Synthesis of nanophase hydroxyapatite/collagen composite

R. Z. WANG, F. Z. CUI, H. B. LU, H. B. WEN, C. L. MA, H. D. LI
Department of Materials Science and Engineering, Tsinghua University, Beijing 100 084, People's Republic of China

One of the most important objectives in biomaterials science is the development of new materials for bone substitution. It has been found that hydroxyapatite (HAp), which is the most widely studied bioceramic, has an excellent biocompatibility and a certain degree of bioactivity [1, 2]. However, HAp is limited in its use because it is brittle compared with natural bone. In order to improve the toughness and bioactivity of bone substitution materials, biocomposites made of HAp and biocompatible organic components or bioactive glass have been developed in recent years [3–10]. Among these materials, HAp/collagen composite is of special interest because it mimics the composition of natural bone [7–10]. Preliminary study reveals that this composite has a better osteo-inductive capacity in cell culture than monolithic HAp [9]. So far, HAp/collagen composite is still at the initial stage of development. It is well known that HAp is nanometre sized and deposits in an orderly way on the collagen matrix in natural bone. However, with the synthetic methods reported, it is not easy to obtain either a uniformly mixed composite [7], or a fine crystal sized material [8–10], while these two factors are important to the bioactivity of the composite [2]. Therefore, new methods are needed to obtain an HAp/collagen composite which mimics not only the composition but also the microstructure of natural bone. This letter reports a new preparation method of HAp/collagen composite in which nanometre sized HAp is homogeneously dispersed in collagen matrix. The results of biological testing will be published separately.

Commercially purchased HAp powder and type 1 insoluble collagen from bovine achilles tendon were used as starting materials. Firstly, 1 g HAp was dissolved in 150 ml, 0.1 N HCl at 20 °C and 450 mg type 1 collagen was added to the solution, which was then ultra-sonicated, using a Model G1125 ultrasonator, until a thoroughly mixed slurry was obtained. Secondly, this solution was diluted to 2000 ml with distilled water, and 23.376 g NaCl was added to increase the stability of the calcium containing solution [11]. This resulted in a 5 mM calcium solution with pH 3.0. At the third step, the solution was gently stirred at 20 °C, and 0.05 M potassium hydroxide solution was added in drops to the calcium solution to adjust the pH to 7.4. When the pH exceeded about 7.0, the solution became supersaturated and HAp started to precipitate onto the collagen. The solution was maintained at pH 7.4 for 10 min, after which the composite was harvested by centrifugation at 5000 rpm and freeze-dried. The weight percentage of collagen in the HAp/collagen composite is 35%, which is similar to that of natural bone. Control material without collagen was prepared in the same way.

A scanning electron microscope (SEM, Hitachi S-550, 25 kV), equipped with energy dispersive spectroscopy (EDS), was used to analyse the microstructures and compositions. X-ray diffraction (XRD) analyses were performed in a Rigaku D/max-RB diffractometer using CuK_α radiation. Infrared (IR) spectra were taken using a Perkin-Elmer system 2000 fourier transform IR spectrometer. In preparing samples for transmission electron microscopic (TEM) observations, the freeze-dried powder was re-suspended in distilled water, a drop of the suspension was placed on a formvar-coated and carbon-reinforced copper electron microscope grid. After air-drying, the sample was observed on a 200 CX TEM at 200 kV.

Fig. 1 shows the XRD spectra of HAp/collagen composite and the control sample. Results on commercially purchased HAp powder with good crystallinity and natural bone are also given in Fig. 1. The inorganic phase in the HAp/collagen composite was determined as apatitic calcium phosphate and no peaks from other Ca–P materials were present in the XRD pattern. The extensive broadening and overlap of the peaks, which is similar to natural bone, indicate that the crystal grains of HAp are

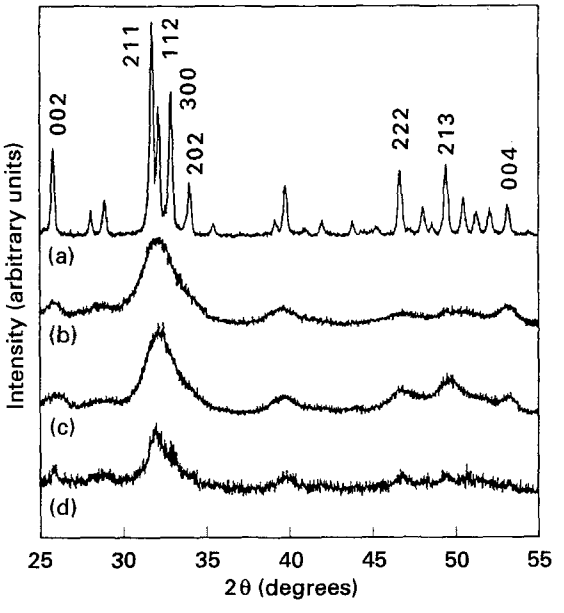

Figure 1 XRD patterns of (a) commercially purchased HAp, (b) synthesized HAp/collagen biocomposite, (c) control sample and (d) natural bone.

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extremely fine. The diffraction peaks of (002) and (004) are sharper and stronger than those of (222) and (213) in the HAp/collagen composite, while in the control sample the latter are sharper and stronger than the former. This difference implies that the presence of insoluble collagen influences the formation process of HAp to some extent.

Analyses of the IR spectra confirm the XRD results. In Fig. 2, the absorption bands at 1087, 1046, 962, 601 and 571 cm^{-1} are due to PO_4 in HAp, 630 cm^{-1} is due to OH, 1419 and 875 cm^{-1} are CO_3 existing in the apatite [12], and the absorption bands at 1660 and 1550 cm^{-1} and the shoulder at 1240 cm^{-1} are due to the NH_2 groups of the collagen in the HAp/collagen composite [2]. Therefore, the inorganic phase developed in the HAp/collagen composite or in the control system is apatite. However, the crystallinity of such apatite is low, as the spectra are not so sharp as those of well crystallized HAp, and the OH and some PO_4 bands appear only as shoulders. The presence of CO_3 bands indicates that the HAp formed here is carbonate-substituted apatite, which is the same as bone apatite. CO_3 is probably incorporated into the solution from the air during material preparation.

The morphologies of the HAp/collagen composite and the control sample are shown in Fig. 3. HAp precipitates are uniformly distributed on the collagen matrix in the HAp/collagen composite (Fig. 3a), and HAp crystal grains can be seen to aggregate into spheroidal particles in both cases. The particle dimension is only about 30–60 nm in the composite, which is only one tenth of that in the control sample (the particle size is in the range of 300–1000 nm in Fig. 3b). This difference clearly indicates that the collagen in the HAp/collagen composite has a function of regulating the distribution of HAp by preventing the aggregation of small HAp particles.

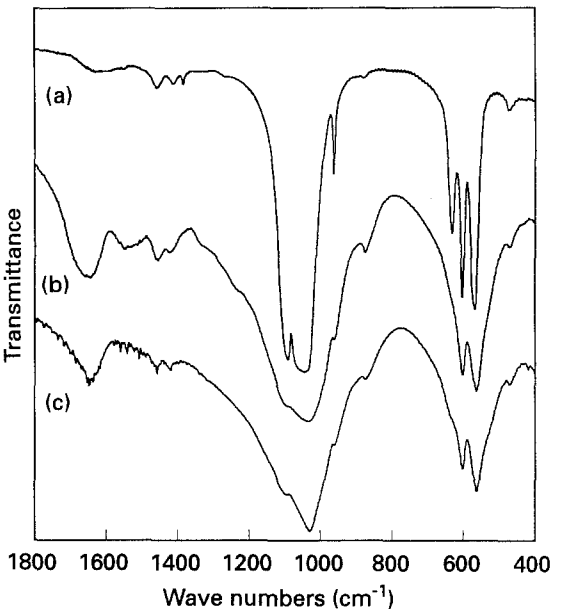

Figure 2 Fourier transform IR spectra of (a) commercially purchased HAp, (b) synthesized HAp/collagen biocomposite and (c) control system.

The negatively charged groups of some amino acids in collagen probably play an important role in this regulating process. They have a good affinity for calcium ions in calcium solution, and therefore are apt to induce HAp nucleation, thus causing a uniform distribution of small HAp particles on the collagen surface. A more detailed study on the regulating mechanism of collagen is under way.

Despite the above differences, the Ca/P ratio of the HAp phase, quantitatively determined by EDS, is the same value in both cases, 1.6, which is lower than the standard value of 1.67. There is also no measurable difference in the crystal size of HAp

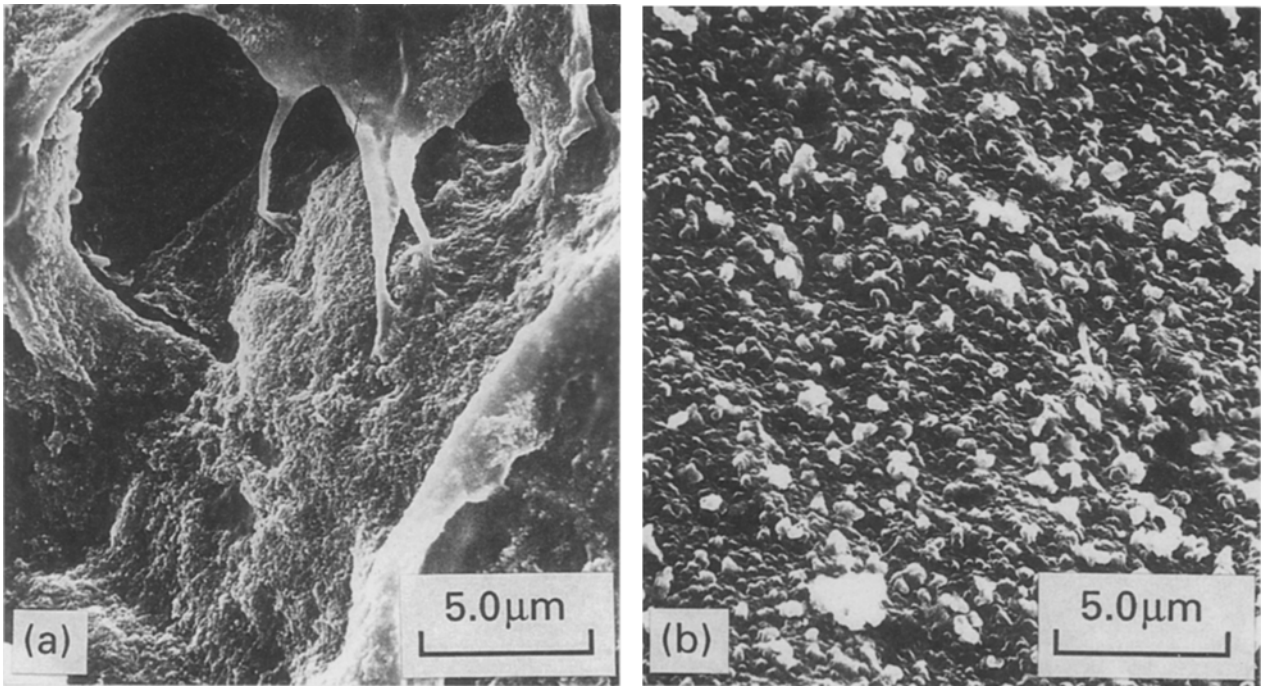

Figure 3 SEM morphologies of (a) synthesized HAp/collagen biocomposite and (b) control sample.

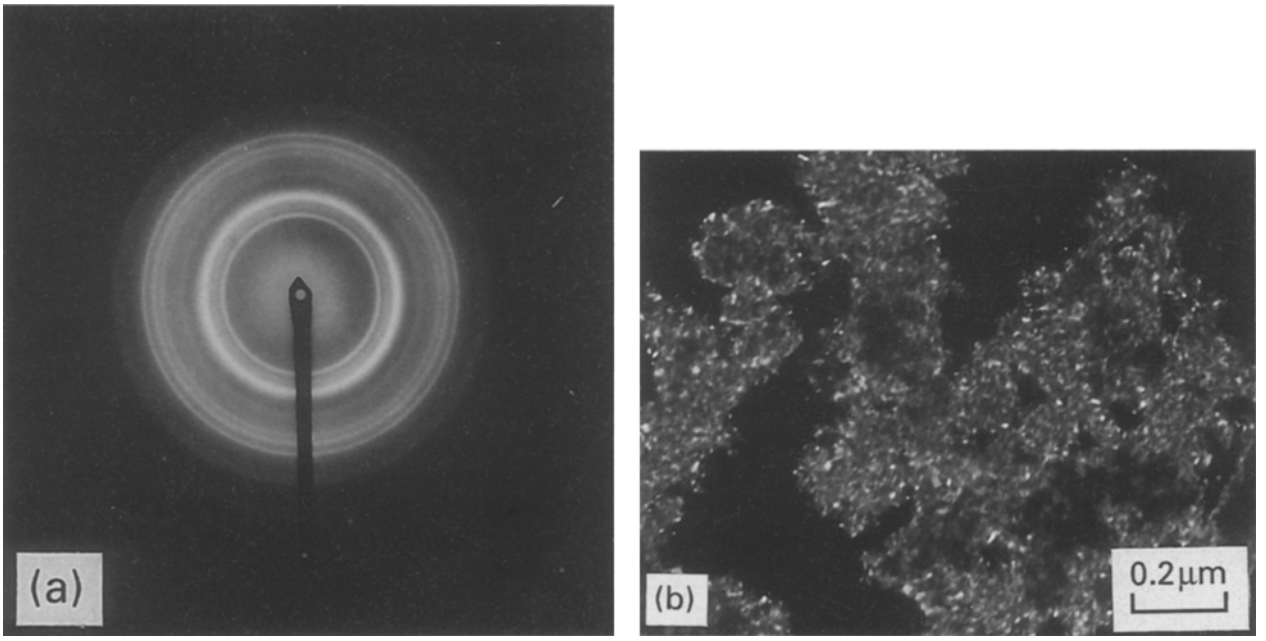

Figure 4 (a) Selected area transmission electron diffraction pattern of HAp/collagen composite and (b) the central dark field image using (0 0 2) reflection showing the nanometre crystals of HAp.

between the HAp/collagen composite and the control sample. Fig. 4 gives a selected area transmission electron diffraction pattern of the composite and the related central dark field image using (0 0 2) reflection, from which the crystal size of HAp was measured to be 2–10 nm. Therefore, the HAp in the HAp/collagen composite is nanometre sized. Such composite is expected to have better bioactivity than those with coarser HAp crystals [2, 5].

In conclusion, HAp/collagen composite with nanometre sized HAp uniformly dispersed on collagen can be synthesized using the method reported in this letter. The presence of insoluble collagen has the function of regulating the distribution of HAp.

Acknowledgements

This study was supported by the National Natural Science Foundation of China.

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Received 22 July
and accepted 28 October 1994