NOVEL BIOCERAMIC SCAFFOLDS FOR REGENERATIVE MEDICINE

Marcelo Henrique Prado da Silva

Calcium phosphate ceramics have been used as synthetic grafts for bone repair. This family of alloplastic grafts is an alternative to allografts (from other individuals from the same specie), autografts (from the same individual) or xenografts (from individuals from other species). Sintered bovine bone is basically composed mainly of hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$ but chemical analyses indicate the presence of Mg. Chemical and heat treatments are generally required to eliminate biological hazard. However, the more crystalline hydroxyapatite is the less resorbable is the product. An approach to have a highly crystalline and still resorbable material is to use additions of alpha or beta tricalcium phosphate, $Ca_3(PO_4)_2$, tricalcium phosphate (TCP). The addition of fractions of some bioactive glasses to hydroxyapatite has shown to be effective in promoting its decomposition to tricalcium phosphate. In addition, glass reinforced hydroxyapatite composite are materials with higher compressive strength due to liquid phase sintering. In this study, novel scaffolds based on hydroxyapatite and tricalcium phosphate are presented.

Keywords: scaffold, bone, hydroxyapatite

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Introduction

Bone is a dynamic tissue, which undergoes healing after severe injuries, since favourable conditions are present. Large bone defects require grafts that can guide bone to grow though their structure or can interact with cells and tissues inducing osteogenesis [1]. Bone grafts can be autogenous, allogenous, xenogenous or alloplastic [2]. Autografts come from the same individual, whereas allografts are bone from other individuals from the same specie. Xenografts are derived from individuals from other species. Synthetic or alloplastic grafts were first designed to be osteoconductive, i.e. e., able to conduct growth of the newly formed bone tissue throughout the pores of the structure [3-6]. Porous bioceramics, bioactive glasses and glass-ceramics are examples of biomaterials used for bone reconstruction. Hydroxyapatite (HA, ($Ca_{10}(PO_4)_6(OH)_2$), is the most well characterised bioceramic. There are several studies on HA bioactivity, i.e. e., the ability to chemically bonding to living bone. However, medical applications of HA are restricted to sites of low-to-medium load-bearing applications. Glass-reinforced hydroxyapatite, GR-HA, is still bioactive and can have higher fracture toughness than pure HA. GR-HA can be produced by mixing HA and bioactive glasses. When a $CaO\text{-}P_2O_5$ based glass is added to hydroxyapatite (HA) and sintered, the glassy phase reacts with HA. The phases present will depend on the sintering temperature and the glass composition [3, 4]. It is particularly beneficial to have bioresorbable phases like beta tricalcium phosphate (β-TCP) and alpha tricalcium phosphate (α-TCP), as these phases are known to be more soluble than HA [3, 4, 7].

Bioactive glass and ceramics can be used as granules or porous scaffolds in applications where bone ingrowth is needed or as scaffolds for tissue engineering [4, 8]. Porous biomaterials based on ceramics and glass ceramics have been produced by several techniques such as the use of polymeric sponge, foaming processes and techniques using organic additives [6, 9, 10]. The main morphological requisites for allowing bone ingrowth are the existence of open and interconnected pores, with pore diameters larger than 100 μm for proper vascularisation and fluid circulation [6, 9, 10]. The interconnectivity of the pores can be achieved by controlling both moulding and sintering processes. However, there is a compromise between interconnectivity and mechanical strength [8, 11].

In this study, two patented scaffolds are presented: polyurethane sponge coated with hydroxyapatite and bovine bone reinforced with bioactive glass[12, 13]. In both cases, there are hydroxyapatite and tricalcium phosphate after sintering. Alpha and beta TCP are more resorbable phases when compared to pure hydroxyapatite. The presence of a biphasic or triphasic structure enhances bio-resorption and is a tool to design scaffolds with resorption rates close to that of new bone formation.

Objective

The aim of this study was to design porous scaffolds with potential to be used as bone fillers and as supports for tissue engineering. Both morphological and microstructural properties were carefully designed to meet the requirements of pore size and distribution, interconnectivity, bioactivity and resorption rate. Ongoing studies are assessing the osteoinductive potential of these materials.

Methodology

The scaffolds derived from polyurethane sponges are obtained by a coating process, which consists of the deposition of monetite, $CaHPO_4$, on porous blocks (scaffolds) with variable dimensions [12]. The scaffolds were hydrothermally coated with monetite and further converted to hydroxyapatite by an alkali treatment with NaOH. The starting solution has the following composition: 0.3M H_3PO_4, 0.5M $Ca(OH)_2$, 1M CH_3CHCO_2HOH. The monetite coating was produced by the immersion of the specimens in the solution at 80℃ during 1 hour. The sponges were then removed, washed in ultra-pure water and dried in an oven at 60℃. The coatings were then converted to hydroxyapatite by immersion in a solution of 0.1M NaOH during 24 hours at 60℃. Specimens were then removed from the alkali solution, washed in ultra-pure water and dried in an oven at 100℃. After drying, the blocks were sintered at 1200ºC. The heating rates were 0.5ºC/min up to 550ºC and 5ºC/min up to the sintering temperature.

The scaffolds of bovine origin were produced by calcinating bovine trabecular bone blocks ($10x10x10mm^3$) at 900ºC with a path at 550ºC and heating rates of 0.5ºC/min and 5ºC/min. The so obtained bone mineral were impregnated with a niobo-phosphate glass [13]. The niobo-phosphate glass has the molar composition of 0.3M Nb_2O_5, 0.3M P_2O_5, 0.2M CaO and 0.2M CaF_2. Reagent grade H_3PO_4 and Nb_2O_5 were used as network former sources and CaF_2 and $CaCO_3$ were used as glass modifiers sources. The reagents were mixed, magnetically stirred overnight and molten in a platinum crucible at 1350ºC. The glasses were rapidly cooled in water, dried in an oven at 100ºC and ground to a medium particle size 0.18μm. The bone mineral blocks were immersed in glass suspensions in ethanol and sintered at 1350ºC. In previous studies, defined fractions of glass were incorporated to bone mineral powder and characterized by XRD [14]. XRD analyses of the impregnated porous blocks confirmed the glass fraction obtained by weighting the blocks before and after impregnation.

The structural analysis of the scaffolds were assessed by X-ray diffraction (XRD) using a Panalytical X”PERT PRO diffractometer with CuKα radiation, a scanning step of 0.05º and a collecting time of 8 seconds per step. Rietveld analyses were performed using the academic version of TOPAS program. Scanning electron microscopy (SEM) analyses of the struts were performed in a Jeol JSM – 5800 LV scanning electron microscope. Fourier transform–infrared spectroscopy (FT–IR Prestige – 21/ Shimadzu) analyses were used to assess the present functional groups on the specimens.

Results and Discussion

Scanning electron microscopy of the scaffolds showed an open porous structure with pore sizes larger than 100μm. This requirement allows not only cell colonization but bone ingrowth and vascularisation of newly formed bone [6, 9, 10]. Figures 1-3 shows images from scaffolds derived from polyurethane sponges.

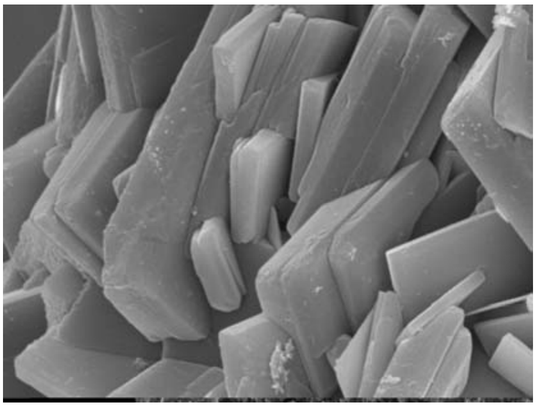


Figure 1 - SEM image of sponge coated with monetite at 4500X.

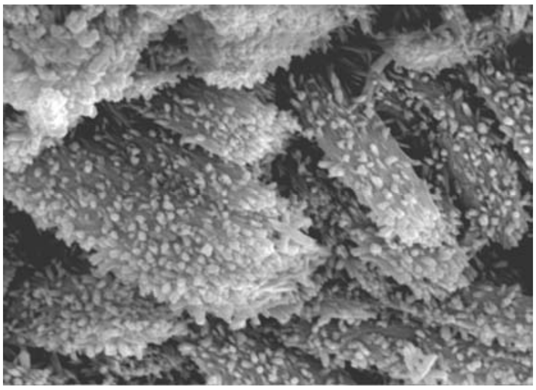


Figure 2 - SEM image of the above coating converted to hydroxyapatite at 10000X.

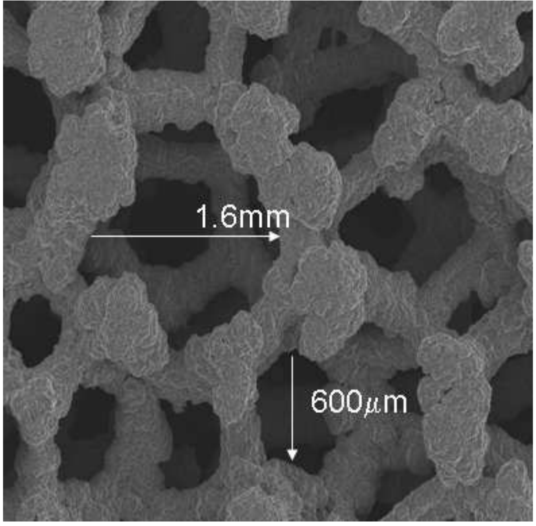


Figure 3 – SEM image of HA coated sponge after sintering at 1300ºC at 33X.

In Figure 3, glass reinforced hydroxyapatite scaffolds derived from bovine bone can be observed. It is clear that the morphological requirements of open and interconnected pores were met. It is worth to mention that one advantage of this route of scaffold production is the maintenance of the natural bone structure.

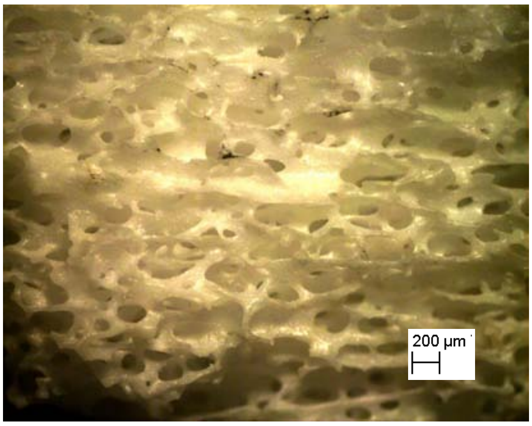
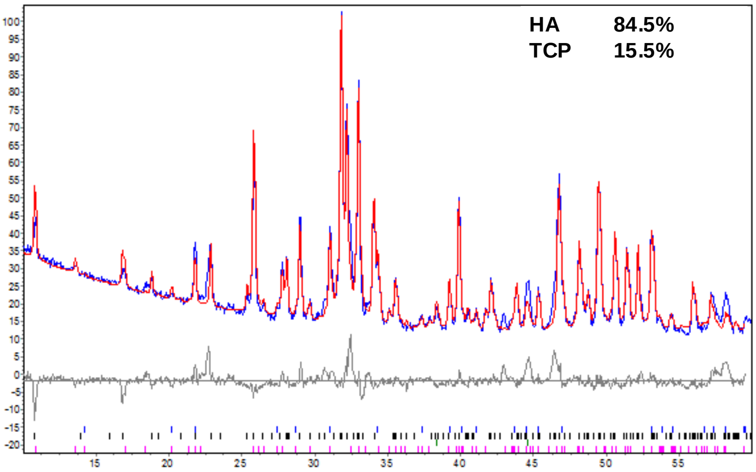


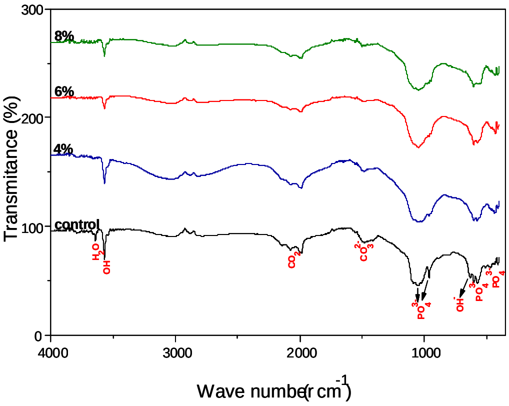
Figure 4 – Light microscopy image of bone mineral after sintering at 1350ºC.

Structural analyses of the scaffolds indicated the presence of hydroxyapatite and β-TCP in the spongy scaffolds, as shown in Figure 5. The glass reinforced scaffolds can develop several different microstructures, depending on the glass fraction added and sintering temperature. Figure 6 shows the XRD result for samples with 4wt% glass and sintered at 1350ºC. The Rietveld analysis identified 84.5 wt% HA and 15.5wt% of β-TCP. The control of the fraction of TCP allows the development of scaffolds with resorption rates compatible with new bone formation rates.



XRD analysis of bone mineral with 4wt% glass after sintering at 1350ºC.

Figure 5 shows FTIR analyses of control samples (without glass) and GR-HA samples. It is clear that the more the fraction of TCP, the less intense is the OH^- band at $3570 cm^{-1}$.



FTIR analyses of bone mineral (control) and glass reinforced bone mineral.

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