Biphasic Calcium Phosphate Bioceramics Modulate Stem Cells and Pre-Osteoblasts` Phenotype

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Introduction: Biphasic calcium phosphate bioceramics (BCP), an intimate mixture of hydroxyapatite (HA) and β-TCP (β-tricalcium phosphate), have been successfully used in orthopedics, cranio and maxillofacial reconstructions for decades (1). BCP can fulfill this very broad range of clinical demands due to their great versatility in terms of mechanical strength, resorption rate, bioactivity and potential osteoinductive property (2, 3). However, the degree and profile of modulation in cell response promoted by BCP scaffolds are not completely understood. This study aims at quantifying and mapping the temporal profile of gene expression of stem cells and pre-osteoblasts when seeded on BCP scaffolds.

Methods: A nanostructured micro and macroporous BCP with 65% HA/35% β-TCP ratio, in a granular form corresponding to 20-40 mesh (Osteosynt®, EINCO Biomaterial Ltda., Belo Horizonte, Minas Gerais, Brazil) was used. Stem cells isolated from 3 different tissues (bone marrow, adipose tissue and dental pulp) as well as pre-osteoblasts (MG-63 cell lineage) were seeded on a monolayer of scaffolds at $3x10^4$cells/cm^2. Cells were purchased from Lonza (USA), except for the dental pulp stem cells which were isolated from extract teeth obtained from the Department of Oral Surgery (New York University College of Dentistry). Stem cells were cultured in control and osteogenic media. The control media for mesenchymal stem cells (bone marrow) and adipose-derived stem cells corresponded to Poietics™ MSCBM and Poietics™ ADSC-BM (Lonza, USA), respectively. Dental pulp stem cells were grown in DMEM/F12 (GYBCO, USA) with 15% FBS, 100units/ml penicillin/streptomycin, 2mM L-glutamine and 2mM non essential amino acids. MG-63 cells were cultured in Minimum Essential Medium Alpha (GIBCO, USA), 10% FBS and 100units/ml of penicillin/streptomycin. Osteogenic media corresponded to control media supplemented with 100nM dexamethasone, 0.05mM ascorbic acid and 10mM β-glycerophosphate. Control groups were also represented by cells cultured directly on polystyrene plates, without BCP. The physical features of the BCP as well as cell adhesion were analyzed by SEM; the modulation of gene expression (RUNX2, PPAR-γ, SOX9, bone sialoprotein, osteopontin, alkaline phosphatase, with GAPDH as housekeeping gene) was quantified by RT-PCR. Statistical analysis was performed using Turkey test ($p \leq 0.05$).

Results: The BCP presented micro and macroporosity with a nanostructured surface to which cells attached and changed their morphology, compared to cells grown on polystyrene plate. Since day 01 of cell seeding, the BCP promoted changes in the pattern and timing of gene expression compared to the control groups (cells on polystyrene plate). Such changes were observed not only within groups, when the control and osteogenic media was considered, but also between groups, when stem cells isolated from different tissues were analyzed (Fig. 1). Increased expression of RUNX-2 was observed but the quantification and presence of association with a higher expression of PPAR-γ and SOX 9 was dependent on the cell type studied. Pre-osteoblasts in control media also showed higher levels of bone sialoprotein and osteopontin when in contact with the BCP.

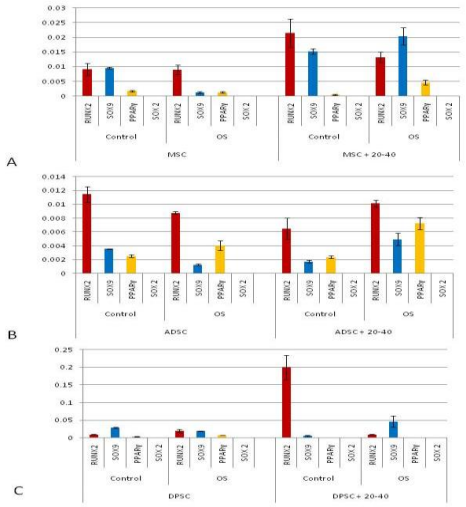

Fig. 1: Modulation of gene expression of mesenchymal stem cells (MSC) (A), adipose-derived stem cells (ADSC) (B) and dental pulp stem cells (DPSC) (C) at day 01 of cell seeding. Control = control media; OS = osteogenic induction media.

Conclusions: This study demonstrated the modulation of stem cells and pre-osteoblasts` gene expression when seeded on BCP scaffolds. The increased level of osteogenic markers promoted by BCP, in control media, is in agreement with studies that have shown their intrinsic osteoinductive capacity. The molecular mechanisms and pathways that determine stem cell fate as well as the concomitant increase of expression of SOX-9 and/or PPAR-γ are being investigated. These results support the clinical success of BCP in the reconstruction of bone defects and provide guidance for the elaboration of new techniques to be used in bone tissue engineering.

References:

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