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Physico-chemical and cell adhesion and differentiation study of 3 coralline species partially transformed to hydroxyapatite

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Abstract –The exoskeleton of some coralline species has remarkably similarities to bone structure. A hydrothermal exchange process for partial transformation to hydroxyapatite of three coralline species: Acropora *palmata,* Montrastrea *Annularis* and Porites *Astroide,* has been used in the present work and its biocompatibility was tested with osteoblast for cellular activity. The samples were characterized by x-ray diffraction (XRD), infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) attached with an energy dispersive analyzer (EDS). The samples were transformed for different periods from 12 h to 48 h and. The best results were obtained for 48h transformation in all three coralline species. The mechanical properties after transformation were also tested showing that the materials kept their strength after transformation.

One of the main requirements for a material to be considered a potential bone graph substitute are adequate porosity of the order of 100 to 200 μm, an osteoconductive structure with a resorption rate which is concomitant with the bone formation process, the adequate mechanical properties and good biocompatibility. The exoskeleton of some coralline species have remarkably similarities to bone structure[1-3]. In the present work a hydrothermal exchange process of the $CaCO_3$ in phosphate solution has been carried out in three coralline species: *Acropora palmata*, *Montrastrea annularis* and *Porites astreoide* with the aim of obtaining partial or total conversion to hydroxyapatite with preservation of the coral skeleton pore architecture. The study of biocompatibility and bioactivity by bone cell adhesion and differentiation were also carried out. The samples were characterized by X-ray diffraction (XRD), infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) attached with an energy dispersive analyzer (EDS). Mechanical properties and porosity before and after transformation were also performed.

Three coral exoskeletons *Porites asteroides, Mastrostea annularis* and *Acropora palmata* were studied. They were thoroughly clean in sodium hypoclorite, washed and sonicated in distilled water for several days. Hydrothermal treatment was carried out in an autoclave by reaction of the coral exoskeletons with stoichiometric $(NH_4)_2HPO_4$ at 280ºC for 0, 12, 24 and 48 h. Osteoblast were isolated from neonatal rat calvarias Sprague Dowley, 2-3 d of age. Cellular adhesion was determined in three time intervals of 4, 6 and 24 h, by direct counting of the remaining cells in suspension after placing in the culture plate $3x10^5$ cells/well. Cell proliferation was determined by direct counting method after 7 days of culture. Culture cells were treated with dexametasone to induced differentiation.

Determination of alkaline phosphatase of the culture media at time intervals of 4, 8 and 14 d was carried out and evaluation of the deposited mineral on coral surface was performed. The samples were characterized by X-ray diffraction (XRD), infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) attached with an energy dispersive analyzer (EDS). Porosity measurements were carried out by water immersion and also by mercury porosimetry.

The results showed that partial transformation of calcium carbonate to hydroxyapatite progressively occurs with reaction time, after 48h the transformation is almost complete. The species with higher porosity (*Montratrea* 60% and *Porites* 56%) showed the fastest and more homogeneous transformation than *Acropora* (35%). Cell adhesion results showed that cell adherence took place in a 100% after 4 h of incubation in comparison with the control. Important differences were observed neither between the different coral species nor with the different thermal transformation periods.

SEM images showed that an appreciable greater number of osteocyte cells on the surface of *Porites asteroide* in comparison with the other species studied, this is consistent with the alkaline phosphatase activity. For all the conditions tested it was possible to see the bone cells growing and establishing a notable cellular network the pore areas of the materials

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