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# Chitosan calcium phosphate: an attempt to induce chitosan scaffolds mineralization

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Resumo:
Osseous reconstruction is a problem of interest for tissue engineering. One approach applied on this issue is the development of biomaterials used to induce osseointegration during osseous repair process, comprising materials from calcium phosphate bone cements to many different phosphorous-containing polymers. The incorporation of phosphorous-containing groups into natural or synthetic polymers as a way to induce the inorganic crystal nucleation and growth and to control its microstructure is an attempt to mimic the function of the organic phase of osseous tissues and promote bone tissue formation. In this study we analyze the effect of chitosan-calcium phosphate (CCP) salt on the mineralization of scaffolds of chitosan (CH). CH was extracted from squid pens and solubilized at 1% in H3PO4 9.4 mol L-1 and stirred for 1h (60ºC) to produce CCP. Then CCP was precipitated with CaCO3 until neutrality, washed with ethanol and dried at 50°C. The product was a yellowish solid soluble in water. A 1% CH solution was prepared by dissolution in 1% acetic acid. Chitosan/chitosan-calcium phosphate (CHCCP) gels were prepared by mixing 2.0 g of 1% CH solution to 0, 5 and 10 mg of CCP under stirring for 3 h, resulting respectively in CHCCP0, CHCCP5 and CHCCP10. Scaffolds were prepared by freeze-drying and the mineralization process was carried out by alternating soaking in 0.2 mol L-1 CaCl2 solution buffered with 0.05 mol L-1 Tris (pH 7.4) and 0.12 mol L-1 Na2HPO4 solution buffered with 0.05 mol L-1 Tris (pH 9.0) at 37°C for 30 minutes each, rinsing with water between the changes and repeating these cycle six times. CH and CHCCP5 before and after mineralization were analyzed by Fourier Transform Infrared spectroscopy. Scaffolds before and after mineralization were analyzed by thermogravimetric analysis and scanning electron microscope (SEM). The CH spectrum showed typical bands for this polymer, such as the O-H stretching band in 3400 cm-1 and the amide I (C=O stretching) at 1657 cm-1, bands also found in CHCCP5 spectra. A P-OH stretching band was observed at 571 and 561 cm-1 for CHCCP before and after mineralization, respectively, indicating the inclusion of phosphate groups in CH. Thermal behavior of scaffolds was characterized by three stages of mass loss; the first one (25-200°C) is associated with release of water and is higher with CCP, the second one (200–380°C) is due to degradation of CH structure and the third stage (380–650°C) is related to the carbonization of polymer. The residue values obtained at 750°C were used to determine the inorganic material contents produced in the mineralization, which were 28.4, 30.2 and 31.4% for CHCCP0, CHCCP5 and CHCCP10, respectively. These values indicate that the presence of CCP did not substantially increase the mineralization efficiency. SEM results showed fully mineralized surfaces with spherical aggregates, which presents a morphology of needle-like, a shape commonly observed in crystalline apatites.