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Microspheres based on hydroxyapatite nanoparticles aggregates for bone regeneration

A.Y. Pataquiva Mateus[1,2,a], M.P. Ferraz[1,3,b] and F.J. Monteiro[1,2,c]

[1]INEB - Instituto de Engenharia Biomédica, Laboratório de Biomateriais, Rua do Campo Alegre, 823, 4150-180 Porto, Portugal

[2]Universidade do Porto, Faculdade de Engenharia, Departamento de Engenharia Metalúrgica e Materiais, Porto, Portugal

[3]Universidade Fernando Pessoa, Praça 9 de Abril 349, 4249-004 Porto, Portugal

[a]yovana@ibmc.up.pt, [b]mpferraz@ufp.pt, [c]fjmont@ineb.up.pt

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Abstract. This study concerns the preparation and characterisation of microspheres associating alginate and two different types of hydroxyapatite (HA), which are intended to be used as drug delivery systems and bone regeneration matrices. Hydroxyapatite nanoparticles (HA-1 and HA-2) were prepared using a chemical precipitation synthesis based on H3PO4, Ca(OH)2 and a surfactant, SDS (sodium dodecylsulphate), as starting reagents. These two powders of nanoHA and alginate were used to prepare two different types of microspheres. Both powders and microspheres were characterised using FTIR, TEM, SEM, mercury porosimetry analysis and X-ray diffraction Results show that pure hydroxyapatite (HA) and mixtures of HA/β-TCP in the nanometre range were obtained from both HA syntheses. Microspheres with different characteristics were obtained from these two types of hydroxyapatite.

Introduction

Bone exhibits natural hydroxyapatite crystals with needle-like or rod-like shapes, well arranged within the polymeric matrix of collagen type I. These natural nanoparticles formed in physiological environment have a more dynamic response when compared to synthetic material with larger particle size [1]. In order to prepare fine HA powders, many chemical processing routes have been employed, including hydrothermal reactions, sol-gel synthesis, pyrolysis of aerosols and micro-emulsion, biomimetic process, and chemical precipitation, which is the most used alternative. Nowadays, several improvements in injectable bone substitutes are being developed as minimally invasive cell carriers for tissue regeneration both for bone and cartilage [2-4]. Desired characteristics of synthesised hydroxyapatite are fine and uniform particle size, in the nanometre range, phase homogeneity and minimized degree of particle agglomeration [5].

This work describes the preparation and characterisation of novel nano-hydroxyapatite-alginate microspheres intended to be used as an injectable bone filling material or as enzyme delivery matrices. The purpose of this work is to characterise the morphology and chemical composition of microspheres based on hydroxyapatite obtained by two different procedures.

Materials and Methods

Aqueous solutions of calcium hydroxide (Ca(OH)2) and ortho-phosphoric acid (H3PO4, 85%), both of analytical grade, were used as reactants for the preparation of two different types of HA nanoparticles. HA powder (HA-1) was synthesised according to a previously reported procedure [6]. Briefly, 1L of an aqueous suspension of H3PO4 (0.6M) was slowly added drop by drop to a 1L of an aqueous suspension of Ca(OH)2 (1M) while stirring vigorously for about 2h at room temperature. Concentrated NaOH was added until a final pH of 10.5 was obtained. The white solution obtained was washed using de-ionized water and dried in oven at 80°C for 24 h. HA

powder 2 (HA-2) was synthesized in the same way, with the difference of the addition of sodium dodecyl sulphate addition (10g) into the calcium solution. The alginate/nanohydroxyapatite microspheres preparation has been reported elsewhere [6].

Chemical characterisation was performed using Fourier transformed infrared (FTIR) spectroscopy. For this purpose, hydroxyapatite and microspheres were reduced to powder and analyzed as KBr pellets using a PerkinElmer System 2000 spectrometer.

The size and morphology of the nanosized HA was determined using a Transmission electron microscope (TEM, Zeiss model EM 902A) at an accelerating voltage of 80kv. TEM images were acquired and observed with Axion Camera Zeiss and AxionCs 40AC Zeiss, v 4.2.0.0 software.

The microspheres morphology was analyzed by digital imaging and scanning electron microscopy (SEM). Using a JEOL JFC-100 fine coat ion sputter device, samples were sputter coated with gold. The HA microspheres were then studied with a JEOL JSM-6301F microscope at an accelerating voltage of 5 kV.

X-ray diffraction (XRD) characterisation of all prepared samples was conducted using a Panalytical X´Pert PRO alpha-1 with a RTMS X´Celerator detector. It used Ni-filtered Cu Kα radiation over the 2θ range of 10-90º at a scan rate of 2.4º /min and with a sampling interval of 0.002º at 40 mA and 45 kV

Results

Figure 1 shows the needle-like shaped morphology of the HA-1 and HA-2 nanoparticles after sintering process. SEM images of the microspheres are presented in Figure 2. After the sintering process their size is decreased due to alginate burn-off, even though their spherical shape is maintained. Higher surface roughness is evidenced in HA-2 microspheres when compared to HA-1 (Figures 2c and 2d). The pores shown in both samples (Figures 2e and 2f), seem to be present in larger amount in HA-1 than in HA-2 microspheres, albeit the interconnectivity is clear in HA-2 microsphere sample. Mercury porosimetry analyses confirmed higher average porous diameter and porosity in HA-2 than HA-1 (results reported elsewhere) [6]. XRD patterns of all samples are shown in Figure 3. HA-1 powder is represented by the pattern in Figure 3a and some undefined humps may be seen, due to the non-sintered state. Figure 3a and pattern c present many similarities even though after the sintering process they revealed the presence of different phases. The pattern in Figure 3b shows clearly the peaks corresponding to pure monoclinic HA, however, Figure 3d shows a mixture of monoclinic HA and β-TCP.

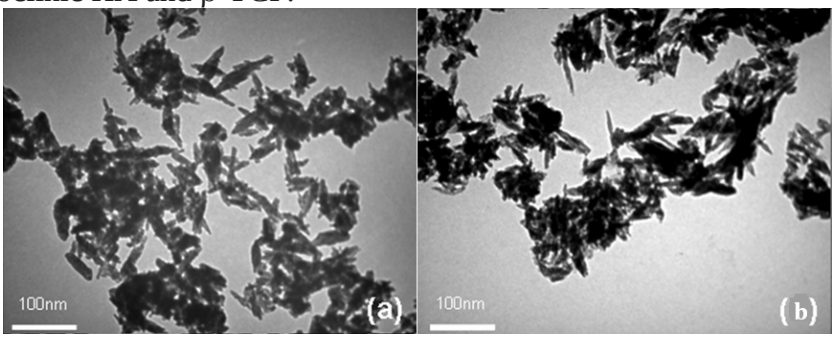

Figure 1 – SEM images from HA-1 and HA-2 powders

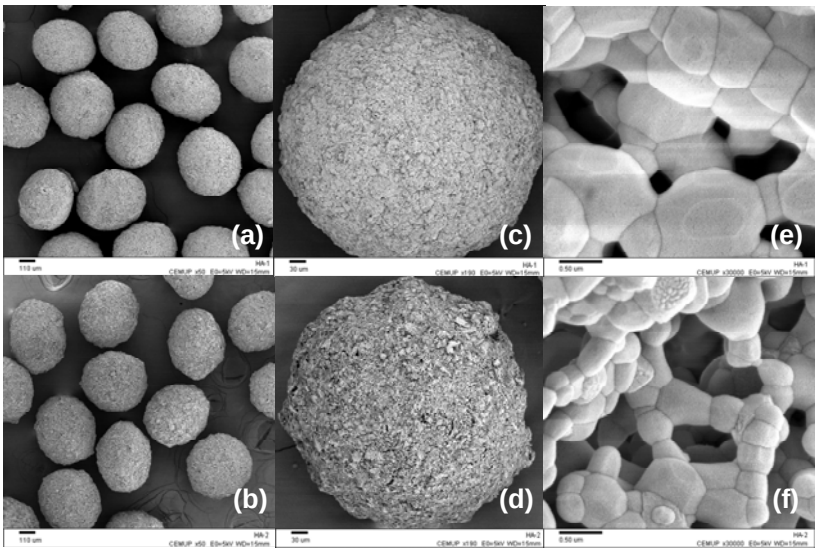

Figure 2 – SEM of HA-1 and HA-2 microspheres 2 (a,b: x50; c,d: x190 and e,f: x30000).

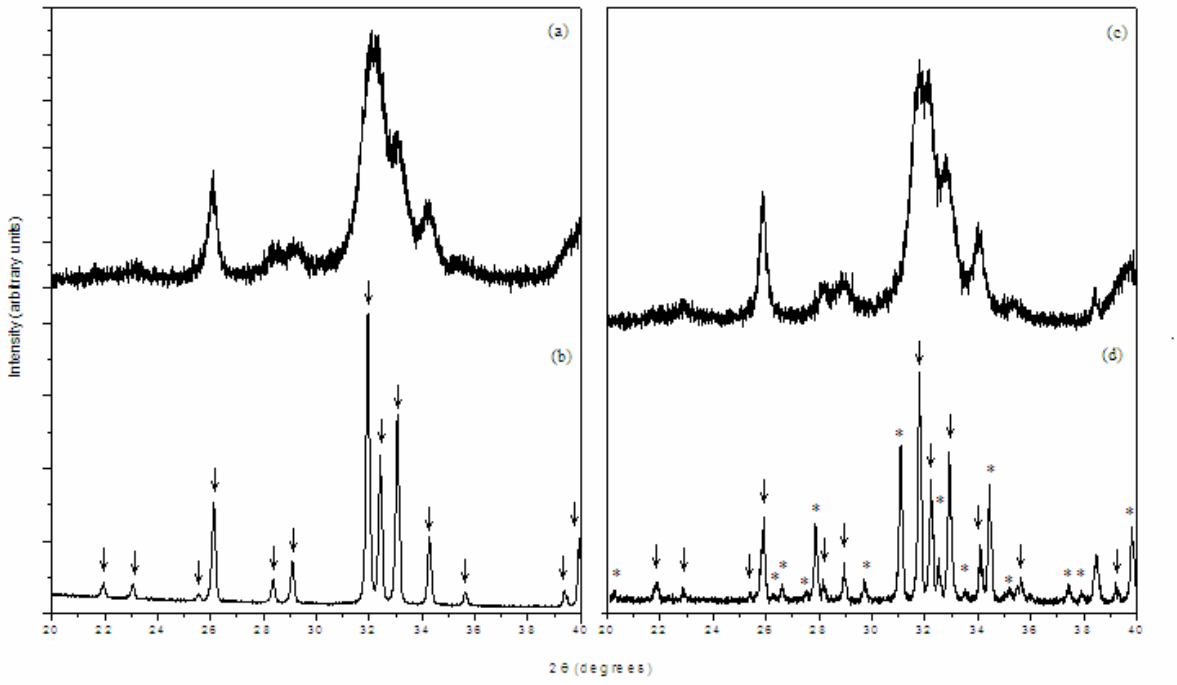

Figure 3 – XRD spectra from (a) HA-1 powder, (b) HA-1 microspheres, (c) HA-2 powder, (d) HA-2 microspheres. ⇣: monoclinic HA, *: β-TCP.

Discussion

TEM and SEM images showed the orientation and size of the nanoparticles aggregates and also verified the traditional dimensions and morphology of the HA nanoparticles are maintained in the microsphere structure due to the fast sintering process, avoiding the complete particles melting. The characterisation analyses included FTIR, TEM, SEM, mercury porosimetry and XRD. FTIR analysis indicated that the characteristic spectra bands of both ceramic powders, HA-1 and HA-2, are maintained in the microspheres, suggesting that sodium alginate did not induce subsequent modifications in the ceramics structures. It is important to point out the multiple binding sites on the microsphere surface (Fig 2e,f) that could allow the binding to several macromolecules such as enzymes or antibiotics, among others. This feature may enhance their role as drug delivery system components to be used in biomedical applications.

Conclusions

Preparation and characterisation of two different types of hydroxyapatite and alginate microspheres, intended to be used as drug delivery systems and bone regeneration matrices, were studied in the present work. Hydroxyapatite nanoparticles (HA-1 and HA-2) were prepared using a chemical precipitation synthesis based on H_3PO_4, $Ca(OH)_2$ as starting reagents, with addition of the surfactant SDS (sodium dodecyl sulphate) in one of these processes. These two powders of nanoHA and alginate were used to prepare two different types of microspheres. Both powders and microspheres were characterised using FTIR, TEM, SEM, mercury porosimetry analysis and X-ray diffraction. Results indicated that pure hydroxyapatite HA and a mixture HA/β-TCP in the nanometer range, were obtained from HA-1 and HA-2 synthesis respectively. Macroporous microspheres with different characteristics but showing adequate shapes and sizes were obtained from these two types of hydroxyapatite.

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