

CHARACTERIZATION OF HAP/CHITOSAN COMPOSITES FOR LONG BONE REPLACEMENT

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Worldwide around 200 million people are annually afflicted by musculoskeletal system problems from accidents or diseases [1]. According to the World Health Organization WHO, the high energy fractures are the highest problem for a hospital due mainly to the slow bone regeneration and expensive health care [2]. Nowadays, many methods and techniques have been developed and applied to design advanced materials for bone replacement. The combined use of synthetic or natural bone grafts, and growth factors play an important role in long bone regeneration. The ideal synthetic biomaterial should be biodegradable, biocompatible, osteoconductive or osteoinductive, cheap, and with high surface area to volume ratio. A strategy for imitating in a tridimensional way the structure, composition and mechanical properties of a bone is mimicking it at nanometric scale. In this work we present the characterization of HAp/Chitosan composites by using scanning electron microscopy SEM, energy dispersive spectroscopy EDS and X-ray diffraction XRD for its application in long bone replacement. The composites were prepared by the electrospinning technique using as precursors hidroxiapatite HAp and Chitosan. Chitosan (MW= 650kD) is a natural, plenty, and biocompatible polysaccharide rich in amine ions, obtained from crustacean shells [3]. At pH's lower than $pK_a = 6.3$ (pK_a : is pH where the non-ionized fraction corresponds to 50%), most of the amine groups (-NH₂) are protonated, becoming the chitosan in a water-soluble compound, and a cationic polyelectrolyte (-NH₃) [3,4]. By its side, hydroxyapatite HAp: Ca₁₀(PO₄)₆(OH)₂, is the most important biological calcium phosphate presents in the vertebrate bones [5]. The HAp nanoparticles used in this work were manufactured by the hydrothermal technique (results do not showed here). HAp/Chitosan fibers were spun from different aqueous solutions, all of them with a pH= 3. The X-ray diffractometer employed in this work use a Cu-K_α line (Siemens D5000 and the samples were analyzed with a 0.2° step, 40 mA and 20 kV, the scanning electron microscope (JSM 6300 JEOL) worked at 5 and 10kV combined with a X-ray Energy Dispersive Spectrophotometer (system NORAN 1100/1110EDX). The EDS microanalysis results were obtained over an area of 100 μm². By this characterization technique we quantified the concentration of C, Ca, P, O and N. Table I shows the Ca and P contents in the composites, and the respective Ca/P ratios. The Ca/P ratio is an important parameter when the obtaining of a bone substitute is attempted, then bone and HAp Ca/P ratios are included in order to compare our results with the standard values. We found that at pH=3, 1% w/v chitosan, and 1% w/v HAp and spun at 20 V, a Ca/P ratio close to bone composition (Ca/P_{bone} = 1.67) was obtained. The HAp hexagonal phase of the samples synthesized was confirmed by the XRD patterns. Fig. 2 shows the morphologic difference between pig trabecular bone the samples A and B and the samples synthesized by electrospinning C and D with different concentration HAp/chitosan ratio and the spun voltaje. From these results, it is evident that, the morphology is strongly influenced by both deposition parameters. Fig. 3 shows the HAp,

Chitosan, and MCP standard patterns, together with the spectra of C and D samples. Three HAp characteristic peaks, (211), (112) and (300) are present in the spectra, but also the (-1-21) and (-121) peaks corresponding to calcium phosphate MCP ($\text{CaH}_4\text{O}_8\text{P}_2$) are present [6]. The presence of the MCP in the composites is justified for being a precursor in the HAp synthesis. According to the spectra, we can say that, the composites synthesized preserve the structural characteristics of HAp.

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TABLE I
CA AND P CONCENTRATION IN HAP/CHITOSAN
SAMPLES ESTIMATED FROM EDS
MICROANALYSIS

Sample	Calcium Ca (%)	Phosphorus P (%)	Ca/P ratio
MA	12.05	9.34	1.30
MB	43.39	10.76	4.04
MC	67.81	32.18	2.10
MD	6.32	12.55	0.5
HAp(teo)	5	3	1.67
Bone	59.29	32.81	1.4 – 1.7

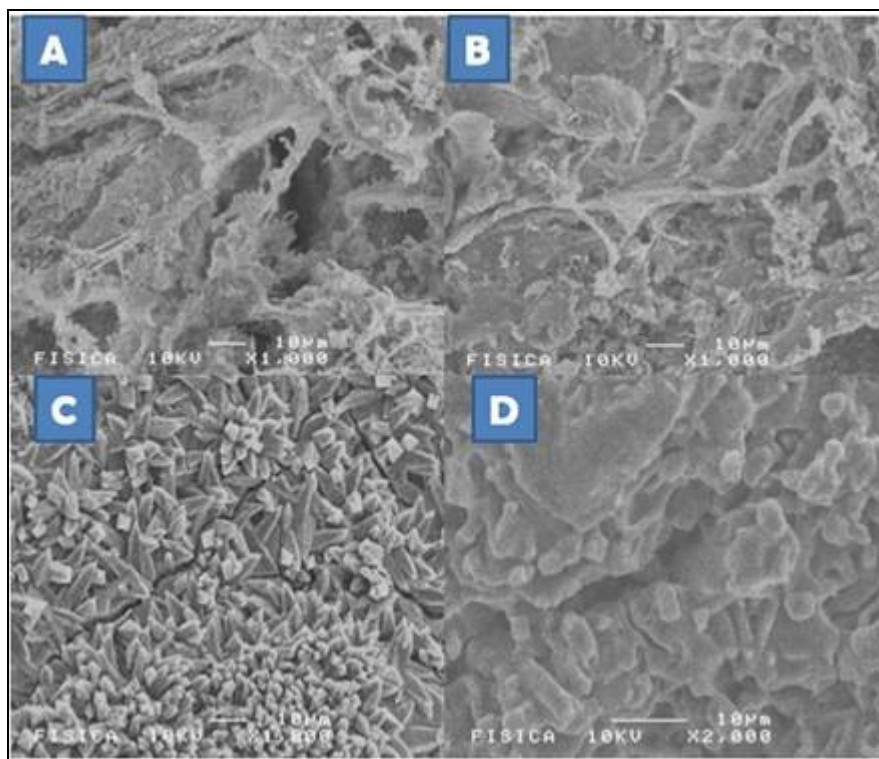


Figure 1. SEM micrographs of samples obtained from solutions containing different HAp/chitosan ratios, and two magnifications and 10 kV.

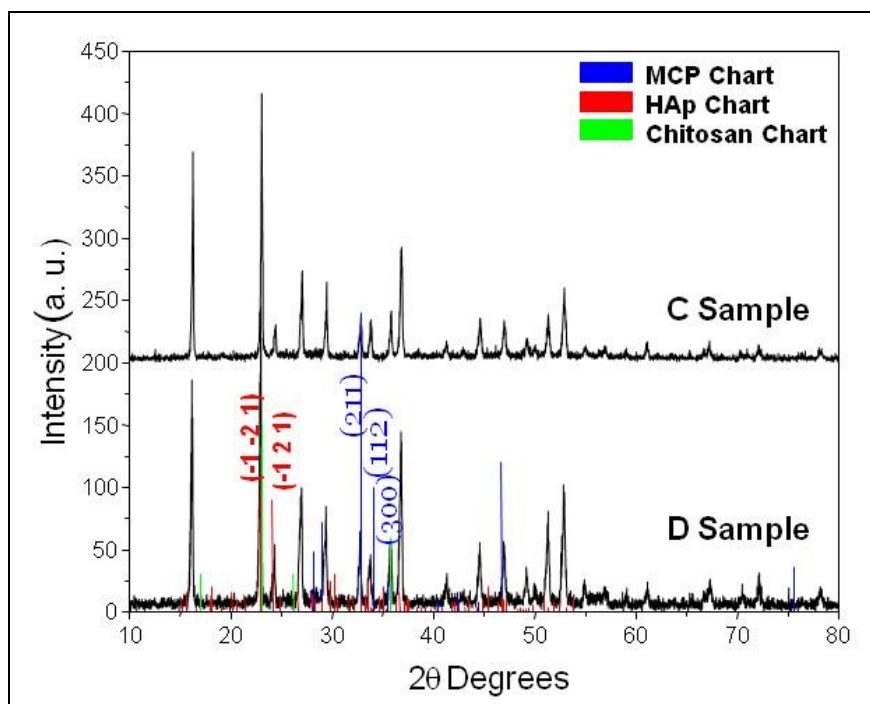


Fig. 3 XRD spectra of the C and D samples

