration in oral surgeries to achieve osteosynthesis, bone augmentation and implant osseointegration. Human mesenchymal stem cells (hMSCs), when combined with hydroxyapatite/beta-tricalcium phosphate (HA/TCP) ceramic scaffolds have been shown to induce bone formation in large or long bone defects. BGMs are currently used in bone regeneration in oral surgery, therefore the aim of our study was to investigate whether biomaterials with different chemical composition and geometry improve the osteogenic capability of the hMSCs in a site of implant.

Methods: hMSCs, derived from pooled bone marrow of three donors, were expanded according to standard protocols and cultured in presence of the different biomaterials for 14 and 28 days.

Four different BGMs were selected: deantigenic equine bone tissue, **BIOGEN** (scaffold A); natural bone mineral (scaffold B); silica tricalcium phosphate and hydroxyapatite (HA) composite, **BONIT-MATRIX**, (scaffold C); bovine bone conjugated with a synthetic peptide, **PEP-GEN P15**, (scaffold D). hMSCs morphology and interaction with BGMs was observed with optical and scanning electron microscopy. Cells proliferation and vitality were determined by MTT analysis. Early and later osteogenic gene expression (OC, BSP, ALP, COLL1) was determined by quantitative real time PCR.

Results: SEM showed an early adhesion of hMSC to the different BGMs within 6-72 hours with a fibroblastic phenotype. An increment of cell density was revealed on the surface of scaffold B respect to the others. Early and later osteogenic markers revealed by quantitative real time PCR (qPCR) throughout the experimental time-course were equally expressed from the hMSC cultured in presence of different BGMs, although an high induction of BSP gene was detected in cell associated to scaffold D and COLL1 gene in scaffold A. However these markers resulted to be less expressed when compared to the cells cultured in osteogenic medium as control. In vivo implant of scaffolds associated to the hMSCs in immunodeficient mouse animal model are in progress to evaluate the ability of cells to reconstitute the bone tissue.

Conclusion: hMSCs express osteogenic markers when cultured on BGMs with different chemical composition and geometry demonstrating the capability of scaffold to support their proliferation and differentiation.

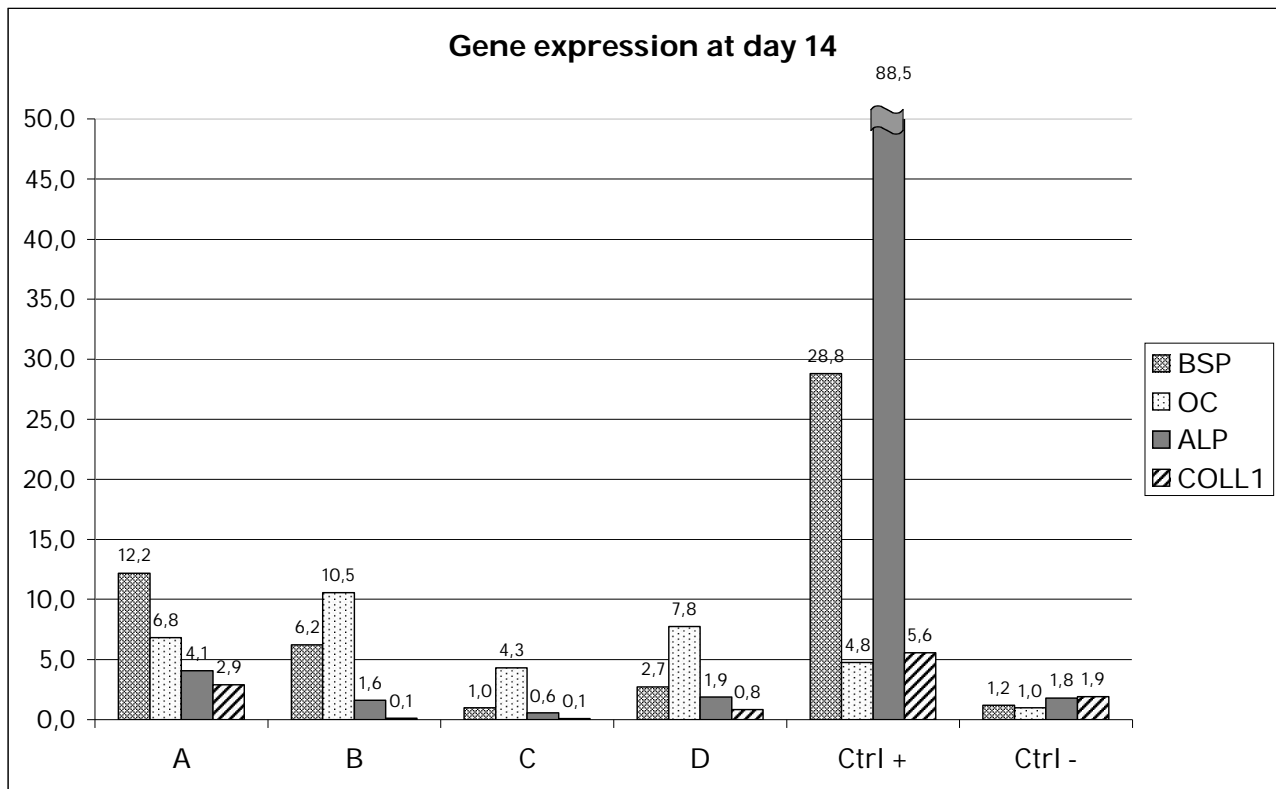


Fig. 1 Bar chart depicting the expression of four genes, significant for osteogenic differentiation, at day 14, of human bone marrow stromal cells, cultured in presence of different bone graft materials. Positive control cells were supplemented with osteogenic medium. Negative control did not receive neither any treatment nor biomaterials.