Effects of pH and temperature on microstructure and morphology of collagen/hydroxyapatite matrix synthesized in vitro

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Abstract

Collagen and hydroxyapatite (Hap.) are co-precipitated by proper ratio under different conditions for 18 hrs to form Hap./collagen complex. SDS-PAGE pattern, FTIR, and microstructure of the complex were analyzed to confirm the bonding of Hap. and collagen and the effects of pH and temperature on the microstructure of the complex. The results were as follows: SDS-PAGE pattern of the Hap./collagen complex was found that α2-chain disappeared from the banding as compared with collagen. FTIR spectra showed a peak appearing at 1338cm-1 which indicated that the complex arose a new bonding of Hap./collagen. From the SEM micrographs for the Hap./collagen complexes were found more compact networks easily formed under the neutral to alkaline conditions at animal body temperature 37 °C than under acidic pH and temperature at 40 °C. However, SEM micrographs showed a leaf-like structure under pH 5 and temperature at 40 °C condition. From these results we conclude that Hap. and collagen composite can be synthesized in vivo under animal physiological conditions.

Introduction

Collagen and hydroxyapatite (Hap.) are mainly constructional components of bone. Hap. is the most stable under the physiological conditions and also possesses an excellent biocompatibility. Therefore, Hap. and collagen are currently used as the biomaterials for artificial bone synthesis. The purpose of this study was to investigate effects of pH and temperature conditions on microstructure and morphology of Hap./collagen complex synthesized in vitro.

Materials and methods

Collagen extracted from pork skin using the method modified from the procedure described by Rousseau and Gagnieu (2002). The purity% of the collagen was determined by the method of Reddy and Enwemeka (1996).Hap./collagen complex was synthesized using the self-organization mechanism described by Kikuchi *et al*.(2001). SDS-PAGE was carried out by the procedure described by Harlow and Lane (1988). Scanning electron micrograph observation: the sample of Hap./collagen complex was dehydrated by freeze drying and coated with gold. The micrographs were observed by SEM (JSM-7401F, JEOL). In order to confirm the reaction of collagen with Hap., fourier transform infrared spectra were recorded by FTIR spectrophotometer (FTIR-8400S Shimadzu, Japan) using the method of Rodrignes *et al*. (2003).

Results and discussion

The yield% and purity% of collagen extracted from pork skin were about 11.83% and 31.42%, respectively. The photograph of Fig. 1 was the appearance of Hap./collagen complex. Fig. 2 indicated SDS-PAGE patterns of the supernatant and precipitate of Hap./collagen complex. From the result we found there was no any protein existing in the supernatant (lane 3). Lane 1 was the pattern of collagen and lane 2 indicated the pattern of Hap./collagen. From lane 2 we found alph 2 disappeared from the gel, it may bond to

Hap. to form Hap./collagen complex.

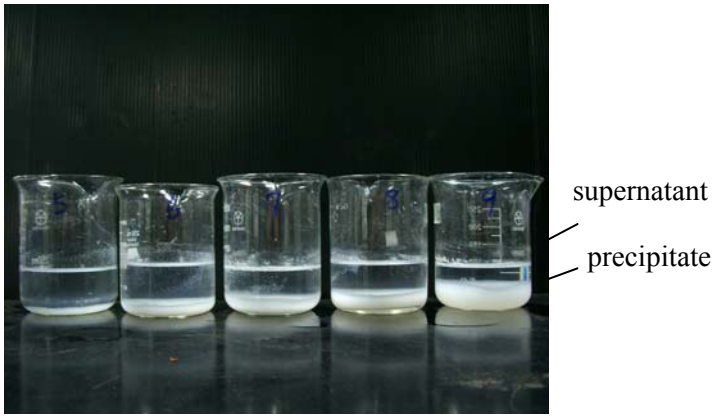


Figure 1. The appearance of Hap./Col complex (precipitate of complex form left side to right side; pH=5 ~ 9)**.**

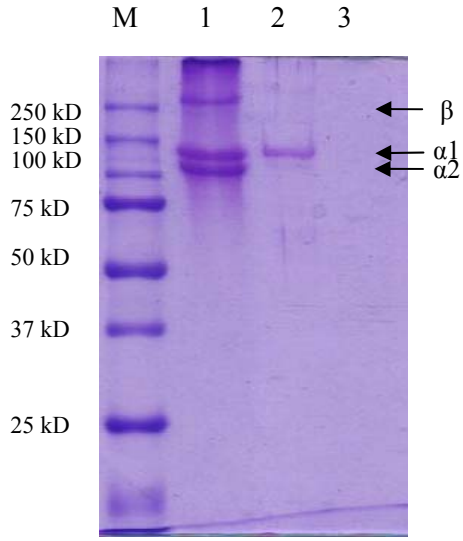


Figure 2. Electrohoretic analysis of our lab's collagen and Hap./collagen complex. (M: marker; Lane 1, our lab's collagen ; Lane 2, Hap./collagen complex's precipitate; Lane 3, Hap./collagen complex's supernatant).

The FTIR spectra obtained from collagen reacted with Hap. to form a Hap./collagen complex were given in Fig. 3. The FTIR spectra between 563 and 604cm-1 indicated the crystal structure of Hap., and the zone between 962 and 1050cm-1 indicated the P-O structure expressing the stretch structure. The wider zone between 1645 and 3500-3200cm-1 was caused by the water molecule adsorbed on the surface of Hap. The stretching and vibration of O-H bonds on the surface of Hap. were located at 3586cm-1, and the chelating of O-H bonds was located at 2980cm-1. However, the peaks at 1470-1400cm-1 as well as 876cm-1 were C-O bond group caused by CO2 in air or water involved in the reaction possibly. If the peak appearing at 1340cm-1 was the peak of pure collagen. However, if the absorbed peak was located at lower wavelength of spectra which indicated bonding formed between Hap. and collagen. The peak at 1338cm-1 on the spectra was a bond between Hap. and collagen, in other words, Ca2+ on Hap. and COO- on collagen formed a chemical bonding (Kikuchi et al., 2001). From the above spectra it can be proved that Hap. and collagen had bonding to form a complex. SEM micrographs of Hap./collagen complexes formed in different pH and temperature conditions were shown in Fig.4 to Fig.6. The micrographs of SEM for Hap./collagen complexes synthesized under neutral to alkaline pH at temperatures 37 °C and 40 °C appeared a coral-like structure. However, the SEM micrographs were looked leaf-like structure when Hap./collagen complexes were formed under acidic pH despite at 37 °C or 40 °C. From these results we conclude that Hap./collagen composite can be synthesized in vivo under animal physiological conditions.

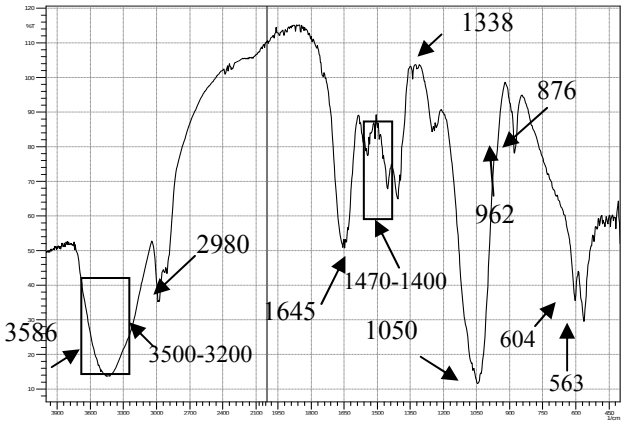
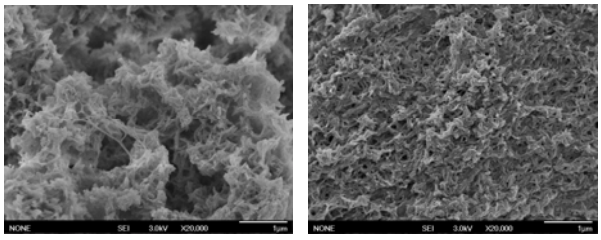
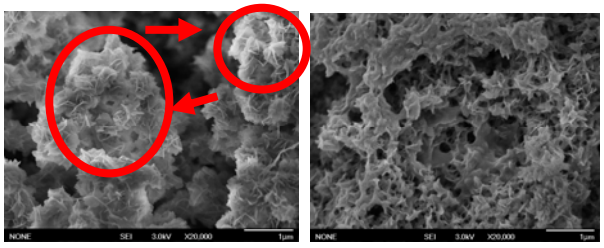


Figure 3. Fourier-transformed infrared spectra of Hap./Col composite.



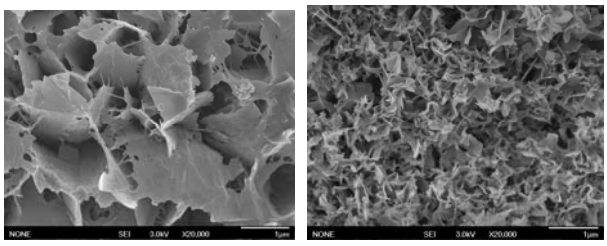
40 °C 37 °C

Figure 4. Scanning electron microscopy (SEM) image of Hap./Col composite.(pH=9).



40℃ 37℃

Figure 5. Scanning electron microscopy (SEM) image of Hap./Col composite.(pH=6).



40℃ 37℃

Figure 6. Scanning electron microscopy (SEM) image of Hap./Col composite.(pH=5).

Acknowledgements

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