Clinical and in vitro evaluation of different nanostructured calcium phosphate-based ceramics for bone regeneration

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INTRODUCTION

Biphasic calcium phosphate ceramics (BCP) have been frequently reported to repair small bone defects in non-load bearing areas. However, it is essential to have BCP with different HA/ß-TCP ratios and distinct mechanical properties that allow for a broader range of clinical applications (1, 2, 3).

OBJECTIVE

To analyze the in vitro osteogenic potential of BCP presenting distinct physical properties, and to report its clinical outcome in the reconstruction of critical size bone defects.

METHODS

In vitro studies: BCP (65% HA/ 35% ß-TCP) in granular form (Osteosynt®, EINCO Biomaterial Ltda., Belo Horizonte, Minas Gerais, Brazil), with 3 different particle sizes (20-40, 40-60 and 60-80 mesh), and blocks composed only of BCP and BCP + Polymethylmethacrylate (PMMA) were seeded with human mesenchymal stem cells (h- MSC) at 3x104cells/cm2 and cultured in 2 medium: control media (DMEM low glucose, 10% FBS and 1% antibiotic-antimicotic) and osteogenic media (control media, 100nM of dexamethasone, 0,05mM of ascorbic acid and 10mM of ß-glycorophosphate). Morphological features of the materials and the cells were examined using scanning electron microscopy (SEM); cell proliferation/viability was determined using XTT Cell Viability Assay (Biotimum, Inc. CA, USA); alkaline phosphatase activity was quantified by the conversion of para-nitrophenyl phosphate to para-nitrophenol (Sigma); cell number was measured by DNA content (Quanti-iT ™ PicoGreen® dsDNA Assay Kit, Molecular Probes, Invitrogen, USA); quantitative gene expression of bone sialoprotein and osteopontin was measured by RT-PCR and osteocalcin expression was quantified using the ELISA Kit (Invitrogen, Inc.). All the results were statistically analyzed by ANOVA ($p<0.05$).

Clinical studies: Large bone defects were filled with granules and blocks and analyzed radiographically and by computed tomography (CT).

RESULTS

SEM analysis demonstrated that all of the scaffolds had a nanostructured surface with pores ranging from 1 to 450µm. The cells were observed growing on the surface of the scaffolds, within the macropores and bridging the openings of the macropore structure (Figure 1). The highest cell numbers were detected on the block BCP + PMMA and the granules 40-60 mesh and 60-80 mesh (up to 420µm) at the latest time point (Figure 2). Higher levels of gene expression were determined for cells grown on the materials, even in the control media, in comparison to cells on tissue culture

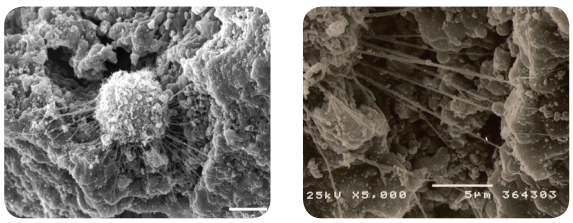


Figure 1: Physical structure of the BCP and the adhesion of a stem cell in a pore (granule 40-60 mesh).

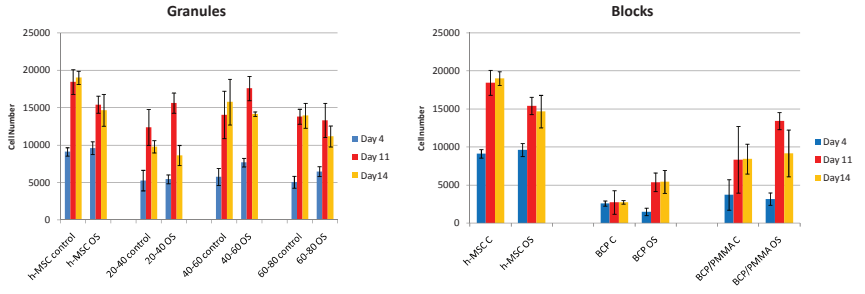


Figure 2: Analysis of the cell viability over 14 days of culture. The cell number and viability was higher for the granules compared to the blocks.

plastic (Figures 3 and 4). Higher levels of alkaline phosphatase activity were observed fro cells on the granules (Figure 5). Cells on BCP blocks had the highest level of osteocalcin production (Figure 6). Clinical data show that the BCP Osteosynt® allows and supports the regeneration of large bone defects in load-bearing areas (Figures 7 and 8).

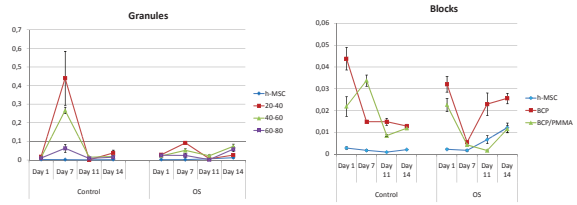


Figure 3 : Analysis of osteopontin showing higher levels of expression in the control media, except for the smallest granules.

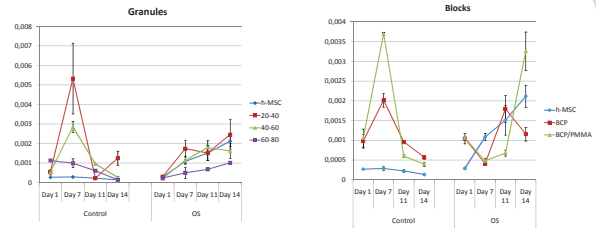


Figure 4 : Bone sialoprotein expression was higher in the control media compared to the induction media (OS). The granules 60-80 mesh showed the lowest level of expression and the blocks BCP/PMMA the highest levels in the OS media at the latest time point.

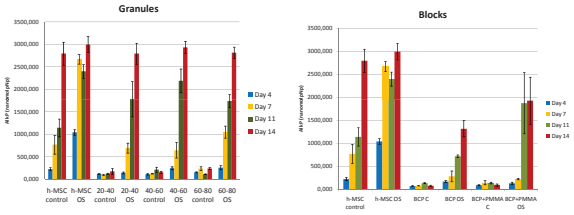


Figure 5: Alkaline phosphatase activity was higher for the granules at the latest time point compared to the blocks.

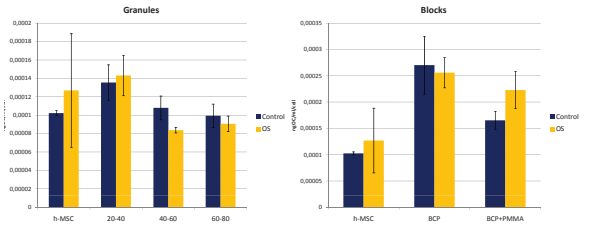


Figure 6: Osteocalcin synthesis after 14 days of culture. The BCP blocks showed the highest level of osteocalcin expression, even in for cell cultured in control media, among groups.

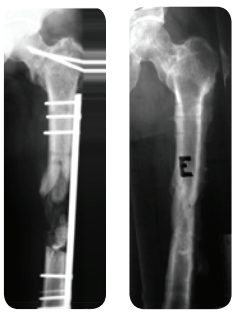
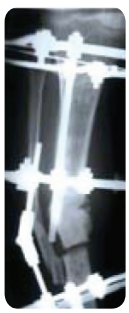
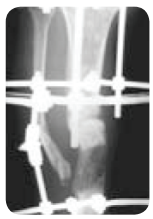


Figure 7: Femoral segmental bone reconstruction prior the correct BCP implantation (A) and 2 years postoperatively (B).





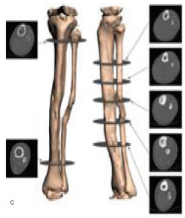


Figure 8: Tibia reconstruction with granules 40-60 mesh followed by bone lengthening. The CT images show the formation of medullar and cortical zones in the area that was reconstructed with the BCP. A-Osteotomy after 18 months of the BCP implantation, B- Bone lengthening, C- The final CT analysis.

CONCLUSION

This study demonstrated the biocompatibility and osteogenic potential of nanostructured biphasic calcium phosphate ceramics alone or in composites.

The in vitro study confirmed that the physical characteristics of the scaffolds, specifically granule size can influence the level of osteogenic marker expression. Different chemical and physical structures can give rise to biomaterials that are able to solve specific clinical situations. Defects can vary from non-load bearing areas where high levels of osteogenic activity may be required, to load bearing areas, where the mechanical support and the maintenaince of the space are of importance, at least in the initial phases of the bone healing process.

REFERENCES

1. Schindler OS, Cannon SR, Briggs TWR, Blunn GW. Composite ceramic bone graft substitute in the treatment of locally aggressive benign bone tumors. J Orthop Surg. 2008, 16(1): 66-74.

2. Lobo SE, Wykrota FHL, Oliveira ACMB, et al. Quantification of bone mass gain in response to the applications of biphasic bioceramics and platelet concentrate in critical-size bone defects. J Mater Sci Mater Med. 2009, 20: 1137-47.

3. Hing KA. Bioceramic bone graft substitutes: influence of porosity and chemistry. Int J Appl Ceram Technol. 2005, 2 (3): 184-99.

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