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APATITE NUCLEATION OF DENSE AND POROUS CHITIN MEMBRANES

[1]Leandro Medeiros Motta, *[2,3]Gláucio Diré Feliciano and [4]Cristina Tristão de Andrade

[1]Unit Pharmacy (UFAR), State University Center Foundation of the West Zone (UEZO), Rio de Janeiro, Brazil.
[2]Laboratory of Chemical and Biological Analysis (LAQB), State University Center Foundation of the West Zone (UEZO), Rio de Janeiro, Brazil.
[3]Estácio de Sá University (Estácio), Health Sciences Area, Rio de Janeiro, Brazil.
[4]Federal University of Rio de Janeiro (UFRJ), Institute of Macromolecules Professor Eloisa Mano, Rio de Janeiro, Brazil

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***Correspondence for Author**
Leandro Medeiros Motta
Unit Pharmacy (UFAR), State University Center Foundation of the West Zone – UEZO.

ABSTRACT

The formation of apatite (CHA) from a synthetic body fluid was induced by the presence of dense and porous chitin membranes. Infrared spectra and thermograms obtained for chitin alone and for the composite biomaterials were compared and indicated the deposition of CHA. Scanning electron micrographs showed the formation of spherical crystals of CHA on the surfaces of both membranes. Images obtained for the cross- sections ceramic material with apparently lower crystallinity was observed for dense membranes.

KEYWORDS: apatite nucleation, dense and porous membranes, chitin, crystallinity.

INTRODUCTION

Polymeric membranes have been developed as matrices for artificial mechanical support of osteogenic cells in bone tissue regeneration. When implemented, many of these materials are encapsulated by fibrous tissue and remain as foreign bodies without adherence to the bone. To overcome this problem, the use of apatite in polymeric composites have been recommended, in order to obtain materials capable of mimicking the extracellular matrix of bone tissue.[1]

Calcification is a natural process that occurs during many situations in the human body, for example, after a bone is fractured. The natural process of calcification can be reproduced and investigated in laboratory biomaterials such as chitin. The calcification of biomaterials can be called deposition "in situ". The deposition studies "in situ" are of great interest in many scientific and technological fields. In the field of materials, they lead to the development of more resistant materials and functional [Cassiano]. In the medical field, they help understanding the natural formation of bones and teeth and allow obtaining osteogenic implants and prostheses for orthopedic correction.[2] as well as help in developing new frameworks for tissue engineering,[3] Moreover, the prostheses generally do not fail due to calcification or pathological calcification. Detailed knowledge of biomineralization processes help prevent the pathological calcification and, consequently, the failure of medical implants. Hydroxyapatite (HA) of the formula Ca_{10} $(PO_4)_6$ $(OH)_2$, consists of the main inorganic component of hard bone tissue. However, the in vivo stability of the synthetic HA slows absorption. In order to improve absorption, porous HAs, or nanostructured fibers have been developed. On the other hand, the HA differs in its stoichiometry, composition and crystallinity of other biological apatites, which have a higher composition carbonate and are deficient in calcium. Synthetic Fluid (SBF), with ion concentrations similar to those of human blood plasma were used for obtaining apatite of a single phase (CHA), neutral aqueous medium, and were considered as the best source for obtaining apatite carbonate.[2,3]

In a study it was observed that the chemical characteristics indicate that the analyzed chitosan has high solubility in moderately acidic solutions, that is relevant to its applications in various areas.The analysis of the healing process under seen macroscopic point discloses that chitosan plays an important role in recovery from acute cutaneous lesions in rats by accelerating the healing process and providing reduction of the width of injury, which enhances their potential for medical application.[5]

In this study, the nucleation of apatite in dense and porous membranes of chitin was investigated.

MATERIALS

PA lithium chloride (LiCl), Vetec Fine Chemicals Ltd., Rio de Janeiro; N, N-dimethyl acetamide (C_4H_9NO), Vetec Fine Chemicals Ltd., Rio de Janeiro, RJ; Chitin powder extracted from shrimp shells Penaeusschmitti, coming from the fishing port of Niterói, RJ.

Trial

SchmittiPenaeus shrimp shells were washed in distilled water at room temperature. After drying, the skins were milled and subjected to treatment with 0.25N HCl for 30 minutes. After neutralization, the demineralized material was treated with 1N NaOH solution at room temperature for 24 hours to deproteinization.

To obtain dense chitin membranes a LiCl solution of 7% was prepared in N,N-dimethylacetamide (DMAc) and used for the dissolution of polysaccharide to 1% (w/w). The solution was poured into Petri plates and conditioned under 88% relative humidity, the 37°C for 3 days. After this period, the membranes were washed with deionized water until complete elimination of the organic solvent.

To obtain porous membranes of chitin, a 5% solution of LiCl in DMAc was prepared. Calcium carbonate 2% chitin and 0.5% were added and the resulting solution was poured into Petri plates and conditioned as described above. The chitin membrane $Ca(CO_3)_2$ were flooded with 1N HCl solution for 1 hour and washed with deionized water.

The simulated physiological fluid (SBF) was prepared according to the literature,[4] by mixing aqueous solutions of NaCl, NaHCO3, KCl, $Na_2HPO_4.2H_2O$, $MgCl_2.6H_2O$, $CaCl_2.2H_2O$, Na_2SO_4, and $(CH_2)3CNH_2$ in Erlenmeyer flask and the volume completed to 700 mL with water. Prior to adding the sixth solution, a volume of 15 mL of 1N HClwas added. The pH was adjusted to 7.4 with additions of 1N HCl and the volume made up to 1 liter.

The chitin membranes were kept in SBF for a period of 7 days, after which they were dried at 50°C to constant weight.

The membranes of chitin/CHA were analyzed by infrared absorption spectroscopy, thermal analysis and scanning electron microscopy.

RESULTS AND DISCUSSION

Figure 1 shows the absorption spectra in the infrared chitin (a) and the powder obtained from chitin dense membrane after immersion in SBF (b). In Figure 1a, three peaks can be observed in the carbonyl group absorption region of the 1652, 1627 and 1558 cm^{-1}. These absorptions, as with that to 1420 cm^{-1} are characteristic of chitin. Although there is interference of the polymeric phase and the characteristic bands of the phosphate group at 1030 cm^{-1} and carbonate group at 1460 cm^{-1} are not resolved, the wider bands in these regions and the -OH group stretching region and the band 870 cm^{-1} indicate the deposition of CHA on the membrane.

The thermal behavior of chitin and composite obtained by the deposition of CHA dense membrane was evaluated up to 900 °C by thermogravimetric analysis. Adsorbed water loss occurred by heating to 150 °C in both samples. Decomposition of chitin sample was performed in two steps, with a significant loss of mass of between 330 and 400 °C and between 400 and 750 °C. For the composite, the range studied, significant weight loss was in the range 250 to 400 °C. From 400 °C, greater thermal stability was observed.

Figures 2 ad shows scanning electron microscopy images (SEM) of dense membranes (a, c) and porous (b, d) after the nucleation of CHA in SBF. In Figures 1a and 1b, the morphology of the surface with spherical CHA crystals can be observed. Figures 2c and 2d show micrographs of cross sections, analyzed after immersing the membrane in liquid nitrogen. For unclear reasons, CHA crystals can be seen in Figure 2d, whereas the dense membrane, the CHA deposited appears as lower crystallinity material.

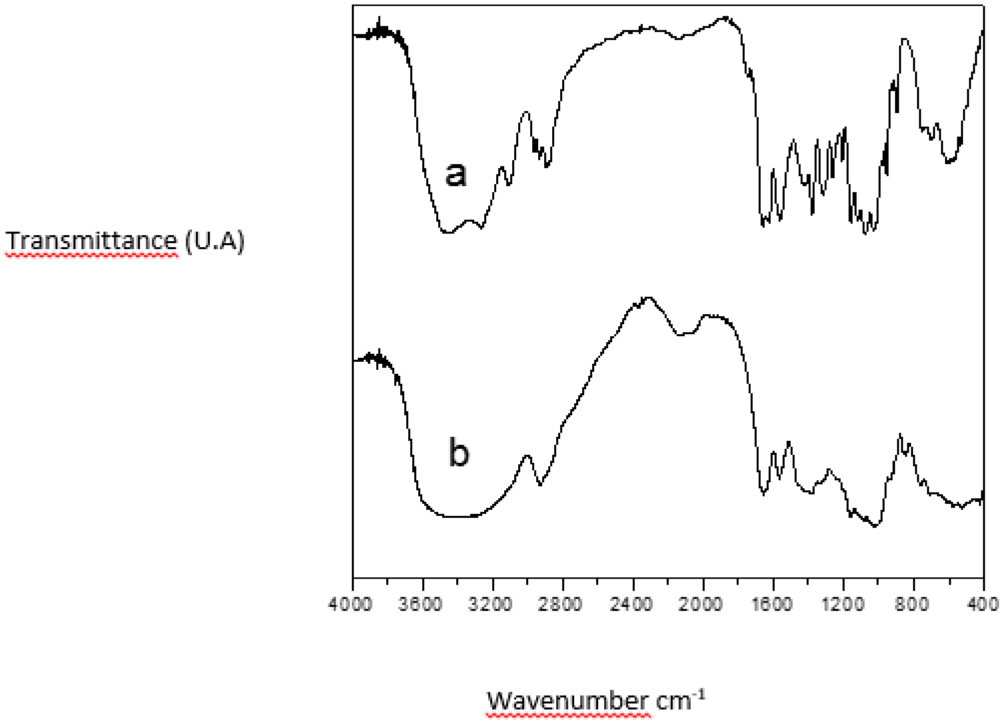
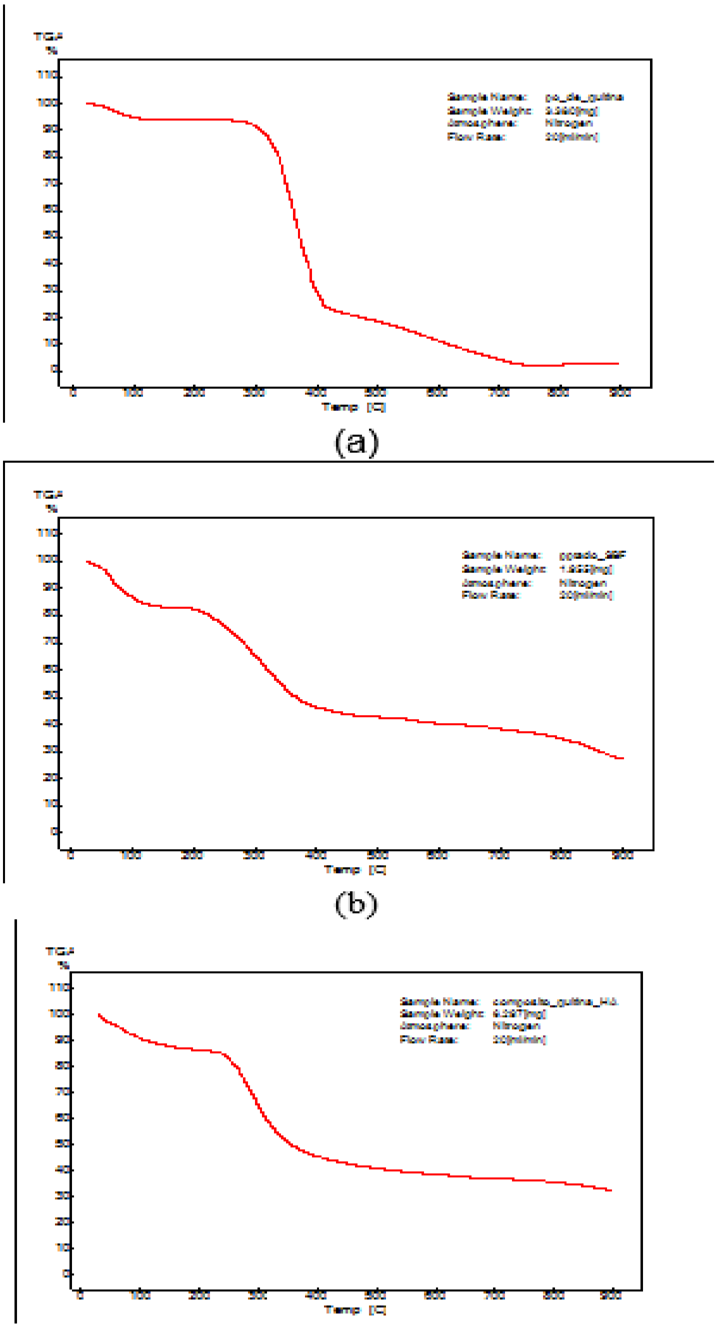


Figure 1 absorption spectra in the infrared chitin (a) and the composite chitin / CHA (b).



(a)

(b)

(c)

Figure 2: chitin alone thermograms of (a) the precipitate filtered FBS, after 7 days in contact with the dense membrane chitin (b) and the dense membrane of chitin after 7 days of immersion in SBF.

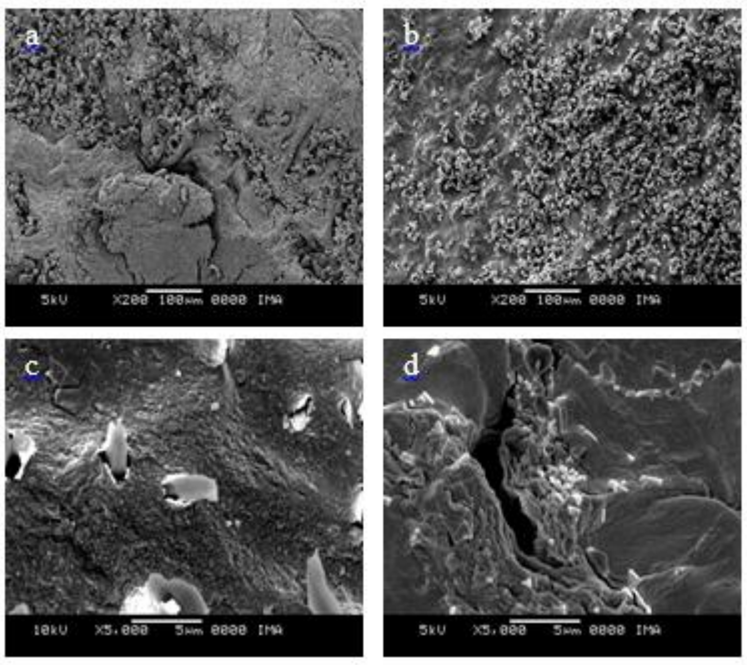


Figure 3: Scanning electron micrographs of the surfaces of dense membranes (a) and porous (b) and cross sections of dense membranes (c) and porous (d), after immersion in SBF for 7 days.

CONCLUSIONS

The results show that the chitin does not act as an inert material SBF and induces the nucleation of CHA. CHA crystals were visualized by scanning electron microscopy on the surface of dense and porous membranes and within porous membranes, after immersion in SBF for 7 days.

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