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Osteogenic effect of tricalcium phosphate substituted by magnesium associated with Genderm® membrane in rat calvarial defect model

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ABSTRACT

Beta-tricalcium phosphate (β-TCP) is one of the most widely employed bioresorbable materials for bone repair since it shows excellent biological compatibility, osteoconductivity and resorbability. The incorporation of divalent cations such as magnesium onto the β-TCP structure (β-TCMP) may improve the biological response to the material through the release of bioactive ions. The objective of this study was to evaluate, on a rat calvarial critical size grafting model, the bone regeneration process using β-TCP and β-TMCP granules by histomorphometric analysis. Results demonstrated that six months after bone grafting, the association of GBR (guided bone regeneration) using a membrane (GenDerm®) and granules of β-TCP and β-TCMP significantly improves bone repair in the treatment of critical-size defect in rat skulls, in comparison to untreated defects or GBR alone, leading to a bone level approximately four to five-fold greater than in the blood clot group. The β-TCMP + GenDerm® membrane group presented 40.5% of the defect area filled by newly-formed bone, even at the central part of the defect, rather than only at the border, as seen in the other experimental groups.

1. Introduction

Bone grafting is a commonly performed procedure for bone tissue reconstruction. It is estimated that more than 500,000 bone-grafting procedures are performed annually in the United States in order to repair bone defects in orthopedics, neurosurgery and dentistry [1]. As an alternative to the bone autografts and allografts, implantations of synthetic bone substitutes have been tried experimentally or performed clinically. The major advantages of using synthetic bone substitutes are the prevention of donor site morbidity and increased operative time to remove autograft bone, and prevention of immunological reactions to the allograft [2].

Calcium phosphate based ceramics are promising synthetic materials used in bone replacement surgery. Among them, beta-tricalcium phosphate [β-$Ca_3(PO_4)_2$, β-TCP] and hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HA] have received particular attention [3]. As a bone substitute, HA is highly osteocompatible and presents osteoconductivity, but stoichiometric HA is not bioresorbable and remains in the body for a long time after implantation. In contrast, β-TCP ceramics show resorbable characteristics during bone regeneration, and can be completely substituted for the bone tissue after new bone formation [4]. Studies in a critical-sized femoral defect model in rats showed significantly

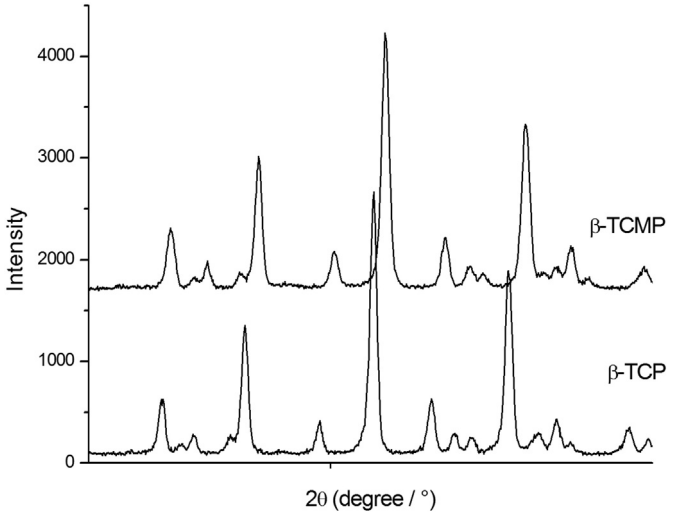

Fig. 1. XRD patterns of β-TCP (JCPDS 09-0169 file) and β-TCMP granules (JCPDS 13-0404 file).

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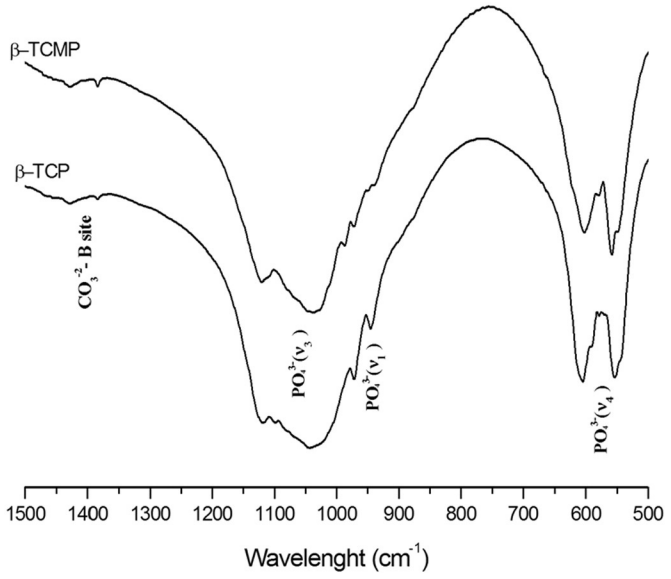

Fig. 2. Details of FT-IR spectra of β-TCMP and β-TCP granules.

higher bone formation in defects grafted with β-TCP compared to HA [5].

Natural bone apatite is poorly crystalline and presents cationic and anionic substitutions in the sites of the hydroxyapatite crystal structure; for this reason its composition is far from the typical stoichiometric HA [6]. Considering cationic substitutes, magnesium (Mg) is undoubtedly one of the most important bivalent ions. In calcified tissue, the amount of Mg is higher in the apatitic phase at the beginning of the calcification process, and decreases with increasing calcification [7]. In calcium-phosphate materials, the substitution of Mg^{2+} for Ca^{2+} decreases the size of the unit cell of apatite lattice giving rise to an apatite with low crystallinity. When replacing Ca, the Mg atom, by having a smaller atomic radius, increases the TCP structural stability, improving its mechanical properties and reducing its solubility providing a stable cell-material interface [8].

Xue et al. [9] showed that the dissolution behavior is crucial key for cell response. The substitution of Mg^{2+} for Ca^{2+} reduces the solubility of Mg-TCP, which is helpful to enhance cell attachment and proliferation. The high solubility of TCP damages the stability of cell-material interface resulting in a low cell density and ALP expression.

Previous studies have shown that β-TCP doped with magnesium (β-TCMP) is biocompatible, and presents higher rates of human osteoblastic attachment and proliferation when compared to β-TCP [10]. Mg doping in beta-tricalcium phosphate (β-TCP) also decreases *in vitro* osteoclast differentiation and osteoclast-mediated degradation of β-TCP [11].

The Mg metabolism after bioresorbable implant placement is still an open question. Magnesium seems to be an important factor controlling *in vivo* bone metabolism since it acts on both bone formation and resorption. Magnesium ions are known to be involved in the initial steps of osteogenesis, and are key co-factors for several enzymatic reactions in bone biology [6,12].

β-TCP based material resorption *in vivo* is controlled by two factors: self-dissolution and cell-mediated dissolution. The evaluation of self-dissolution behavior aimed to control the release rate of Mg, which will help to study the effects of Mg release from β-TCMP on the cell-mediated dissolution or cell function [13]. Bioceramic degradation is also possible by osteoclastic bone remodeling. The local acidic environment, which is produced by these cells, leads to an increased solubility of the ceramic material [14].

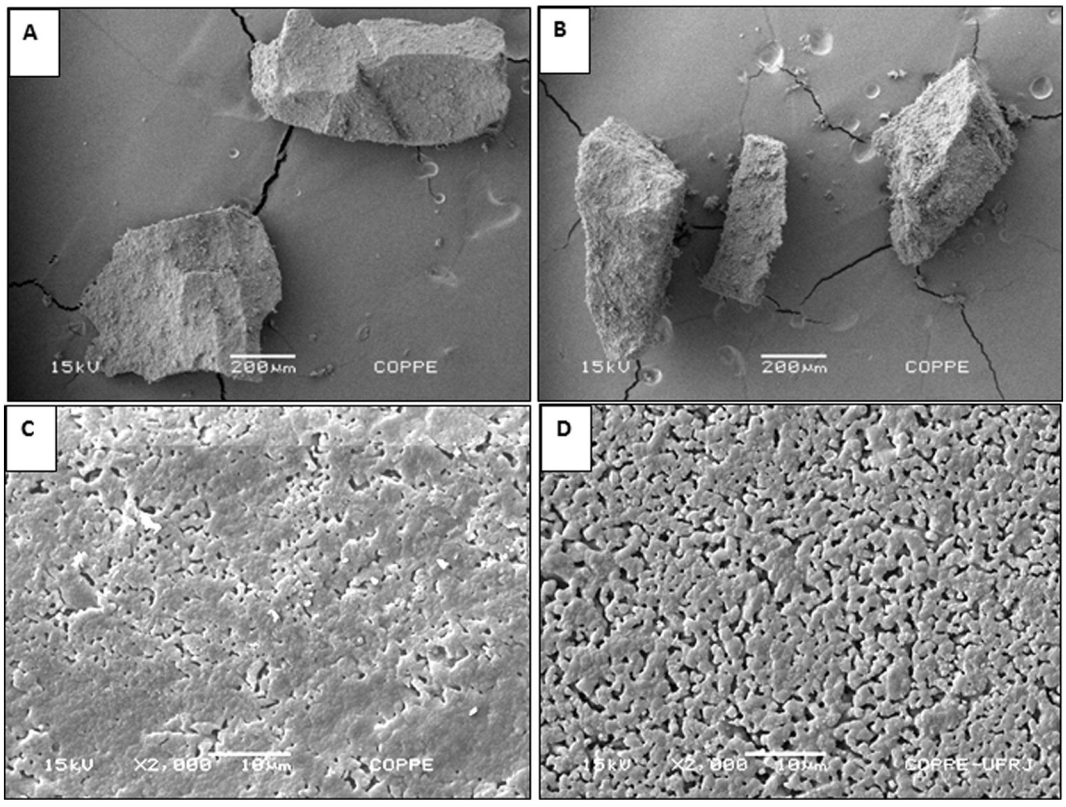

Fig. 3. Scanning Electron Micrographs of granules. (A) β-TCMP granules (×80). (B) β-TCP granules (×80). (C) β-TCMP granules presenting lower microporosity (×5000). (D) β-TCP granules (×5000).