Synthesis of bioceramic foams from natural products

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Abstract

Bioceramic foams obtained through agri-waste products, such as egg shell and white egg recycle, represent an interesting way for waste prevention and waste management developing potentially commercial products. The aim is to design and investigate a new method to shape foams in a easy and environmental viable process. The use of egg shell as eco-compatible reactant instead of commercial ones, and the egg white as foaming agent to produce a tridimensional macroporous structures were optimized by a sol-gel route.

The crystalline and quantitative phase composition were studied by Rietveld refinement, optimization of the foaming process and porosity determination by SEM and Hg porosimetry. The work demonstrated that the use of egg shell and white egg allows to obtain bioceramic foams with composition of 60wt.% HA and 40wt.% β-TCP with a total porosity of 70% and a porosity ranging from 5 to 300 μm. These features are ideal for bone regeneration scaffolds and heavy metal capture from polluted waters.

Keywords: Bioceramics, regeneration bone defects, biological waste, reclycling.

Introduction

Natural ceramics such as skeletons of marine organisms (coral, sea urchins, oyster shells, conch and clam shells, etc.), fish bone and egg shells, useless after the utilization of their contents, are often considered as environmental pollution since they favor bacterial growth.[1] This waste is available in all kind of developed and undeveloped societies in huge quantity from food processing, egg baking and hatching farms. They have been previously used as Ca source for producing calcium phosphates ceramics to be applied on biomedical and tissue engineering as bone substitutes.[2,3] This fact fulfills the European societal challenge of turning a waste product into a potential material for improving life quality of bone disease patients.

Calcium phosphates produced from natural sources have been synthesized using various sources of Ca and P and through several methods,[4-7] including wet chemical precipitation, sol-gel, hydrothermal synthesis procedure, etc. and one of the most attractive Ca sources because of its abundance is the eggshell. It is composed in its majority (94%) by calcium carbonate, having other components in a minority range (less than 4%), such as calcium phosphate, proteins and magnesium carbonate. But calcium phosphates produced by the commonly used synthesis routes such as precipitation or hydrothermal route have several drawbacks, being the main one that the obtained materials are in the form of powders.

The main interest of proposing new routes is to produce these bioceramics in a final form which by means of its porosity, chemical and structure gets as similar to the natural bone as possible, to be directly used on the application with no further synthesis procedures needed. Moreover, there is a high interest on substituting the chemical reactants for natural products contributing to environmental preservation. This fact has become a major challenge to be met using the recycling of materials discarded by all these productive sectors.[8]

This study proposes a new upfront, economic, and reproducible technique for fabrication of calcium phosphates foams with a porosity range similar to the natural bone, using egg shell as Ca source. The main objective of the work is to propose a technology highly competent in terms of cost and quality. We have combined together two conventional methods to produce a tridimensional macroporous structure by using a sol-gel route submitted afterwards to a microwave and thermal treatment. This synthesis offers fast reaction, easy reproducibility, narrow particle distribution, high yield and throughout volume heating.

2. Experimental

2.1 Bioceramic *foams preparation*

Bioceramic foams (BF) were prepared as follows. The egg shells were washed with tap water several times and a last wash with distilled water. Afterwards, H_2O_2 was added to eliminate the organic matrix from the egg shell. The inorganic resulting product was dried at 50°C. The dried egg shells were milled and sieved, keeping the fraction of particles with sizes lower than 63 μm. 4 grams of $CaCO_3$ powder was added to 5.54 mL of nitric acid HNO_3 and 10 ml of MiliQ water and stirred at room temperature for 1 h, leading to a 4M solution of calcium nitrate.

$$CaCO_3 + 2HNO_3 \rightarrow Ca(NO_3)_2(aq) + CO_2 + H_2O \quad \text{(eq. 1)}$$

Meanwhile, a solution of 4.3 ml of triethylphosphite $P(OCH_2CH_3)_3$ (TIP), Sigma Aldrich) was diluted in 1.8 ml of water and stirred during 15 min. Afterwards, the calcium nitrate solution was added to TIP solution under magnetic stirring and the resulting sol was aged for 6 h at 60ºC.

The aged sol was then mixed with fresh egg white in a 1/1 and 2/1 ratio sol/egg white, respectively under a vigorous mechanic agitation to provoke homogeneous foam. After producing it, the foam is quickly dried using microwave heating for 8 min divided in two cycles of 5 and 3 min. Finally, the dried foam was calcined at 700ºC during 15 h leading to BF foams.

2.2 Bioceramic *foams characterization*

To evaluate the inorganic and organic fraction of the egg shells they were characterized by thermogravimetric (TG) analysis and X-ray diffraction (XRD) was used to confirm the crystalline phase. TGs were carried out in a Perkin-Elmer Pyris Diamond TG/DTA instrument, between 30 and 1000 ºC in air at a flow rate of 100 ml/min and a heating rate of 10˚C/min and XRD in a Philips X'Pert diffractometer using Cu K radiation.

To determine the quantitative phase composition, the BF sample diffractogram was refined by the Rietveld method using FullProf software.[9] Previously reported structural data for HA[10] and β-TCP[11] were used as the initial model for Rietveld refinements.

Scanning electron microscopy (SEM) was performed in order to evaluate the foams morphology in a JEOL JSM 6335F field emission scanning microscope with an ISIS Oxford LINK EDX analyzer. Elemental analysis was performed in a Perkin Elmer 2400 CHN and Analyzer. Foams porous range was assessed by Hg porosimetry measurements in an AutoPore IV porosimeter (Micromeritics Instrument Corporation, Norcross, GA, USA).

2. Results and Disscusion

Scheme in Fig 1 describes the detailed novel synthesis and conformation of BF foams from natural products. It consists in three steps: (1) agri-waste processing of egg shell, (2) calcium phosphate (CaP) sol synthesis and (3) conformation of macroporous BF foams by egg white stirring, microwave heating and annealing treatment.

Fig. 1. Scheme of synthesis and shaped of BF foams from natural products

XRD difractograms are depicted in Fig. 2 and confirm the calcite crystalline phase present on the natural egg shell used as source of calcium. The crystalline species present in the egg shell were identified comparing the characteristic peaks shown in the XRD pattern with the database from the International Center for Diffraction Data, Powder Diffraction File, International Center for Diffraction Data, Pennsylvania, USA, 2002. The distinctive peaks of the XRD pattern were identified as the crystalline phase of calcium carbonate in the calcite phase. This result corroborated that the egg shell sample is mainly composed of calcium carbonate.

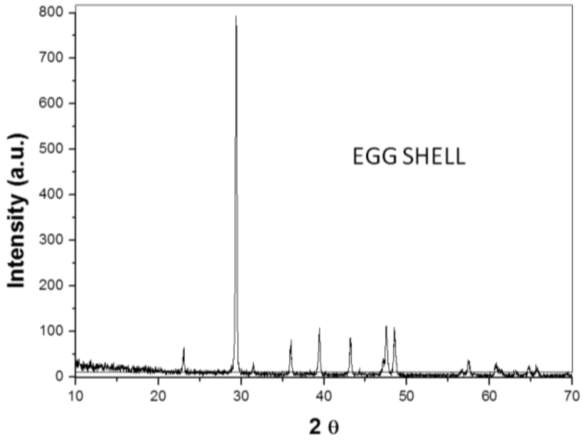

Fig. 2. XRD difraction of calcite phase from egg shell, natural reactive to obtain BF foams.

Fig. 3 shows the XRD patterns collected for the produced BF. The calculated pattern and difference plot obtained by Rietveld refinement are also shown. The BF phase composition after calcination at 700ºC corresponds to a hydroxyapatite and β-TCP phases. This pattern shows well-deconvoluted maxima with low profile broadening, which indicates that a crystallization process has occurred. The phase composition of BF foams calculated by XRD Rietveld analysis, corresponded to 60 wt.% HA and 40 wt.% β-TCP. The refinement of the appropriate size and strain parameters, using different models, provides automatically an output file with the apparent sizes along the different [hkl]* reciprocal directions[12]. We can reconstruct the average crystallite shape using the apparent sizes along the different directions with a crystallite size of 115 nm. The [100]* and [001]* directions reveals 80 and 180 nm, giving rise to needle shaped crystallites. These crystals are two fold bigger than apatite bone mineral with similar neddle shaped[13].

The biphasic composition of BF after its calcination is due to the presence of calcium deficient hydroxyapatite. That is because chicken egg shell presents cations substitutions (EDS in Fig. 4) and amorphous phases as carbonates and phosphates[14] that favoured calcium deficient apatite leading to a biphasic calcium phosphate HA/β-TCP composition after thermal treatment[15,16].

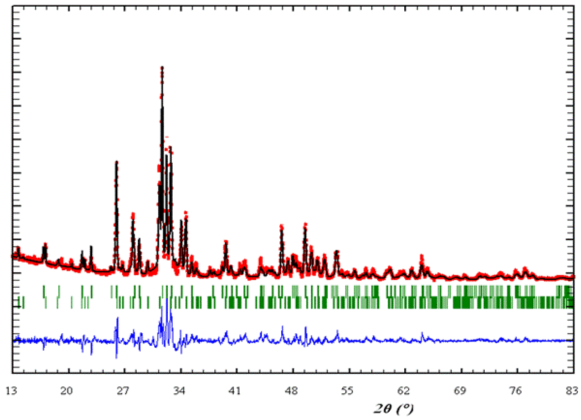

Fig. 3. Experimental (symbols) and calculated (solid line) powder X-ray diffraction patterns for HA/β-TCP foams. The lower trace is the difference between observed and calculated patterns. The vertical lines mark the position of the calculated Bragg peaks for an apatite and β-tricalcium phosphate phases, respectively.

To confirm the total elimination of organic matrix of bioceramic foam, elemental analysis was carried out. The analysis indicated no presence of carbon and nitrogen in their composition suggesting a total elimination of initial organic components of the natural product (organic egg shell matrix and egg white).

Hg porosimetry of the final BF foam (Fig. 4) shows a total pore volume ranging from 1-600 µm (being 600 µm the maximum range measured in the Hg porosimeter), what was also seen by SEM images Fig.5.

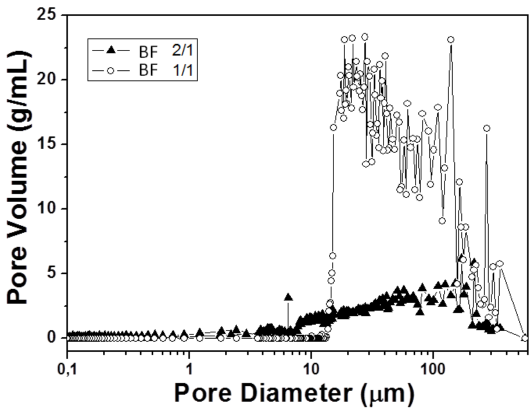

Fig.4. Pore size distributions by Hg intrusion corresponding to BF foams with a 2/1 and 1/1 relation of egg white/sol, respectively.

For a fixed sol amount, the connectivity between pores can be modified by decreasing the egg white amount. This allows the compression of the pore walls during drying and as the binder burns out. That process can be seen in the mercury porosimetry plots from BF foams at 2/1 and 1/1 sol/egg white ratio, respectively (Fig.4). The 2/1 ratio for BF sample have an excess of egg white on its composition and the most of the structure collapse during annealing, leading to a five-fold lower porosity than for the 1/1 ratio. Following these results, the 1/1 white egg/sol ratio has been selected as the optimal BF foams. All foams maintain their porous structure after drying and calcination process, preserving a total porosity measured by Hg intrusion of aprox. 70 %. Their specific surface area lays in the 1-600 µm range and surface area of 2.4 m^2/g. The macroporosity in the range of 1 to 20 µm is 21% of total porosity. As it is well known in tissue engineering applications, the macropore range is important as it allows cellular development, influences on the type of cells attracted, and plays an important role on the orientation and directionality of cellular ingrowth. SEM images in Fig. 5 exhibit an interconnected macroporosity ranging from 5 to 80 µm with some pores of around 300 µm in the fracture surface of the BF foams. The porosity higher than 100 µm, plays an important role in cellular and bone ingrowth, being necessary for blood flow distribution and having a predominant function in the mechanical strength of the substrate. Therefore, interconnected porosity of three-dimensional foams is a very important matter due to its great influence on the implant final behavior for bone regeneration[17]. In case of heavy metal immobilization these porosity is crucial for maximize the surface in contact with polluted water[18].

EDS measurements performed at the SEM observations, show a value of Ca/P ratio = 1.6 describing a biphasic calcium phosphate with HA (Ca/P 1.67) and β-TCP (Ca/P 1.50).

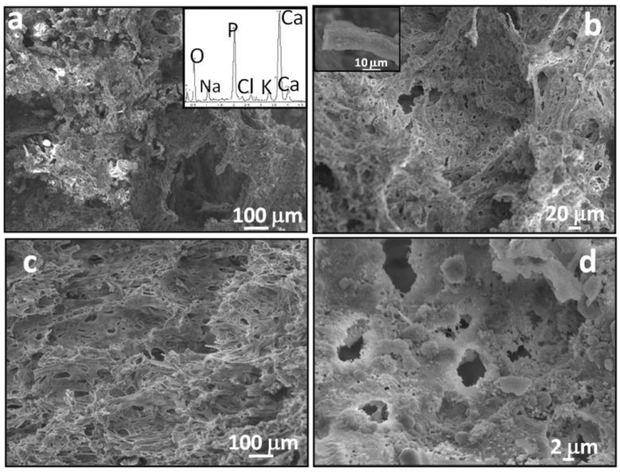

Fig. 5. SEM micrographs and EDX analyses of surface (a-b) and fracture (c-d) of BF foams with interconneted macropores.

These results are highly promising as currently, HA/β-TCP bioceramics are recommended for their use as alternatives or additives to autogenous bone for orthopedic and dental applications. Their bioactive behavior and excellent biocompatibility make them a very useful material for bone tissue replacement[19-21].

This is justified due to the higher solubility of the β-TCP component. The reactivity is therefore increased with the β-TCP/HA ratio. Consequently, the bioreactivity of these compounds can be controlled through control of the percentage of their phase composition[22-24] . Thus, it has been considered that 40% β-TCP/60% HA composition of the BF foams, has the optimal ratio of bioceramic phases. This is supported by the existence of already commercialized products containing this ratio, manufactured using synthetic chemical reactants for their use in biomedical applications. They show optimal bioreabsortion and bone regeneration. Some of this commercial examples are Triosite™,4Bone™, Hatic™ , OptimMX etc[25].

Another application of BF foams is as a heavy metal captor from polluted waters. It is consisted of immobilize toxic metals (Pb, Ag, Cu etc.) through the formation of metal phosphates form calcium phosphates with reduced solubility in a wide range of environmental condition[26]. That is energetically and economically profitable for treating consumable waters in underdeveloped areas. That method of inmobilization is more efficient when compared to other proposed materials such as zeolites, peat moss or aluminum oxide[27].

Therefore, the increase of sensibility to environmental issues together with the orientation towards the reuse of waste materials opens a wide range of new eco-compatible products for substituting the synthetic chemical reagents. The recovery leads to benefits, such as saving of natural raw materials and energy, and even, to an improvement of the quality of the final products[28].

Regarding the benefits of using egg shell Oliveira et al. have estimated the respective economic & environmental benefits and investments[29]. The different applications for the use of egg shell waste as a base material for bioceramics or as heavy metal captor could suppose an economical benefit of fivefold cost of the conventional treatment of egg shell residues. From the point of view of environmental benefit, the reduction of energy consumption in a direct method for 3D conformation of these bioceramics is the main parameter to be evaluated when compared with the environmental impacts of the applications using chemical treatment and high temperatures on the formation of powders used in previous processes. Using the proposed method and agri-waste products, it could be reduced over 90%[30].

Conclusions

A novel technology for the preparation of the BF foams has been carried out using a simple and economic technology. Agri-waste products have been used in the manufacturing of bioceramic foams, instead of chemical comercial reactives with high contents on calcium. The greatest novelty lays in the direct manufacturing of 3D bioceramic foams from natural resources (egg shell and egg white) with minimized environmental impact and contributing to the recycling cycle. Their physico-chemical characteristics, composition, high and interconnected porosity and consistency are adequate to proceed with the application of these BF foams as bone regenerative scaffolds as well as heavy metal immobilization applications. This strategy must be tested in pilot scale studies prior to seeking to expand into commercial scale.

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