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Formation of nano-hydroxyapatite on recombinant human-like collagen fibrils

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Abstract

Mineralization of recombinant human-like collagen is an important biomaterial for bone tissue engineering. Features of the formation of nano-hydroxyapatite on recombinant human-like collagen surface were investigated by SEM, XRD, FTIR and TEM. Formation mechanism of the nano-crystals is also discussed. The nano-HA is about 6–25 nm in size and with their *c*-axis parallel to the fibrils orientation.

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1. Introduction

The hydroxyapatite (HA)-collagen composites widely exist in nature, especially in the bone [1] of vertebrate animals, which play special roles in helping bone exhibiting its mechanical property. Investigations indicate that the composites in bone are a complex assembly composed of type I collagen nanofibrils with HA precipitated on their surface. The *c*-axis of the HA is parallel to the longitudinal axis [2–4] of collagen fibrils and the mineralized fibrils are assigned to be parallel to each other.

The HA-collagen composite shows great promise in clinical application because of its compositional (and partly structural) analogy to natural bone through nucleation growth of Ca–P ions on collagen matrix. Yet, the collagen derived from animals has many problems in quality and purity, especially diseases of animals such as mad cow disease, and of effects of immunological reactions [5–9]. Distilling collagen directly from human body is impossible, so recombinant human collagen might be a good choice.

We have studied biomineralization of calcium phosphate on recombinant human-like collagen. Here results on formation of nano-HA crystals on the recombinant human-like collagen were reported. The nano-HA is about 6–25 nm in size and with their *c*-axis parallel to the fibrils orientation.

2. Experimental

Water-soluble recombinant human procollagen was obtained from JuZi biogene technology Ltd. Co., which was produced by using fermentive *E. coli* [10]. $CaCl_2$, NaH_2PO_4, NaOH were analysis grade. As a solvent, deionized water was used.

The procollagen was diluted in water at a concentration of 0.6 mg/ml at room temperature for 5 h. $CaCl_2$ solution (2.8 ml 0.1 M) was added into 20 ml of

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procollagen solution and maintained for 20 min after mixture. NaH_2PO_4 solution (1.68 ml 0.1 M) was then added slowly and the pH was adjusted to 7.0 by 0.1 M NaOH solution. When the pH exceeded about 6.0, the solution became supersaturated and calcium phosphate started to precipitate with collagen. The saturated solution was maintained at pH 7.0 for 24 h, after which the precipitate was harvested by centrifugation at 5000 rpm and suspended in deionized water to remove salts. The centrifugation and suspension cycle was repeated 3 times. After the last suspension the sample was freeze-dried. The precipitate was ground into fine powder for later examination. The reaction was maintained in N_2 atmosphere.

We used scanning electron microscopy (SEM) to examine sample morphology and microstructure. The sample was sputter-coated with a layer of gold about 10 nm thick for SEM (JSM-6301F) observations. The structure and crystallinity of the sample was investigated by X-ray powder diffractometer (D/max-rA Rigaku diffractometer, CuKα radiation ($\lambda = 0.15418$ nm), Japan). The sample was scanned from 10° to 60° with a scan speed of 4°/min. Transmission electron microscopy (JEOL 200CX) was used to examine the structure of the sample which was prepared by transferring precipitate powder onto carbon-coated copper grids. In order to investigate the composite, infrared spectra were taken using a Perkin-Elmer system 2000 Fourier transform IR (FTIR) spectrometer in the range of 4000–400 cm^{-1}.

3. Results and discussion

The mineralized collagen is a three-dimensional network of collagen fibrils on which crystals of calcium phosphate have settled. Scanning electron microscopy of the sample shown in Fig. 1 demonstrated the flower-like morphology of the mineralized collagen fibrils on the powder surface and the 'leaves' of the flower are entangled and perpendicular to the surface. The fibrils are about 30 nm in width and 500 nm in length, which are composed of recombinant human-like collagen covered with nano-HA.

The crystallographic structure of the calcium phosphate nanocrystals was investigated by X-ray diffraction. Fig. 2 shows the XRD pattern of the mineralized collagen and commercially purchased HA powder. Fig. 2A exhibits the pattern of purchased HA powder with sharp peaks implying its good crystallinity. Fig. 2B shows the pattern of the mineralized recombinant human collagen with the peak intensity proportional to the one of pure HA without any other peaks. It suggests that the inorganic phase in the mineralized collagen is HA and there is no other phases of calcium phosphate in the sample. There is no texture because the crystals in the mineralized collagen have random orientations in the powder during XRD diffraction.

The average crystallite size of the hydroxyapatite in the mineralized collagen can be estimated by Sherrer formula [11,12]

$$D_{hkl} = \frac{0.89\lambda}{\beta_{hkl}\cos\theta}$$

The individual size of crystallite was calculated as 23.7 nm where (002) plane peak was used with $2\theta = 25.9°$, as 6.4 nm where (310) plane peak was used with $2\theta = 39.68°$ and as 8.7 nm where (222) plane peak was used with $2\theta = 46.76°$.

The samples were further characterized by TEM. Fig. 3 shows an example of TEM morphology and selected area electron diffraction (SAED) patterns of the mineralized recombinant human collagen. As can be seen in the TEM image, morphology of the mineralized collagen is nano-rods with diameters of about 6 nm with varied length. Considering the average individual size of the

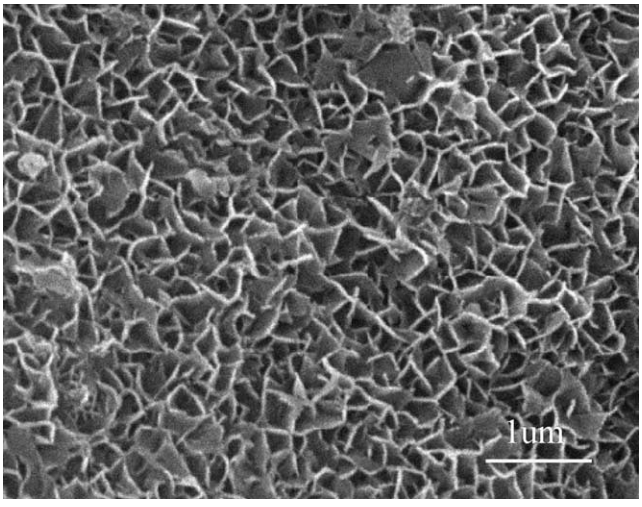

Fig. 1. SEM of mineralized recombinant human-like collagen fibrils.

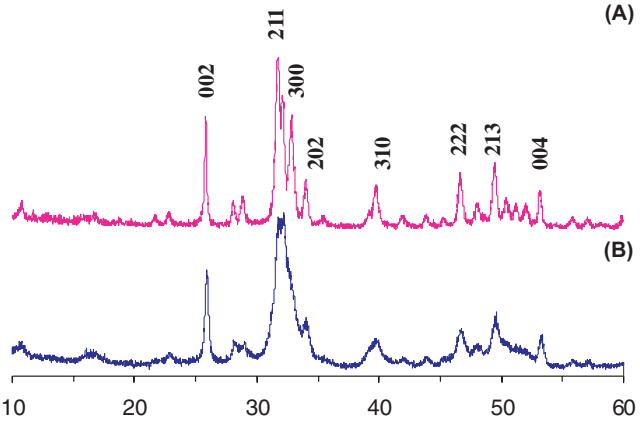

Fig. 2. X-ray diffraction patterns of mineralized collagen fibrils (B) and commercially purchased HA (A).