MINERALIZATION OF CHITOSAN : COLLAGEN : GELATIN HYDROGELS

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ABSTRACT

Hydrogels are macromolecular networks defined as capable of absorbing large quantities of water and other fluids. Various hydrophilic polymers are used in the preparation of hydrogels for use in pharmaceutical and medicinal fields. Hydrogels have been widely studied in tissue regeneration with great interest in the regeneration of bone tissue in an attempt to overcome problems of grafting. Collagen, chitosan, and other natural polymers may be used to produce such hydrogels. This work reports the preparation of collagen:chitosan:gelatin hydrogels, crosslinked with grape seed extract and subsequently mineralized *in vitro*. Chitosan was prepared by alkaline deacetylation of chitin obtained from squid pens and a 1% chitosan solution was prepared by dissolving chitosan in acetic acid (1%). A 1% gelatin (Sigma-Aldrich) solution was prepared in 1% acetic acid and gelatinized at 60°C for 30 min. Anionic collagen was obtained by treatment of bovine tendon in aqueous alkaline solution containing salts (Cl^- and SO_4^{--}) of K^+, Na^+ and Ca^{++} (pH ~ 13), for 24 h at 20°C. Collagen gel was obtained by extraction with acetic acid (pH 3.5) aqueous solution and concentrations (1wt%) were determined by lyophylization. The hydrogels were prepared by mixing chitosan/collagen/gelatin (1:1:1) and incorporated with 0.35% of grape seed extract and named QCGE and QCG (without extract). The hydrogels were cooled and immersed in 0.12 mol L^{-1} Na_2HPO_4 buffered with 0.05 mol L^{-1} Tris buffer (pH 9). The *in vitro* mineralization process was carried out by soaking the hydrogels in 0.2 mol L^{-1} $CaCl_2$ buffered with 0.05 mol L^{-1} Tris buffer (pH 7.4) at 25°C for 30 min, rinsing with deionized water and then soaking in 0.12 mol L^{-1} Na_2HPO_4 solution buffered with 0.05 mol L^{-1} Tris buffer (pH 9.0) for 30 min and rinsing with deionized water. The alternate soaking cycle was repeated 6 times to obtain the mineralized hydrogels which were then frozen, lyophilized and named QCGEM and QCGM, respectively. The evaluation of the *in vitro* mineralization ability has been performed by DSC, TGA, SEM, EDX and X-ray diffraction. DSC curves showed the denaturation temperature (Td) of collagen present in the samples being 48.3°C (QCG) and 51.5°C (QCGE), indicating the crosslinking effect. TGA curves showed residues higher than 50%, which correspond to the formed calcium phosphate. EDX spectra showed Ca/P ratio of 1.48 ± 0.07 (QCGM) and 1.52 ± 0.01 (QCGEM). The surface and cross-section morphology was analyzed by SEM that indicated a porous structure, with interconnected pores throughout the lyophilized hydrogels and with extensive mineral deposits, homogeneously distributed on the hydrogels surfaces and internally. Powder X-ray diffractograms were performed in a Rigaku RU200B equipment, using CuKα radiation, 50 kV, 80 mA, rate scaning 2^o min^{-1} and 2θ between 5 to 80^o. The diffraction peaks are well defined and assigned to monophase crystalline HA. Although the diffraction showed that in both cases only HA formation occurred, it was observed by SEM that the extract morphologically modifies this deposition.

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