INFLUENCE OF DIFFERENT PORE FORMING AGENTS ON BIPHASIC CALCIUM PHOSPHATE BIOCERAMIC SCAFFOLDS

L. F. Cóta¹, L. F. S. Paiva²*, J. N. Lunz², A. A. Ribeiro², L. C. Pereira¹, M. V. de Oliveira²

¹ Metallurgical and Materials Engineering Program, COPPE, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil; ² Powder Technology Laboratory, Materials Processing and Characterization Division, National Institute of Technology, Rio de Janeiro, RJ, Brazil.
* Av. Venezuela, 82 - Sala 602. Praça Mauá. CEP 20081-312 Rio de Janeiro, RJ, Brazil. lilian.fernanda@int.gov.br

Abstract. Bone formation may be induced by adjusting the bioreactivity and biodegradability of biphasic calcium phosphate (BCP), which vary according to their phase composition and porosity. This work aimed to manufacture BCP scaffolds, composed of hydroxyapatite (HAp) and β-tricalcium phosphate (β-TCP), from HAp powders synthesized by sonochemical technique. HAp powders were mixed with pore forming agents (40 wt.%), such as ammonium bicarbonate (AB) or polyethylene wax (PE), and uniaxially cold pressed. In air furnace, AB and PE were vaporized by heat treatment, and sintering at 1000°C for 2h. The samples were characterized by X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and geometric method for porosity determination. XRD spectra presented HAp (20%) and β-TCP (80%) phases. FTIR spectra proved characteristic bands of phosphate groups. BCP/AB and BCP/PE samples exhibited porosity of 67% and 72%, respectively. The type of pore forming agent did not influence significantly on scaffolds porosity.

Keywords: Biphasic calcium phosphate, Hydroxyapatite, Beta-tricalcium phosphate, Scaffolds, Biomaterials.

1. INTRODUCTION

Synthetic materials based on calcium phosphates (CaP), such as hydroxyapatite [HAp - Ca₁₀(PO₄)₆(OH)₂], β-tricalcium phosphate [β-TCP - Ca₃(PO₄)₂] and biphasic HAp/β-TCP ceramics have been frequently studied and clinically tested in the biomaterial research field (1). The main reason is that CaP based materials promote a direct chemical connection between bone tissues and ceramic implants, because of their bioactivity. HAp sintered ceramics are widely used due to promotion of osteointegration, which is defined as the ability
to create a physico-chemical bond in the interface of materials and bones. However, HAp bone remodeling and osteoconductivity are low in contrast with TCP ceramics. Therefore, TCP ceramics have been suggested as scaffold materials for supporting bone regeneration, and substitution for bone tissue \(^{(2-4)}\).

The main idea of a biphasic calcium phosphate (BCP) ceramic is determined by a balance of more stable phases (e.g., HAp) and more soluble phases (e.g., any type of TCP). The usual way to prepare these biphasic ceramics consists of sintering non-stoichiometric calcium orthophosphates, at temperatures above \(\sim 700^\circ C\), followed by its partial decomposition into TCP phase \(^{(5)}\). Important properties of BCP bioceramics relating to their medical applications include: macroporosity, microporosity, bioreactivity, dissolution and osteoconductivity \(^{(6)}\). Hence, the HA/β-TCP proportion between phases with different dissolution rates is crucial for controlling BCP properties for bone regeneration. The rate of bone formation can be induced by adjusting the bioreactivity and biodegradability of BCP scaffolds, which vary according to their phase composition and porosity.

In structural aspects, porosity and the interconnection of microspores, both macro and micro-levels, are important factors for bone healing \(^{(7)}\). Manufacturing processes produce porous materials, based on pore forming agents that are removed either by heating or by dissolution. All techniques lead to samples presenting pores with amount, size and shape that are close to those of the initial pore formers. Thus, it is possible to predict the volume and pore size of a scaffold by controlling the particle size and % volume of the pore forming agents \(^{(8)}\).

Then, this work aimed to compare two pore forming agents in the production of BCP scaffolds, composed of hydroxyapatite (HAp) and β-tricalcium phosphate (β-TCP), from nanosized HAp powders synthesized by sonochemical technique.

2. MATERIALS AND METHODS
Hydroxyapatite powders were synthesized by previously developed sonochemical method (9), from the following precursors: Calcium Chloride dehydrate (1 M) and Sodium Phosphate dodecahydrate (0.6 M), Eq. (A), with estimated Ca/P molar ratio of 1.5.

\[
10 \text{CaCl}_2 \cdot 2 \text{H}_2\text{O} + 6 \text{Na}_2\text{PO}_4 \cdot 12 \text{H}_2\text{O} \rightarrow \text{Ca}_{10} (\text{PO}_4)_6 (\text{OH})_2 + 18 \text{NaCl} + 90 \text{H}_2\text{O} + 2 \text{HCl} \quad (A)
\]

The nanosized HAp powders were mixed with two different types of pore forming agents, such as 40 wt.% of ammonium bicarbonate (AB) or 40 wt.% of polyethylene wax (PE), in order to produce biphasic calcium phosphate (BCP) scaffolds. Particle size of the pore forming agents, AB and PE, was within the range of 250-300 µm. BCP/AB and BCP/PE samples were uniaxially cold pressed at 100 MPa, using a cylindrical mold of ø 6.17 mm and pre-load of 17 MPa (500 N), in a universal testing machine (EMIC – DL 3000), load cell of 20 kN (LAMAP/INT).

Both pore forming agents, AB and PE, were vaporized from the BCP samples when submitted to heat treatments at 170°C for 2h and 550°C for 4h, respectively, with heating rate of 0.5°C/min, in order to obtain porous materials (scaffolds). In the air furnace Jung (Model 0212), the samples were further processed by sintering at 1000°C/2 hours with 2 °C/min heating rate.

The HAp powders and BCP scaffolds were characterized by X-Ray Diffraction (XRD, Panalytical X’Pert Pro Diffractometer), in the range of 10°<2θ<90° with a scan speed of 50 (s/step), and step size of 0.05°.

As a biphasic material was obtained after sintering, the XDR analyses were used to semiquantitatively calculate the volumetric percentage, considering the strongest peaks of each phase as reference. Relative Intensity Ratio (RIR) of HAp and β-TCP phases was quantified according to Eqs. (B) and (C), respectively. Where \( I_{HAp} \) and \( I_{βTCP} \) represent the strongest peaks of each crystalline phase, in which HAp was described as 2θ = 31.774°, according to JCPDS card #9-432 and β-TCP was described as 2θ = 31.023°, according to
The samples were also analyzed by Fourier Transform Infrared Spectroscopy (FT-IR, Shimadzu IR Prestige-21 spectrometer). All FT-IR spectra are averages of 128 scans performed in the range of 4000-400 cm\(^{-1}\), with resolution of 4 cm\(^{-1}\).

The scaffold microstructures were also evaluated by Scanning Electron Microscope/Field Emission Gun (FESEM, Quanta FEG 450). SEM micrographs with magnification of 10,000 and 50,000 X were acquired at 20kV.

Porosities were determined by geometric method for green and sintered samples, in order to observe modifications undergone after the heat treatment. Theoretical density of the biphasic material formed by HAp and \(\beta\)-TCP was a weighted value of the theoretical densities of each phase, Eq. (D), according to volumetric fraction of those phases in the samples. \(RIR_{\text{HAp}}\) and \(RIR_{\beta\text{TCP}}\) are the volumetric percentage calculated for each phase, estimated by (B) and (C), respectively. \(d_{\text{HAp}}\) and \(d_{\beta\text{TCP}}\) are the theoretical densities of each phase, 3.16 g/cm\(^3\) (JCPDS card #9-432) and 3.07 g/cm\(^3\) (JCPDS card #9-169), respectively.

\[
RIR_{\text{HAp}} = \frac{i_{\text{HAp}}}{i_{\beta\text{TCP}} + i_{\text{HAp}}} \quad (B)
\]

\[
RIR_{\beta\text{TCP}} = \frac{i_{\beta\text{TCP}}}{i_{\beta\text{TCP}} + i_{\text{HAp}}} \quad (C)
\]

\[
d_{\text{theoretical}} = RIR_{\text{HAp}}d_{\text{HAp}} + RIR_{\beta\text{TCP}}d_{\beta\text{TCP}} \quad (D)
\]

To determine the relative density and porosity were made three measurements of each dimension (height and diameter) of the cylindrical samples using digital caliper (Marathon Management Model CO030150). With
these data, Eq. (E) was used to calculate the volume of the samples, where $V$ is the volume, $D$ and $h$ are the diameter and height, respectively.

$$V = \pi \left( \frac{D}{2} \right)^2 h \quad \text{(E)}$$

Moreover, the samples were weighed on SHIMADZU scale Model AUY220 to determine the mass, and density was calculated using Eq. (F), where $d$ is the geometrical density of the material, $m$ is the mass and $V$ is the volume.

$$d_{\text{geometric}} = \frac{m}{V} \quad \text{(F)}$$

For calculation of relative density of the samples the Eq. (G) was used. Next, the porosity was deduced based on Eq. (H).

$$d_{\text{relative}} = \frac{d_{\text{geometric}}}{d_{\text{theoretical}}} \times 100 \quad \text{(G)}$$

$$P = 100 - d_{\text{relative}} \quad \text{(H)}$$

3. RESULTS AND DISCUSSION

3.1 X-ray Diffraction (XRD)

Fig. 1 shows the X-ray diffraction patterns of HAp powders and both scaffolds, BTP/AB and BTP/PE. The results indicate that the HAp powder presented exclusively hydroxypatite crystalline phase, since all peaks are attributed to stoichiometric hydroxypatite and no other calcium phosphate diffractions were detected. Also, pre-sintered HAp powder exhibited low crystallinity, which was inferred from the broad width of peaks $^{(10)}$. By the scaffolds analysis, a second phase identified as $\beta$-TCP was observed along with HAp phase. Crystalline
Phases are distinguished in Fig. 1, according to the X-ray diffraction patterns of HAp (JCPDS card #9-432) and \( \beta \)-TCP (JCPDS card #9-169).

After sintering, decomposition of HAp into \( \beta \)-TCP was expected due to the low Ca/P ratio of initial HAp powder, as it was already described by several authors (11-14). By the XRD analysis, it was possible to obtain the phase proportion of each group of scaffolds, BTP/AB and BTP/PE, as shown in Table 1, with the Relative Intensity Ratios (RIR) of HAp and TCP extracted from Eqs. (B) and (C), respectively, and expressed in %. It is noted that these samples have similar phase proportion of commercially available bioceramics.

![XRD spectra of the Hap powder and BCPs scaffolds.](image)

**Fig. 1** XRD spectra of the Hap powder and BCPs scaffolds.

<table>
<thead>
<tr>
<th>Sample</th>
<th>RIR HAp</th>
<th>RIR ( \beta )-TCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPC/AB</td>
<td>21 %</td>
<td>79 %</td>
</tr>
<tr>
<td>BPC/PE</td>
<td>22 %</td>
<td>78 %</td>
</tr>
</tbody>
</table>

3.2 Fourier Transform Infrared Spectroscopy (FT-IR)
FT-IR spectra of samples in the range 4000–400 cm$^{-1}$ are displayed in Fig. 2. Identification of the peaks aimed to compare HAp powder with both sintered BCP samples. FT-IR data are in agreement with XRD patterns (Fig. 1). The broad absorption band between 3700 and 2500 cm$^{-1}$ assigned to the O-H stretch vibration of OH$^-$ groups, together with the absorption peak at 1637 cm$^{-1}$ ascribed to H-O-H bending mode, both are evidence of the presence of absorbed water in the HAp powder that disappeared after thermal treatment at 1000°C. The sharp peaks observed at 3570 cm$^{-1}$ are attributed to the characteristic stretching modes of structural OH$^-$ groups, and it is correlated with crystallinity of HAp phase $^{13,15}$.

In the HAp powder spectrum, the double peak at 603, 563 cm$^{-1}$ belongs to PO$_4^{3-}v_4$, 940 cm$^{-1}$ for PO$_4^{3-}v_1$ and the peaks at 1095 and 1031 cm$^{-1}$ for PO$_4^{3-}v_3$ vibrational modes. In the BCP scaffolds, the sharp bands at 1120, 1080 and 1035 cm$^{-1}$ correspond to $v_3$ asymmetric stretching modes of PO$_4$ in $\beta$-TCP phase. After sintering, the peaks first observed in HAp powder at 603 and 563 cm$^{-1}$ were shifted to 605 cm$^{-1}$/590 cm$^{-1}$ and 570 cm$^{-1}$/550 cm$^{-1}$ for PO$_4^{3-}v_4$ vibrational modes of $\beta$-TCP phase, suggesting a partial HAp$\rightarrow$$\beta$-TCP phase transformation. The absorption bands at 971 cm$^{-1}$ is related to $v_1$ symmetric stretching mode of PO$_4^{3-}$ and confirms crystallinity of the $\beta$-TCP phase. In the HAp powder spectrum, bands at 1415, 1460 and 870 cm$^{-1}$ suggest the presence of CO$_3^{2-}$ vibrational modes, respectively CO$_3$$v_3$ and CO$_3$$v_2$, from the carbonate HAp structure. Those bands disappeared after the sintering $^{16-19}$.
Thermal decomposition of HAp is known to occur when a critical dehydration point is achieved. In the temperatures less than the critical point, crystal structure of HAp remains unchanged in spite of the stage of dehydration. Achieving the critical point, a complete and irreversible dehydroxillation occurs, which results damage of HAp structure, decomposing onto tricalcium phosphate (β-TCP under 1200 °C and α-TCP at higher temperatures) and tetracalcium phosphate (TTCP) \(^{(5)}\). Moreover, residual contaminations from pore forming agents were not observed in FTIR spectra of BCP scaffolds, suggesting that AB and PE were effectively eliminated by heat treatment.

### 3.3 Scanning Electron Microscope/Field Emission Gun (FESEM)

The significance of variables related to the composition of the pore forming agent on porosity of both materials was analyzed. Fig. 3 displays FESEM images of the BCP scaffolds. Figs. 3A and 3C display comparatively BCP/AB scaffold microstructure to that of BCP/PE scaffold in Figs. 3B and 3D.
Both pore forming agents, ammonium bicarbonate or polyethylene wax, assigned a porous structure to the scaffolds. Both BCP scaffolds showed fully interconnected micropore structures with submicron-sized grains. Microstructures of the samples are similar in both magnifications (10,000 X and 50,000 X), with uniform pore size distribution.

3.4 Relative Density and Porosity

The porosities determined by geometric method are shown in Table 2. Differences in porosity among the samples are not significant, with values...
ranging from 68 to 72 % for BCP/AB and BCP/PE, respectively. Thus, results are within the range found in the literature of porous bioceramics for bone regeneration \(^{(1)}\).

**Table 2** Density and porosity of BCP scaffolds determined by geometric method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pore forming agent</th>
<th>Particle size (µm)</th>
<th>Proportion (% wt.)</th>
<th>Density (Green)</th>
<th>Porosity (Green)</th>
<th>Density (Sintered)</th>
<th>Porosity (Sintered)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCP/AB</td>
<td>Ammonium bicarbonate</td>
<td>250-300</td>
<td>40</td>
<td>46.42</td>
<td>32.19</td>
<td>53.58</td>
<td>67.81</td>
</tr>
<tr>
<td>BCP/PE</td>
<td>Polyethylene wax</td>
<td></td>
<td></td>
<td>39.15</td>
<td>27.93</td>
<td>60.85</td>
<td>72.07</td>
</tr>
</tbody>
</table>

Microporosity enhances the specific surface area, thereby promoting protein adsorption to the material. Zhu *et al.* \(^{(20)}\) suggest that microporous distribution on the macroporous surfaces is important to low molecular weight protein adsorption, such as TGF-β1 (tumoral growth factor). Li *et al.* \(^{(21)}\) reported BCP disks with high specific surface area (almost 50 % of porosity) tend to have more adsorbed proteins than those of low specific surface area (24 % of porosity). In recent study \(^{(22)}\), donut shape BCP bone substitutes made of a central macro-pore (about 300 ~ 400 µm) and micro-pores (about 20–60 µm) showed greater new bone formation when compared with similar BCP composition with micro-pores. This aspect is of great importance since the adsorption of proteins to the material facilitates cell proliferation and differentiation.

4. **CONCLUSIONS**

In this study, it was observed that the utilization of a pore forming agent, followed by sintering of the HAp powder, is a suitable technique for manufacturing BCP scaffolds. XRD and FT-IR data indicated that both HAp and
β-TCP phases are present in the sintered scaffolds, suggesting a partial HAp→β-TCP phase transformation. FT-IR spectrum showed the presence of carbonate hydroxyapatite only in pre-sintered HAp powder.

In addition, analysis of the microstructure and porosity of the scaffolds proved satisfactory pores interconnectivity in both BCP scaffolds, indicating that the type of pore forming agent did not influence significantly on the porosity features.

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