Effects of pH and Temperature on Microstructure and Morphology of Hydroxyapatite/Collagen Complex Synthesized in vitro

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ABSTRACT

Collagen is the main structural protein in connective tissue of animal. Hydroxyapatite (HAp.) is a natural inorganic substance of hard tissue in vertebrate, which has excellent biological compatibility. This research mainly was divides into two parts. The first part, collagen was extracted from pork skin using salting out method, and then analyzed its yield, purity, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis and scanning electron microscope (SEM) observation. The second part, collagen and hydroxyapatite were coprecipitated by proper ratio under different temperature and pH conditions for several hours to form HAp./collagen complex. The SDS-PAGE behavior, fourier transform infrared spectrophotometer (FTIR) spectra, X-ray diffraction (XRD) spectra and SEM microstructure of the complex were analyzed. The results were found as the follows： The yield and purity of collagen extracted from pork skin were 11.38% and 31.42%, respectively. The SDS-PAGE patterns and SEM micrographs were observed, collagen prepared by our lab was similar with the products purchased fom Sigma Co. and National Chung Hsing University (NCHU). The SDS-PAGE pattern of the HAp./collagen complex was found that alpha 2 chain disappeared as compared with collagen itself. In alkaline condition, alpha 2 chain disappeared slowly. The phenomenon indicated that the synthesis reaction proceeded. In acid condition, it happened synthesis reaction obviously after precipitating for 36 hours. The FTIR spectra showed the complex arose a new bond of HAp./collagen, but it had confused absorbed peak at pH 5. The X-ray diffraction spectra, HAp. had crystal reaction. But at pH 5, it produced much confused crystal. The SEM micrographs for the HAp./collagen complexes were found leaf-like structure when the synthesis pH value was lower. When the synthesis pH value was higher, the complex showed a compact coral-like shape.

Keywords : collagen ; hydroxyapatite ; pH and temperature ; synthesis

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