JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 5 (1994) 252–255

Preparation and characterization of nanograde osteoapatite-like rod crystals

L. YUBAO*, J. DE WIJN, C.P.A.T. KLEIN, S. VAN DE MEER, K. DE GROOT
Department of Biomaterials, University of Leiden, Rijnsburgerweg 10, bld 55, 2333 AA Leiden, The Netherlands
**Institute of Materials Science and Technology, Sichuan University, Chengdu 610064, PR China*

In this paper, nanograde osteoapatite-like rod crystals are made from wet synthesized calcium phosphate precipitates by hydrothermal treatment at 140 °C under 0.3 MPa pressure for 2 h. The morphology, crystal structure, crystallinity and phase composition of these nanograde rod crystals are similar to those of thin apatite crystals in bony tissues of the body. This analogy provides an opportunity in the near future to build bone-like substitutes which consist of the nanograde rod crystals and special organic matrices and cells.

1. Introduction

Hydroxylapatite (HA) and tricalcium phosphate (TCP) are two calcium phosphate biomaterials with an excellent biocompatibility and bioactivity [1, 2], which is probably due to their similarity with the hard tissues of the body. So far, calcium phosphate biomaterials have been widely used clinically in the form of powders, granules, dense and porous blocks and various composites [3, 4]. As is known, calcium phosphate materials form the main mineral part of calcified tissues. The apatite crystals in bone are formed as thin needles, about 5–20 nm by 60 nm with a poor crystallinity in the collagen fibre matrix, as measured by X-ray diffraction and transmission electron microscopy [5, 6]. While the apatite crystallite sizes in dentin and cementum are roughly comparable to those in bone, the crystallites found in enamel grow much larger, over 100 nm in length. Recently a few reports have been presented concerning the synthesis of HA fibres [7–9], whiskers [10] and needle-like crystals [11, 12] using an agar gel system, homogeneous precipitation method and hydrothermal processing, respectively. These thin crystals could find usage as a chemical catalyst, ion-exchangers of heavy metal ions, an absorbent in column chromatography and in the manufacture of fine sugars, but also as fillers for bone repair and substitute materials. However, because of either too small an amount of product obtained or the complexity of their methods, further adequate biocompatibility tests and experiments to evaluate their usefulness in polymer composite cannot be performed. In this paper, nanograde osteoapatite-like calcium phosphate rod crystals were prepared by hydrothermal treatment in a simple and reproducible way and characterized by X-ray diffractometer and transmission electron microscopy.

2. Materials and methods

Fully washed calcium phosphate precipitates were used in the hydrothermal synthesization of nanograde rod crystals. The precipitates were made by dropping an $(NH_4)_2HPO_4$ aqueous solution into a stirred $Ca(NO_3)_2$ aqueous solution. The pH value for both solutions was 11 to 12, adjusted with ammonium solution and reaction was set at room temperature or at 70 °C for 2 h. The hydrothermal treatment of the precipitates' aqueous solution was carried out at 140 °C under a pressure 0.3 MPa for 2 h in an autoclave to form the nanograde rod crystals. A small amount of glycerine was used as a dispersing agent for the nanograde rod crystals in some cases.

The morphology of the precipitates and the nanograde rod crystals was observed by transmission electron microscopy (TEM). The crystal structure and the Ca/P molar ratio were measured by X-ray diffractometer (XRD), atomic absorption spectrometer (AAS) and ultraviolet spectrophotometer (US) before and after sintering at 1100 °C.

3. Results

TEM photographs of the calcium phosphate precipitates and the nanograde rod crystals are shown in Fig. 1. Fig. 1a is that for starting precipitates, 1b is for rod crystals formed without the addition of glycerine and 1c is for rod crystals formed with the addition of glycerine as dispersing agent. From the photographs, it can be seen that the starting irregular small particles with a mean size of about 15 nm have turned into uniform and non-aggregated rod-like crystals after hydrothermal treatment. The rod-shaped crystals have a basic length from 40 nm to 150 nm and a width (20 nm) similar to the size of the starting small particles. Comparison of Fig. 1a and 1c leads to a conclu-

0957–4530

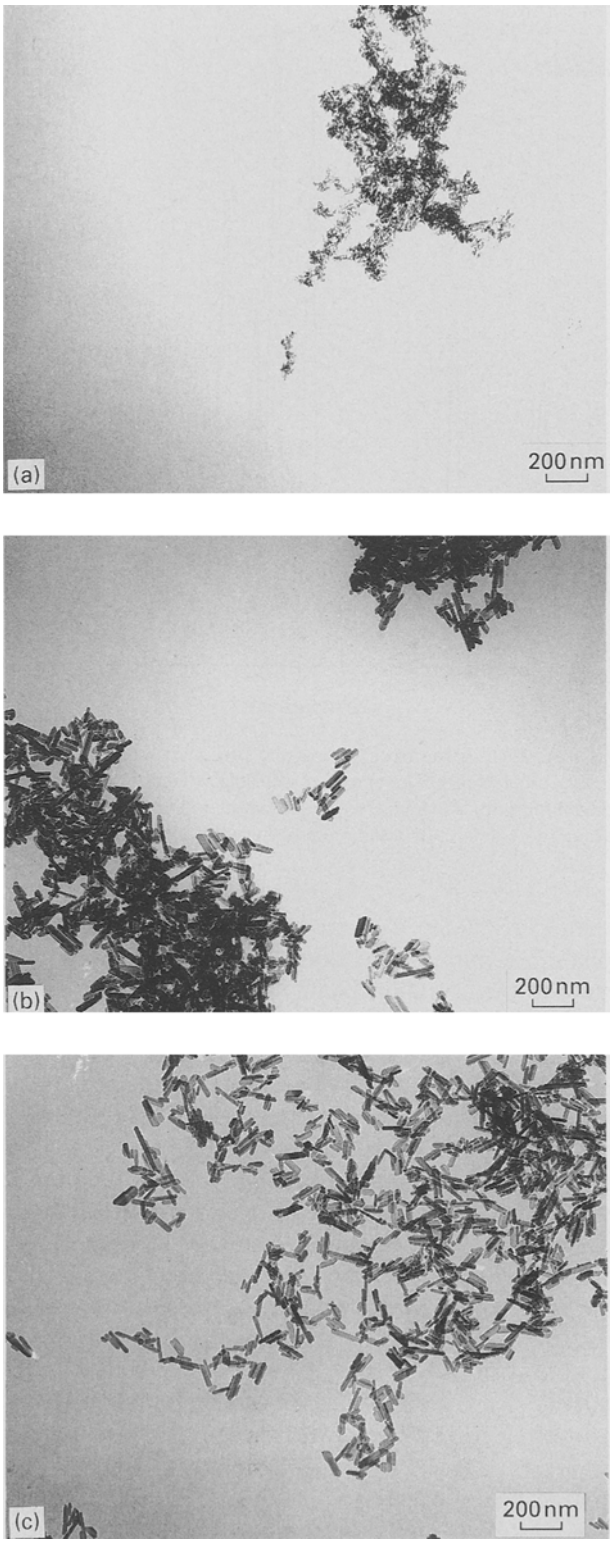


Figure 1 TEM photographs of the starting precipitates and the nanograde rod crystals after hydrothermal treatment: (a) starting precipitates; (b) nanograde rod crystals without the addition of glycerine; (c) nanograde rod crystals with the addition of glycerine as dispersing agent.

sion that higher dispersity of the nanograde rod crystals is obtained by using glycerine as the dispersing agent.

Fig. 2 shows the relevant XRD patterns, in which 2a is that for starting precipitates, 2b is for the same precipitates sintered at 1100 °C, 2c is that for nanograde rod crystals and 2d is for the same rod crystals sintered at 1100 °C. The starting precipitates have a poorly crystallized apatite structure as shown in Fig. 2a. After sintering a well-crystallized structure of biphasic calcium phosphate material which contains nearly 43% HA and 57% β-TCP is obtained. Fig. 2c exhibits an apatite structure with a higher crystallinity than Fig. 2a. This proves that after being treated hydrothermally the poorly crystallized starting precipitate particles have turned into relatively better crystallized apatite rod crystals. When sintered at 1100 °C, the apatite rod crystals become biphasic calcium phosphate crystals with a HA/β-TCP content ratio of about 54% to 46%. The change of the HA/β-TCP ratio between the sintered starting precipitates and the sintered rod crystals is noticeable. The Ca/P molar ratios of the starting precipitates and the rod crystals (both of them have an apatite structure) are estimated from the XRD patterns of their sintered products as 1.57 and 1.59, respectively, which is coincident with the analytical results of AAS and US.

4. Discussion

Since there are no other basic and acidic additives present in the aqueous solution of the starting precipitates during hydrothermal treatment, the Ca/P molar ratio of the starting precipitates basically determines the Ca/P molar ratio of the final product, the nanograde rod-shaped crystals. The change of the HA/β-TCP content ratio from the starting precipitates to the nanograde rod-shaped crystals results from the dissolution of some PO_4^{3-} ions into the aqueous solution during hydrothermal treatment. This has been confirmed by the small decrease in the pH value of the aqueous solution after hydrothermal treatment. The Ca/P molar ratio of the starting precipitates can be easily controlled by adjusting the pH value of the reaction solutions and the reaction temperature and time, as well as the standing time of the wet synthesized precipitates, according to the results given by Jarcho *et al.* [13]. This makes it possible to prepare hydrothermally nanograde calcium phosphate rod-like crystals with a different Ca/P molar ratio or phase composition corresponding to different application purposes.

The starting precipitates have an XRD pattern of poorly crystallized apatite envelope with only one characteristic peak of (0 0 2) crystal plane ($2\Theta = 25.9°$) as shown in Fig. 2a. This means that even the as-prepared calcium phosphate powders have a tendency for preferential growth or crystallization along the c-axis (0 0 2) direction. The same phenomenon can be seen in the XRD patterns of various hydrothermally synthesized calcium phosphate nanograde rod crystals, which have a different Ca/P molar ratio but a similar apatite structure to that in Fig. 2c, only by comparing the intensity of the (0 0 2) peak with the intensity of the highest (2 1 1) peak ($2\Theta = 31.8°$); normally the ratio is 40%. Because of this trend, the irregular small particles in Fig. 1a are able to grow continuously along the c-axis (0 0 2) direction into the nanograde rod crystals in Fig. 1c. The width of the rod crystals is around 20 nm, which is close to the original size (about 15 nm) of the starting particles. This infers

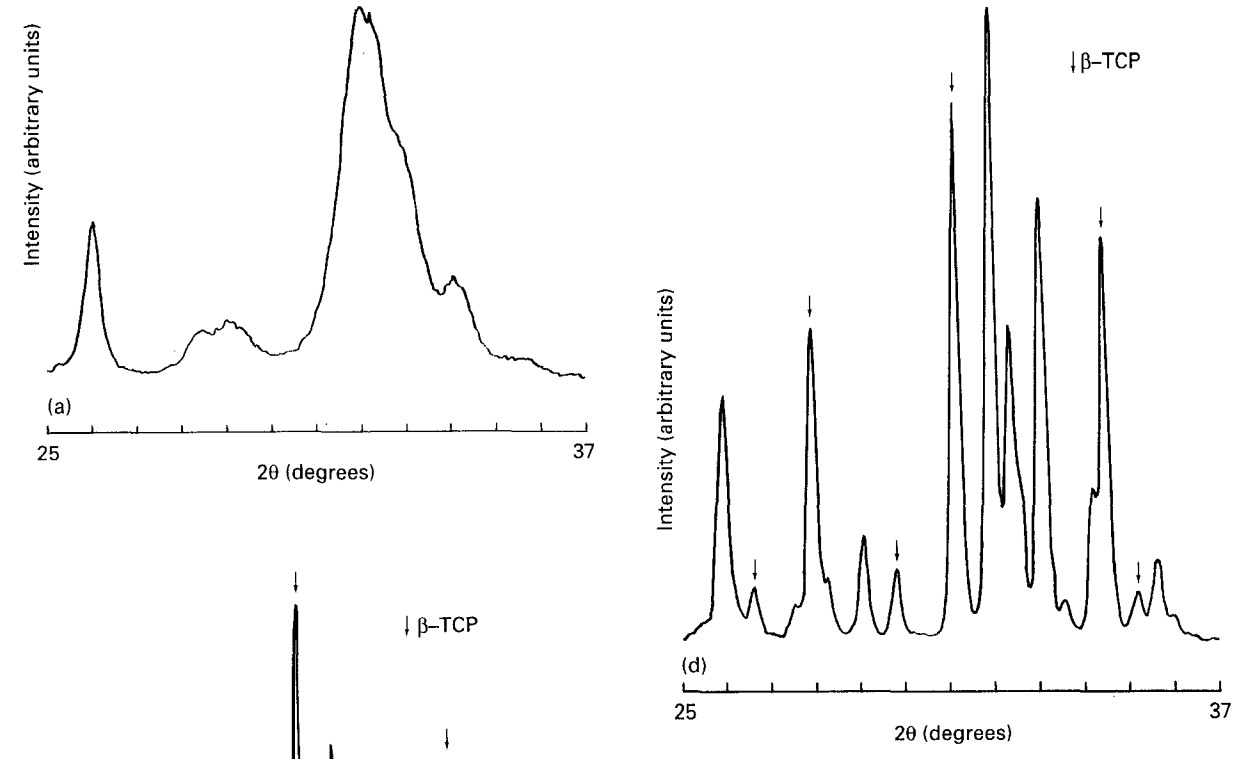
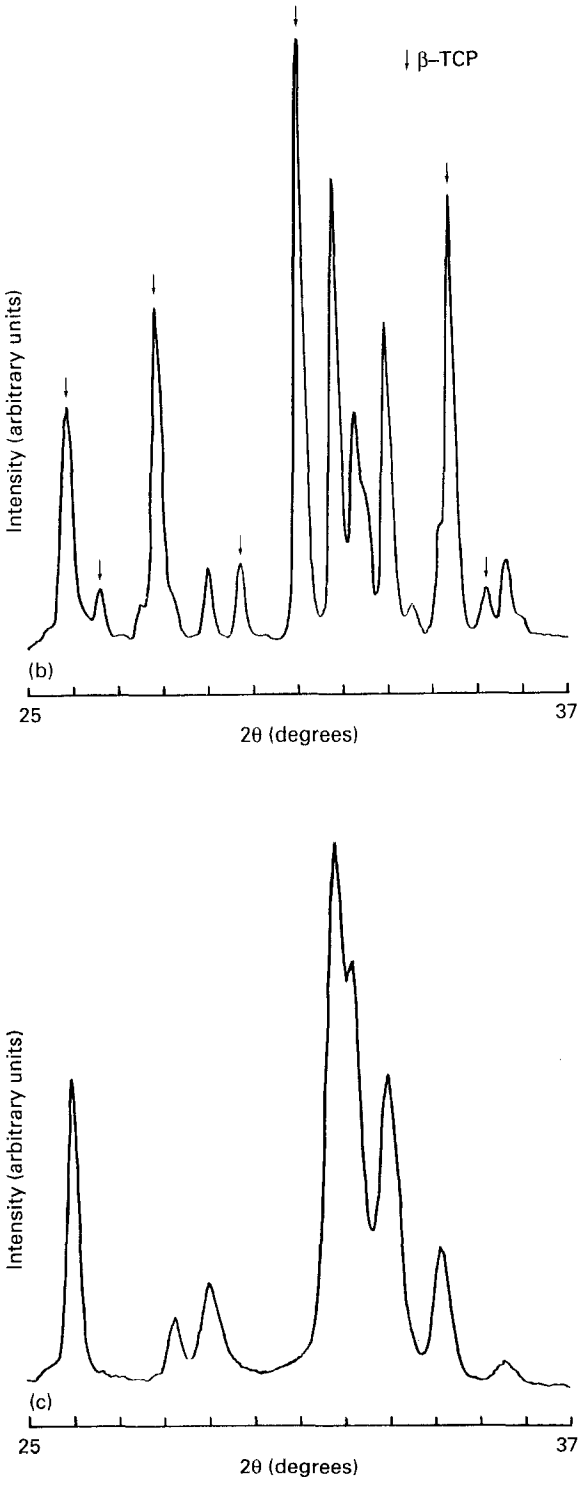


Figure 2 XRD patterns of the starting precipitates and the nanograde rod crystals before and after sintering at 1100 °C: (a) starting precipitates; (b) starting precipitates sintered at 1100 °C; (c) nanograde rod crystals; (d) nanograde rod crystals sintered at 1100 °C.



that 15 to 20 nm is the lower limit of calcium phosphate precipitates that can turn from the amorphous state to the crystalline state.

After sintering at 1100 °C, a crystal structure reflecting the Ca/P molar ratio of the starting precipitates and the nanograde rod-like crystals appears. This indicates that XRD patterns or TEM photographs of the unsintered nanograde rod crystals, such as Fig. 1c and Fig. 2c, are not sufficient evidence that these nanograde rod crystals are pure and well-crystallized hydroxylapatite crystals. In fact, the nanograde rod crystals with a Ca/P molar ratio between 1.67 and 1.5 are non-stoichiometric apatite, or "deficient apatite", which consists of pure HA ($Ca_{10}(PO_4)_6(OH)_2$) and apatite TCP ($Ca_9(HPO_4)(PO_4)_5(OH)$) [14, 15]. The apatite structure in Fig. 2c of the nanograde calcium phosphate rod crystals with a Ca/P molar ratio of 1.59 comes from such a non-stoichiometric apatite. The apatite phase ($Ca_9(HPO_4)(PO_4)_5(OH)$) of the TCP composition in non-stoichiometric apatite comes from the hydrolysis of the amorphous TCP precipitates (Ca/P = 1.5) in aqueous medium. When sintered at high temperature until 1100 °C, the apatite TCP phase turns into β-TCP by loss of water. At this time, the apatite structure of the nanograde rod crystals drops into a biphasic calcium phosphate (HA plus β-TCP) structure. The more the Ca/P molar ratio is below 1.67, the richer the β-TCP is in the mixture.

As mentioned above, calcium phosphate is the main mineral phase in bones and teeth. The apatite crystals in hard tissues are formed as thin needles with a size of about 5–20 nm by 60 nm in bone and over 100 nm long in enamel. The apatite crystals in bone are found to be poorly crystallized non-stoichiometric apatite

with a Ca/P molar ratio of 1.61 [14]. The similarity in morphology, crystal structure, crystallinity and phase composition of natural thin apatite crystals and the hydrothermally synthesized nanograde calcium phosphate rod crystals provides an opportunity to build a bone-like substitute in the future, which is composed of the osteoapatite-like rod crystals and special organic matrices and cells.

Acknowledgements

The authors would like to thank Mr Joop Wolke and Mrs Jolanda de Blieck for their help in the measurements of XRD patterns and AAS and US data. We are also grateful to the Commission of the European Communities for partly subsidizing this study.

References

1. K. de GROOT (ed), in "Bioceramics of calcium phosphate" (CRC Press, Boca Raton, 1983).
2. P. DUCHEYNE and W. HASTING (eds), in "Metal and ceramic biomaterials", Vol. II (CRC Press, Boca Raton, 1984)
3. P. DUCHEYNE, *J. Biomed. Mater. Res.* **21** (A2) (1987) 219.
4. W. BONFIELD, M. D. GRYNPAS, A. E. TULLY, J. BOWMAN and J. ABRAM, *Biomaterials* **2** (1985) 85.
5. J. L. KATZ and R. A. HARPER, in M. B. Bever, (ed) "Encyclopedia of materials science and engineering" (Pergamon Press, NY, 1986) 474–476.
6. J. B. PARK and R. S. LAKES, in "Biomaterials: an introduction" (Plenum Press, New York and London, 1992) 192–196.
7. M. TANAHASHI, K. KAMIYA, T. SUZUKI and H. NASU, *J. Mater. Sci. Mater. Med.* **3** (1992) 48.
8. E. E. BERRY, *J. Inorg. Nucl. Chem.* **29** (1967) 322.
9. E. C. KREIDLER and F. A. HUMMEL, *Amer. Mineral* **55** (1970) 170.
10. M. KINOSHITA, K. ITATANI, F. S. HOWELL and A. KISHIOKA, *Phosphorus Res. Bull.* 1 (1991) 21.
11. K. IOKU and M. YOSHIMURA, *ibid.* 15–20.
12. A. PERLOFF and A. S. POSNER, *Science* **124** (1956) 583.
13. M. JARCHO, C. H. BOLEN, M. B. THOMAS, J. BOBICK, J. F. KAY and R. H. DOREMUS, *J. Mater. Sci.* **11** (1976) 2027.
14. J. L. LACOURT, in "Biomaterials-hard tissue repair and replacement" (Elsevier Science Publishers B.V., Amsterdam, 1992) 81–95.
15. J. C. HEUGHEBAERT and G. BONEL, in "Biological and biomechanical performance of biomaterials" (Elsevier Science Publishers B.V., 1986) 9–14.

Received 8 March
and accepted 11 October 1993