Synthesis and Synchrotron characterisation of novel dual-template of hydroxyapatite scaffolds with controlled size porous distribution

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ABSTRACT

Hydroxyapatite (HAP) scaffolds with a hierarchical porous architecture were prepared by a new dual-template (corn starch and cetyltrimethylammonium bromide (CTAB) surfactant) used to cast HAP nanoparticles and development scaffolds with size hierarchical porous distribution. The Powder X-Ray diffraction (XRD) results showed that only the HAP crystalline phase is present in the samples after calcination; the Scanning Electron Microscopy (SEM) combined with Small Angle (SAXS) and Ultra-Small Angle X-ray Scattering (USAXS) techniques showed that the porous arrangement is promoted by needle-like HAP nanoparticles, and that the pore size distributions depend on the drip-order of the calcium and the phosphate solutions during the template preparation stage.

Keywords: Biomaterials; Nanoparticles; Porous materials.

1. Introduction

In the last few years, many studies have reported the development of new calcium phosphate scaffold types with random or ordered porosity [1,2], specifically for in-situ gradual release mechanisms of therapeutic anticancer, antibacterial and anti-inflammatory substances [3,4]. Sophisticated methods able to combine different templates, two or three casts of different features, are grouped to obtain a single unified and consolidated template to achieve hierarchical porosity [5,6]. Despite being efficient in obtaining mesoporous ($2\text{ nm} < \varnothing < 50\text{ nm}$) and/or macroporous ($\varnothing > 50\text{ nm}$) [7], some of the methods have important limitations such as polymers with high costs or inorganic silicate templates, which lead to the formation of additional silica phases in porous calcium phosphate.

The purpose of this work is to study the synthesis of hydroxyapatite (HAP) scaffolds using an organic combination of starch/CTAB as porous dual-templates and their characterisation by means of XRD and SEM combined with SAXS/USAXS. Once the starch is able to obtain a macroporous system, CTAB was selected to produce mesoporous on hydroxyapatite by micellar distribution [2].

2. Material and methods

2.1. Hydroxyapatite synthesis and template preparation conditions

The dual-template was prepared as follows: 4 g of commercial cornstarch was added to 100 mL of distilled water, and the mixture was gelatinised at 74°C without agitation. The gelatinised cornstarch was added to a 60 mM CTAB boiling solution at 74°C .

Hydroxyapatite particles synthesised with pre-nucleation solution of starch/CTAB/Calcium was called HAP-ACC sample. In this sample the hydroxyapatite pre-nucleation solution is prepared by a Ca^{2+} ion source at 0.167 mol $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$

dissolved into starch+CTAB solution at 74°C. Then, the previously mixed solution was slowly added to the phosphate (PO_4^{3-}) solution source containing 0.1 mol $(\text{NH}_4)_2\text{HPO}_4$ to maintain the stoichiometric ratio $\text{Ca/P} = 1.67$. pH= 10.4 is buffered by NH_4OH (VTEC) solution. After drying at 100°C, the HAP-ACC sample by dual-template is formed. The procedure to obtain the HAP-ACP, sample with pre-nucleation solution of starch/CTAB/Phosphate is quite similar to that described for the HAP-ACC samples. The only differences are the order of mixing of the Ca^{2+} and phosphate sources. To the HAP-ACP samples, phosphate solution was first added to the starch+ Ca_{16}TAB solution at 74° C and at pH= 10.4, followed by the slow addition of the Ca^{2+} source. The final calcination under air takes place at 450° C/4h °C in a muffle furnace, EDG-1800.

2.2. General characterisation techniques

The crystalline phases of all samples were analysed via powder X-ray diffraction (XRD) in a Rigaku, Ultima, equipped with a $\text{CuK}\alpha$ X-ray source operating at 40 kV/40 mA. The measurements were performed in the 2θ interval from 10–70° in step scan mode. The Small-Angle X-ray Scattering (SAXS) data were acquired for powder samples in the scattering vectors range $0.26 \leq q \leq 0.6 \text{ nm}^{-1}$ collected with incident beam $\lambda = 1.488 \text{ \AA}$. Ultra Small-Angle X-ray Scattering (USAXS) results for the ultra-low angle region ($q \leq 0.05 \text{ nm}^{-1}$) were measured at the USAXS beamline setup with incident beam $\lambda = 0.72932 \text{ \AA}$ [8]. The software package Irena for USAXS was used for data reduction and fitting of the experimental data to different models [9].

The particles' morphology as well as the porous systems were analysed using JEOL JSM-7500F with accelerating voltages between 1–5 keV; a small amount of graphite was deposited on the sample surface.

3. Results and Discussion

3.1 Characterisation of hydroxyapatite scaffolds with dual template

Fig. 1(a) shows the SEM images of starch dried at 100°C/24h. It is possible to see the lamellar morphology promoted by the sedimentation of amylose and amylopectin microchains. The results of SAXS data in the high-angle region are shown in Fig. 1(b) for the dried starch sample at 100°C/24h. The two peaks at $q_{(1) \max} = 2.41(\text{\AA}^{-1})$ and $q_{(2) \max} = 4.84(\text{\AA}^{-1})$ with intensity ratios $\left(\frac{2\pi/q_{(2)\max}}{2\pi/q_{(1)\max}} \sim 2.0\right)$ confirmed the lamellar structural organisation of the dried cornstarch reported by [10]. This lamellar structure is used to obtain macropores template.

Additionally, XRD powder patterns of the HAP-ACC and HAP-ACP samples are quite similar to the hydroxyapatite reference hexagonal pattern (ICDD No. 15-1414) [11], as shown in Fig. 2(a). This means that no additional phases due to either starch or CTAB combination were observed, indicating that after calcination, no crystalline form of these two organic additives was present. However, even if there were some organic traces, the amount was so small that they were not seen via XRD (or that they are not crystalline).

Figs. 2(b) and 2(c) present SEM images at different magnifications of the HAP-ACC and HAP-ACP samples, showing pores sizes from 20 μm to 350 nm. These images indicate the great potential of cornstarch in developing low-cost porous systems. Fig. 2(c) shows the ordering of the hydroxyapatite agglomerated needle-like particles around macroporous with a very well defined interface, particle lengths ~ 120 nm and widths ~ 12 nm. However, the SEM-FEG analysis supports the pore sizes being larger than 120 nm; this range is too large for SAXS, but can be resolved with USAXS analysis.

Fig. 3(a) shows the experimental USAXS intensity plotted on a double-logarithmic scale as a function of q with the best-fitted model results to the HAP-ACC and HAP-ACP samples. The following are some considerations about the USAXS data analysis:

- (i) very large scatterers, $q < 0.00075 \text{ (}\text{\AA}^{-1}\text{)}$, can equally be considered to be from aggregates, and thus can be expected to contribute only to the low- q scattering [12,13];
- (ii) the $q > 0.00075 \text{ (}\text{\AA}^{-1}\text{)}$ region in the experimental USAXS data is considered to be dominated by the porous structure, as evidenced from SEM imaging; (iii) therefore only the q -range evaluated at $0.00075 \text{ (}\text{\AA}^{-1}\text{)} < q < 0.25 \text{ (}\text{\AA}^{-1}\text{)}$ was analysed as strictly “small pore” scattering in the USAXS experimental data.

The method of maximum entropy [14,15] is used in this study to analyse the experimental USAXS data results. The maximum entropy method uses different distributions – until a target minimum chi-square is reached – to estimate the pore size distribution, which maximizes the entropy of the system distribution [15].

The porous system was modelled as a distribution of slightly polydisperse oblate spheroid pores with an aspect ratio (length/width) of 0.8 based on previous SEM images. No structure factor $S(q)$ was used in our model, since the system appeared polydisperse [13], as reported by SEM analysis.

The $P(q)$ form factor equation for oblate spheroid pores used to fit the results takes the unified spheroid model from the Irena package [9]:

$$P(q) = \int \left[\left(3 \left(qr \sqrt{1 + (AR^2 - 1)Const} \right)^{-3} \right) \left(\sin \left(qr \sqrt{1 + (AR^2 - 1)Const} \right) - \left(qr \sqrt{1 + (AR^2 - 1)Const} \right) \cos \left(qr \sqrt{1 + (AR^2 - 1)Const} \right) \right) \right] \quad (1)$$

where q is the momentum transfer wave vector, r the radius of the scattering centre, and AR the aspect ratio of the scattering objects.

The fitted curve results are given in Fig. 3(b) with $\text{cm}^3/\text{cm}^3 (\text{\AA}^{-1})$ y-axis unit, that presents the volume-fraction size distributions found by the maximum entropy routine. [14]. The Fig. 3(b) showing a shift in the pore size distribution between the samples prepared in the presence of the dual template, HAP-ACC and HAP-ACP. The first has a well-defined mesoporous population ($\emptyset_{\text{pore}} < 50 \text{ nm}$), while the HAP-ACP sample achieves hierarchical porosity at the nanometric scale with a size distribution for the small mesoporous and macroporous populations ($6 \text{ nm} < \emptyset_{\text{pore}} < 80 \text{ nm}$). The porosity is only achieved by a combination of particle agglomeration driven by CTAB and starch gelatinisation. The pore size distributions are indeed smaller for HAP-ACP. In this synthesis set, the solution drip order combined with the presence of CTAB therefore affects the final pore sizes after the calcination process.

For the HAP-ACP sample, the PO_4^{3-} group is linked electrostatically first with CTAB cationic micelles, forming a CTAB- PO_4^{3-} complex. For this sample when Ca^{2+} ion solution is dropped into the CTAB- PO_4^{3-} complex, some close-packed blocks of hydroxyapatite nucleation are formed, since the PO_4^{3-} group is already hard-linked to the CTAB micelle structures. These close-packed blocks do not appear for the HAP-ACC sample. As for the HAP-ACC sample preparation procedure, the CTAB and Ca^{2+} ions run-up to bind with the phosphate groups and, hypothetically, make a dispersive hydroxyapatite nucleation system.

One can postulate, however, that CTAB surfactant plays an important supporting role in controlling the final pore sizes and keeping the hydroxyapatite particles polydisperse attached to the cornstarch lamellar structure. The use of this dual template provides no locally ordered porous system, but rather a polydisperse porous hierarchical distribution.

4. Conclusions

The SEM-FEG results and USAXS data show that the nanoparticles are aggregates with a needle-like morphology. In addition, it was shown that the order of the addition of the calcium and phosphate solutions infer on the final pore size distributions for the samples synthesised in the CTAB medium. As a general conclusion, hierarchical porosity seems to be achieved via the aggregation of the resultant particles, ordered by the combination of CTAB and cornstarch gelatinisation.

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Figure Captions :

Fig. 1. (a) SEM image and (b) SAXS patterns of lamellar dried starch at 100°C/24h.

Fig. 2. (a) XRD pattern of hydroxyapatite with dual template calcined at 450°C/4h and hexagonal reference pattern [11]. SEM-FEG images: (b) HAP-ACC and (c) HAP-ACP powder sample.

Fig. 3. (a) Synchrotron X-ray ultra-low angle scattering data and best-fitted model by the maximum entropy method. (b) Plot of the maximum entropy volume-fraction size distributions from pore sizes of the HAP-ACC and HAP-ACP samples for the oblate spheroid pores model.

Figure 1

Figure 2

Figure 3