Osteogenesis and Chondrogenesis on Biphasic Calcium Phosphate Custom Made Constructs: *In vitro* study

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1 - Introduction

The complexity and extension of bone defects caused by trauma and tumors frequently require special therapeutic approaches. Bone grafting using particulate or pre-shaped pieces may not provide complete and accurate reconstruction. Therefore, custom made pieces, prepared specifically for each patient after CT scan imaging, represents a better alternative as they provide an anatomical reconstruction of the affected area (Fig. 1) [1]. Biphasic calcium phosphate (BCP) bioceramics, consisting of an intimate mixture of hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP), have been successfully used in different presentation forms. Variations in the HA/β-TCP ratios in BCP allow for the preparation of a material that fulfills the biological and mechanical properties of the axial and appendicular skeleton including load-bearing areas [2]. Fabrication of custom-made pieces may also be a promising approach for the reconstruction of articular surfaces.

2 - Objectives

The aim of this study was to investigate the potential of BCP constructs having different HA/β-TCP ratios in supporting osteogenesis and chondrogenesis.

3 - Methods

BCP custom made constructs were fabricated and provided by EINCO Biomaterial Ltda (Belo Horizonte, Minas Gerais, Brazil). The HA/β-TCP ratios of the BCP scaffolds were: BCP 1, 65%HA/35%β-TCP; BCP 2, BCP 1 + PMMA; BCP 3, 20%HA/80%β-TCP. Human mesenchymal stem cells, MSC (Lonza, USA) were expanded until passage 2 and seeded at $3x10^4$cells/cm^2 (for osteogenesis) and $2.5x10^5$cells/cm^2 (for chondrogenesis). Control groups were represented by cells cultured on polystyrene plates (osteogenesis) or in pellets cultures (for chondrogenesis). Cells were cultured in the following media: control (DMEM low glucose, 10% FBS and 1% antibiotic-antimicotic); osteogenic induction (control media, 100nM of dexamethasone, 0.05mM of ascorbic acid and 10mM of β-glycerophosphate); and chondrogenic induction (DMEM high glucose, 200mM L-glutamine, 1mM dexamethasone, 170mM ascorbic acid, 1% ITS, 1% antibiotic-antimicotic, 100mM sodium pyruvate, 350mM proline, 0.01µg/ml TGF-β3). The topographic features were analyzed using SEM. Cell proliferation and cell viability assays were determined using Quanti-iT ™ PicoGreen® dsDNA Assay Kit (Molecular Probes, Invitrogen, USA) and XTT Cell Viability Assay (Biotimum, Inc. CA, USA), respectively. Quantitative gene expression (bone sialoprotein, osteopontin, aggrecan and collagen type II) was determined using RT-PCR, with RPLP0 as housekeeping gene. Statistical analysis, Tukey ($p<0.05$).

4 - Results

The SEM images of the scaffolds showed a nanostructured surface with an intricate and interconnected micro-, meso- and to a lesser extent, macroporosity, with higher meso and macroporosities observed in the BCP 2 scaffolds (Fig. 2). Cells were observed growing in mono- and multi-layers, and penetrating the macroporosity (Fig. 2). The highest optical density values that indicate viable cells as well as higher cell number were observed at days 7 and 11, mostly within the BCP2 scaffolds (Figs. 3 A and B). The bone markers expression was observed in early time points in both, osteogenic induction media and control media (Figs. 4 A and B). For chondrogenic differentiation, the expressions of collagen type II and aggrecan were observed only in the induction media (Figs. 5 A and B).

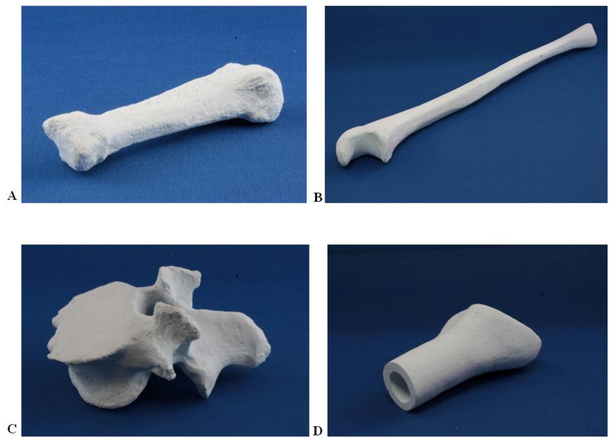

Fig. 1: Custom made constructs made of biphasic calcium phosphate ceramics. A – phalanx, B – ulna, C – vertebra, D – section of a tibia showing the space corresponding to the medullar zone.

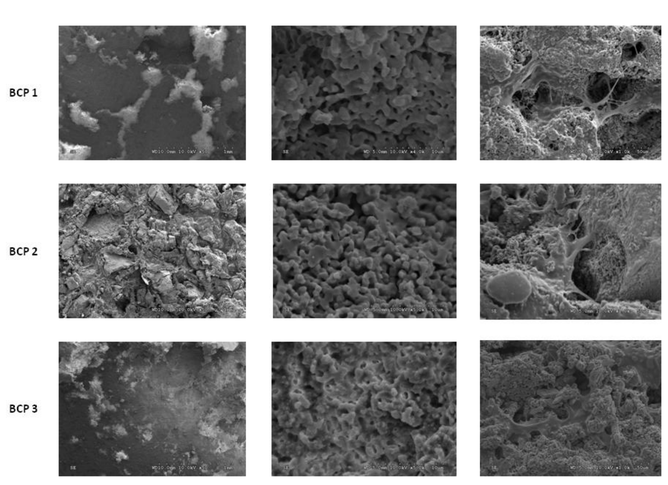

Fig. 2: SEM images of the three scaffolds at lower and higher magnifications (left and middle columns, respectively), as well as at day 07 of cell culture (right column).

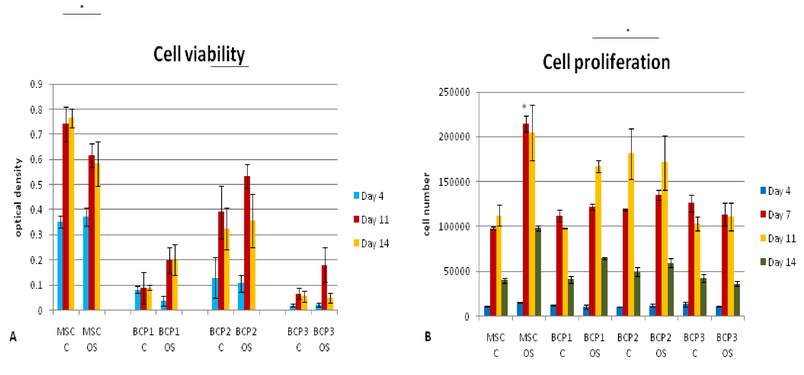

Fig. 3: BCP 2 scaffolds showed the highest values of viable cells (A). BCP 2 in both media and BCP 1 in control media favored cell proliferation more than BCP 3 ($p<0.05$).

5 - Discussion

The advantages of BCP ceramics over autografts and allografts have been extensively described [1, 2]. BCP scaffolds have the potential to promote new bone formation through intramembranous and endochondral processes [3]. The cell response is directly dependent on the BCP chemical composition (HA/β-TCP ratio) and the physical features (nanostructure, surface topography, and interconnecting micro- and macro-porosities). The better performance of the BCP 2 scaffolds may be attributed to these features as shown by higher cell viability, proliferation and differentiation.

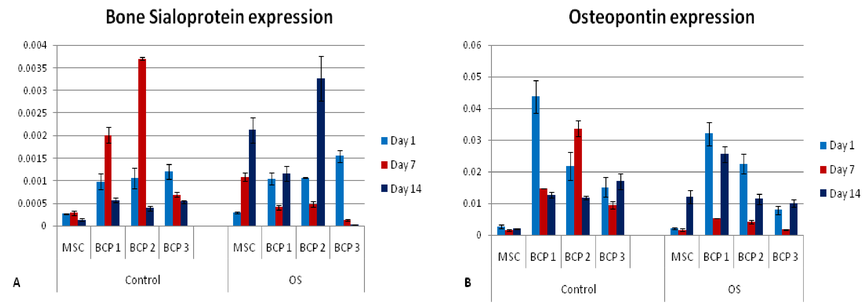

Fig. 4: Analysis of osteogenic differentiation through the expression of bone sialoprotein (A) and osteopontin (B), in control and osteogenic induction media (OS). The three scaffolds modulated the MSC phenotype. BCP 2 showed the most promising results, followed by BCP 1 scaffolds. Values were normalized to RPLP0 housekeeping gene.

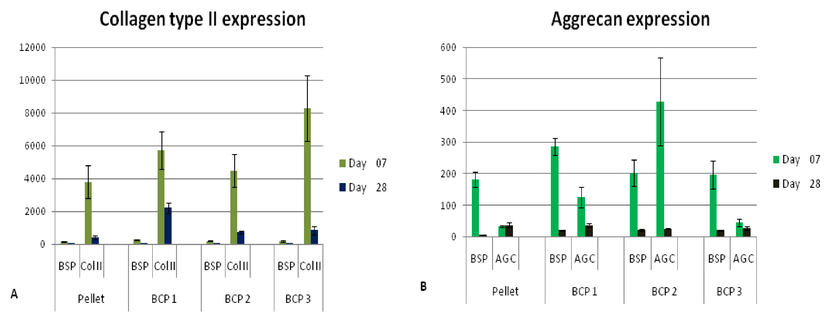

Fig. 5: Analysis of chondrogenic differentiation. The levels of collagen type II expression (A) were higher than the expression of aggrecan (B). Values were normalized to RPLP0 housekeeping gene. BSP: bone sialoprotein.

The high expression of osteogenic markers, even in control media, is consistent with BCP's potential to promote and sometimes induce bone formation. Regarding chondrogenic differentiation, the expression of collagen type II and aggrecan was shown in the induction media, indicating that BCP-based scaffolds can support, but not necessarily induce chondrogenesis.

6 - Conclusion

This study shows that BCP-based custom made pieces can be prepared to substitute and reconstruct the skeleton. More importantly, BCP/polymer constructs with specific topography can promote and induce osteogenesis and support chondrogenesis thus opening new perspectives for the treatment of defects that include articular surfaces. Engineering a multilayer BCP/polymer scaffold may be useful for the reconstruction of articular surfaces.

References

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Acknowledgments

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