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Influence of concentration of hydroxyapatite surface modifier agent on bioactive composite characteristics

J.K.M.C. Gonsalves [a,*], J.N.S. Ferro [b], E.O. Barreto [b], R.S. Nunes [c], M.E.G. Valerio [a,d]

[a] Post Graduate Program in Materials Science and Engineering, Federal University of Sergipe, São Cristóvão, SE, Brazil
[b] Cell Biology Laboratory, Federal University of Alagoas, Maceió, AL, Brazil
[c] Department of Pharmacy, Federal University of Sergipe, São Cristóvão, SE, Brazil
[d] Physics Department, Federal University of Sergipe, São Cristóvão, SE, Brazil

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ABSTRACT

The characterization of chitosan – hydroxyapatite (CH – HAp) composite sponges prepared via freeze-drying methodology is reported in this study. Stearic acid (SA), added as a surface modifier of the HAp nanoparticles, induced changes in the TG/DTG results, particle size distribution and particle morphology. Composite sponges prepared with SA coated HAp demonstrated enhanced biocompatibility and structural properties, as compared to the composites prepared with uncoated HAp. SA coating modified the morphology of the composite, promoting a better dispersion of HAp particles within the composite sponges, and better homogeneity of the polymeric cover with HAp particles. The viability of the composites for cell culture applications was analyzed, and the results suggest that the sponges are biocompatible. Therefore, SA proved to be a good candidate for surface coating of HAp nanoparticles prevent agglomerations, and could be used effectively in the preparation of biocompatible composite sponges with chitosan.

1. Introduction

Hydroxyapatite (HAp), chemical formula $Ca_{10}(PO_4)_6OH_2$ and molar ratio Ca/P 1.67, is one of the most bioactive ceramics used in biomedical applications due to its similarity to the mineral phase of human bone [1–4]. Its unique surface properties, such as reactivity, porosity, polarity and hydrophobicity, and the presence of active functional groups Ca–OH and P–OH (as anchoring points of various substances), are the main reasons why HAp is commonly applied in bone tissue repair and reconstitution [1].

The formation of composites by combining the properties of HAp with the inclusion of a polymeric agent, can improve biocompatibility, osteoconductivity and osteoinductivity, and provides a structural arrangement for tissue growth and cell proliferation [5,6]. A wide range of biocompatible polymers is available, however chitosan (CH) is notable for its flexibility and malleability (it can be shaped into various physical forms), a toxicity, biocompatibility and biodegradability. It also prevents the formation of bacterial biofilms in a biological environment and can be obtained from renewable resources [5,7,8].

HAp particles tend to agglomerate when attached to polymeric meshes due to their high surface energies and polarity mismatch with the polymeric chains [2,3]. The interface established between the inorganic particles and the polymer is one of the main factors that affect the structural properties of the composites [3,9], thus affecting their usability.

In order to provide a better dispersion of the HAp particles in the biopolymeric net, avoiding segregation, modifications to the surface of the bioceramic can be carried out to improve the performance of the material. This may be done through addition of surfactants, coupling molecules, fatty acids or solvents (such as ethanol) [3,10]. Thein-Han et al. (2009) [11] reported that the surface modification of a HAp-silicon composite favored the cellular matching process, with increased cell attachment, viability and proliferation.

Surface modification can be done via biological, chemical, physical or mechanical methods [2], and the use of adhesion-modifying agents to the surface of HAp is based on the ionic interactions between the hydroxyl and the anions of the coating molecules [1].

The insertion of fatty acid-derived carbon chains is mostly done by chemical methods, in which a solvent is added to catalyze the reaction and induce the formation of chemical bonds between the

* Correspondence to: Materials Science and Engineering Department, Federal University of Sergipe, Av. Marechal Rondon, s/n, Prof José Aloísio de Campos City University, 49100-000 São Cristóvão, Sergipe, Brazil.
E-mail address: jkmarinheiro.cg@gmail.com (J.K.M.C. Gonsalves).

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acid and the active functional groups of the HAp surface [12]. Stearic acid (SA) is a medium-size fatty acid that is biocompatible and biodegradable, being excreted by the natural metabolism of the body [13]. SA is also an anti-agglomeration agent and has been used in the calcium carbonate industry to assist the flow properties [13,14].

Zhang et al. [12] modified the surface of HAp particles with SA in order to use them as a coating agent of titanium prostheses. They noted that the HAp-SA composite, obtained by physical adsorption, attenuated the corrosive process of titanium prostheses as compared to a HAp-only coated surface.

In this context, the aim of the present work was to produce HAp-Biopolymer composite sponges with well dispersed HAp particles in the polymeric chains. In order to overcome the agglomeration trend of the HAp nanoparticles, a surface modification was done using SA. Chitosan was chosen as the biopolymer, and the contribution of SA content in the interaction between the HAp ceramic powders and the polymer were evaluated. The cost effective methodology of heating under reflux was applied for HAp surface modification. The biocompatibility of the produced composite sponges was also evaluated via cell viability assays.

2. Experimental

2.1. Synthesis of hydroxyapatite particles and surface modification

The synthesis of hydroxyapatite (HAp-P) was done via wet precipitation route. A solution of 0.167 mol L^{-1} calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$] (NEON, São Paulo, Brazil) was slowly added under a flow rate of 2 mL min^{-1} to 0.1 mol L^{-1} solution of ammonium phosphate dibasic [$(NH_4)_2HPO_4$] (NEON, São Paulo, Brazil). The pH of the reaction medium was maintained at 10.4 by adding a solution of ammonium hydroxide (NH_4OH) (NEON, São Paulo, Brazil). The recipient was matured for 24 h at room temperature. After this period the resulting solution was filtered, and the precipitate was dried at room temperature and calcined at 600 °C for 1 h; obtaining the HAp nanopowders.

The modification of the HAp surface was done by stearic acid (SA) (Sigma Aldrich, St. Louis, USA), at mass fractions of 7% and 15% (weight/weight, w/w) to HAp content (HAp-M7% and HAp-M15% samples respectively). SA was solubilized in 250 mL of ethanol (C_2H_5OH) (NEON, São Paulo, Brazil) under heating. The HAp nanopowders were added to the solution and the SA/ethanol/HAp mixture was allowed to reflux for 48 h. Subsequently, the entire reflux system content was transferred to a beaker and stirred at room temperature until complete evaporation of the organic solvent [13].

2.2. Preparation of HAp/CH/HEC composite

Highly pure chitosan (CH) was purchased from Sigma Aldrich (St. Louis, USA), and deacetylation was checked by conductometric titration. The results indicated that the purchased CH had 95.25% of Degree of Deacetylation (DD). CH was solubilized in dilute acetic acid, forming a polymeric hydrogel with a concentration of 1% (weight/volume, w/v). It was observed that the viscosity of the CH only hydrogel was too low to guarantee a good homogeneity in terms of particle dispersion. Several tests conducted with this hydrogel confirmed that after adding the HAp nanoparticles, they precipitated to the bottom of the flask producing heterogeneous composites. One possible way to overcome this problem was slightly changing the polymeric chains by adding a small amount of hydroxyethyl cellulose (HEC) (PharmaNostra, Rio de Janeiro, Brazil), at a concentration of 0.3% (w/v). Under continuous magnetic stirring, the HAp was then slowly added to the CH/HEC hydrogel and the mixture was kept under stirring for 24 h at room temperature, producing homogeneous mixtures. After the homogenization the composites were frozen at −20 °C for 48 h and then subjected to lyophilization (Liofilizador LS3000, TERRONI, São Carlos, Brazil) for 24 h.

The composite sponges had an inorganic/organic concentration ratio of 66:34 (w/w), close to the average concentration ratios found in human bones [11,15,16]. Although it is not present in human bone tissue, CH has a structure that resembles the biological compounds, such as hyaluronic acid, and its biological properties favor the regeneration of human bones [17]. CH and hyaluronic acid are both polysaccharides with quite similar structures. CH is formed by random β-(1-4) links of N-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit), and the amount of deacetylated to acetylated units gives the DD degree. Hyaluronic acid is formed by repeated units of D-glucuronic acid and D-N-acetylglucosamine, linked via alternating β-1,4 and β-1,3 glycosidic bonds [18].

By keeping the inorganic/organic concentration ratios of the composite sponge close to the concentration ratio of the bone tissue, it was thus possible to focus the work on the influence of the surface modification of the inorganic HAp on the general structural properties of the composites. Table 1 describes the various composites investigated in this study with their composition.

2.3. Characterization techniques

2.3.1. Dynamic Light Scattering (DLS)

The dynamic light scattering technique was used to estimate the average hydrodynamic radii of the HAp-P, HAp-M 7% and HAp-M 15% samples and these values can be used to estimate the average particle sizes. The measurements were performed using a Malvern Zetasizer ZS analyzer (Malvern Instruments, UK), with a fixed detector at an angle of 173°, and a 35 mW He-Ne laser with wavelength of 633 nm. The measurements were done with the nanopowders, in suspensions that were prepared as follows: 3 mg of each sample was dispersed in 40 mL of acetic acid under ultrasonication (500 W Cole Parmer Ultrasonic Processor Model CV18), with 35% amplitude for three cycles of 5 min. An aliquot was taken at half height of the beaker and deposit in a quartz cuvette. Origin Pro version 8 software was used to analyze the data.

2.3.2. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra were obtained on a Shimadzu IR Prestige-21 spectrometer (Kyoto, Japan) in the wavenumber range of 4000–400 cm^{-1}, with 8 cm^{-1} spectral resolution and the accumulation of 32 spectra per scan for each sample. The samples were mixed to KBr (VETEC, Rio de Janeiro, Brazil) and conformed into pellets by uniaxial pressure.

2.3.3. X-ray diffraction analysis (XRD)

Powder XRD patterns were obtained on a Rigaku Ultima+ PCDMAX diffractometer (Tokyo, Japan) in continuous scan mode at

Table 1
Composition of inorganic/organic (66/34 w/w) composites in the presence of HEC. *HAp-P:* pure hydroxyapatite obtained by wet synthesis. *HAp–M7% and HAp–M15%:* hydroxyapatite with the surface modified by 7% and 15% stearic acid, respectively. *CH:* chitosan. *HEC:* hydroxyethyl cellulose.

COMPOSITES	
SAMPLE NAME	COMPOSITION
Group I (GI)	HAp-P + CH + HEC
Group II (GII)	HAp-M7% + CH+ HEC
Group III (GIII)	HAp–M15% + CH+ HEC

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