In vitro Stem Cell Response to BCP and BCP/Polymer Scaffolds

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INTRODUCTION

Biphasic calcium phosphate (BCP) ceramics of varying hydroxyapatite and ß-tricalcium phosphate (HA/ß-TCP) ratios are gaining acceptance as bone substitute materials (1). Customized pieces, made in accordance to the requirements of each patient can also be obtained with such biomaterials and represent an important tool for the reconstruction of acquired and congenital facial defects (2) **(Fig. 1)**. In orthopedic surgeries, polymethylmetacrylate (PMMA) has been extensively applied as spacers or cements but with controversial clinical results, including the formation of fibrous tissue at the interface with bone. In spite of that, it has been used for decades (3). The search for a scaffold that possesses the advantages of both biomaterials without their disadvantages justifies this study.

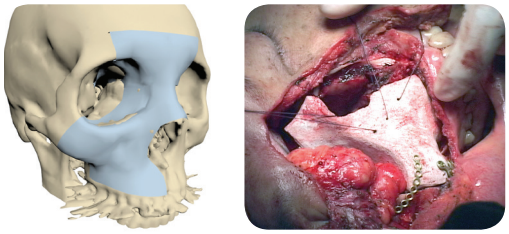

Fig.1:Example of the application of a customized piece for the reconstruction of a hemifacial defect. A: the 3D analysis of the piece (blue area) that will be constructed using BCP; B: transoperative view of the piece in the surgical site.

OBJECTIVE

This study aims at evaluating the biocompatibility and osteogenic potential of two BPCs of different HA/ ß-TCP ratios (ceramic 1 and ceramic 2) and BCP + PMMA composite scaffolds that may be used as customized pieces in the reconstruction of large maxillofacial bone defects.

METHODS

Human mesenchymal stem cells (h-MSC) were expanded until passage 2 (P2) and seeded at 3x104cells/cm2 onto the scaffolds: ceramic 1 (65% HA/ 35% ß-TCP), ceramic 2 (10% HA/90% ß-TCP) and composite (ceramic 1 + PMMA). The scaffolds were provided by EINCO Biomaterial Ltda. (Belo Horizonte, Minas Gerais, Brazil). The constructs (scaffolds + stem cells) were kept in 2 different media: control (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-glycerophosphate). As control groups, stem cells were seeded directly on polystyrene plates. Morphological features of the materials and the cells were examined using scanning electron microscopy (SEM); cell 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) and quantitative gene expression of bone sialoprotein and osteopontin was measured by RT-PCR. All the results were statistically analyzed by ANOVA ($p<0.05$).

RESULTS

The SEM analysis showed that all the scaffolds have similar nanostructured surface but different porosities. All surfaces allowed cell attachment and proliferation. Formation of layers penetrating the pores was observed **(Fig. 2)**.
The number of viable cells was increased in the composite scaffolds, mainly in osteogenic media **(Fig. 3)**. Most of the groups reached their highest cell number

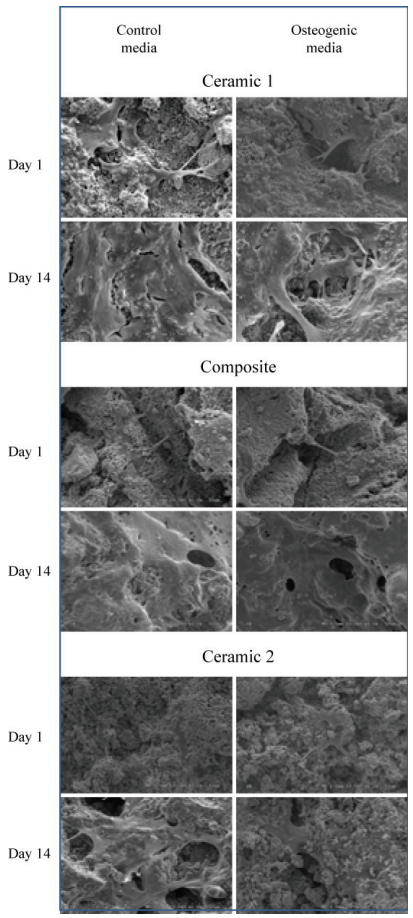

Fig. 2: SEM images showing the nanostructured surface of the scaffolds and the morphology of the cells at days 01 and 14. C: control media; OS: osteogenic media; Cer 1: ceramic 1 (HA/ß-TCP ratio: 65/35); Comp: composite (cer 1+PMMA); Cer 2: ceramic 2 (HA/ß-TCP ratio: 10/90).

by the 11th day. The ceramic 1 and the composite presented higher number of cells compared to the ceramic 2 **(Fig. 4)**. The alkaline phosphatase activity was elevated in all groups at day 14. Interestingly, the composites also showed increased activity by day 11 in the control media, which was higher than all other groups at the same time point **(Fig. 5)** The gene expression, normalized to the housekeeping gene RPLP0, showed higher modulation of osteopontin for the ceramic 1 (day 1), while the composite showed higher modulation of bone sialoprotein (day 7). Although these values were observed at different time points, they were higher in the control media compared to that in the osteogenic media **(Fig. 6)**.

DISCUSSION

The era of regenerative therapies relies on providing alternatives that allow bone reconstruction and not only bone substitution (2). Therefore, the use of materials that support and promote osteogenesis and maintain its stability over time has been preferred over the inert materials such as PMMA (1).
It is known that PMMA can promote a fibrous tissue formation and has weak mechanical properties. However, variations of its components, formulations ratios and molecular weights have been shown to improve its in vivo performance (3). This was demonstrated in our present study which has shown that special fabrication of a BCP/PMMA composite created positive effects on cell viability, proliferation and differentiation towards the osteogenic lineage. The same study also showed that HA/ß- TCP (ceramic 1) with a higher ratio elicited greater cell response than the one with lower HA/ßTCP ratio (ceramic 2). The molecular mechanisms that explain the modulation of different genes, i.e., osteopontin for the ceramic 1 (HA/ ß-TCP = 65/35), and bone sialoprotein for the composite (BCP/PMMA), need to be further investigated. Studies to determine if the same phenomenon is observed with BCP and other polymers (e.g., PLGA, PCL) are underway.

CONCLUSION

This study demonstrated the in vitro biocompatibility and osteogenic potential of nanostructured biphasic calcium phosphate (BCP) ceramics alone or in composite form with PMMA. It provided evidences that, depending on the manufacturing process and pre-treatment of its components, the composite BCP/PMMA can not only overcome the disadvantages of using such polymer alone but also can improve its interaction with stem cells. Ultimately, this study encourages a new perspective for the treatment of patients with maxillofacial deformities.

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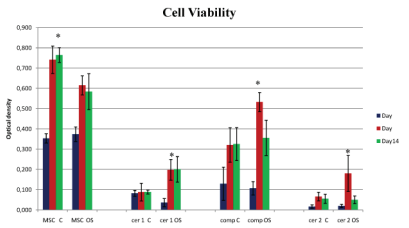

Fig. 3: Graphs showing higher cell viability in the composites. MSC: mesenchymal stem cells; Cer: ceramics; Comp: composites; C: control media; OS: osteogenic media.

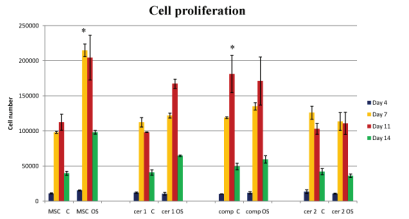

Fig. 4: Higher cell number was observed after 11 days in culture. MSC: mesenchymal stem cells; Cer: ceramics; Comp: composites;C: control media; OS: osteogenic media.

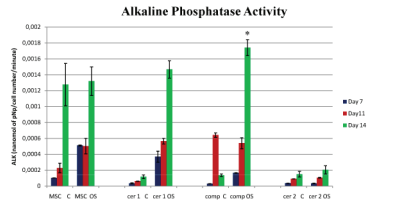

Fig. 5: Alkaline phosphatase levels were elevated in OS media in all the scaffolds. However, composites at day 11, in the control media showed higher levels even when compared to that in the osteogenic media, in the same time point. MSC: mesenchymal stem cells; Cer: ceramics; Comp: composites; C: control media; OS: osteogenic media.

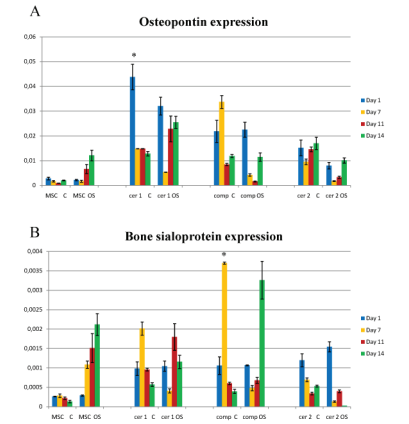

Fig. 6: The gene expression evidences a higher modulation of osteopontin (A) with the ceramic 1 and of bone sialoprotein (B) within the composite, both in control media. MSC: mesenchymal stem cells; Cer: ceramics; Comp: composites; C: control media; OS: osteogenic media.

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