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Hydrothermal synthesis of biocompatible whiskers

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The preparation of non-toxic and biocompatible fibres or whiskers is one of the most urgent tasks today, because most of the fibrous materials which have been used (including asbestos which has been used for many years) are thought to be biohazardous. Whiskers of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$: HAp), which is expected to be one of the best biocompatible materials, have been successfully synthesized by hydrothermal treatments of beta-tricalcium phosphate (beta-$Ca_3(PO_4)_2$: beta-TCP) with citric acid. These whiskers were single crystals, elongated along the *c*-axis, with a length of 20–30 μm and a width of 0.1–1 μm. They were slightly calcium deficient (Ca/P molar ratio = 1.63) and they contained a trace of CO_3^{2-} in their structure.

1. Introduction

Whiskers and fibres are required in the development of modern composite materials, particularly in reinforced composites based upon polymers, metals and ceramics. Asbestos fibres have been widely used as natural fibres because of their high tensile strength, high thermal and chemical stability, low cost and abundance [1]. Now, however, the research and development of new asbestos-substituting materials is an urgently needed task, because asbestos is thought to be seriously biohazardous and to cause diseases such as asbestosis, carcinomas of the bronchus, malignant mesotheliomas and pleural plaques [1, 2].

The main factors to be considered when addressing carcinogenicity are believed to be the morphology and the chemical composition of the materials. Stanton and Pott have postulated a hypothesis [3, 4] (on morphology) that fibres with lengths longer than 8 μm and widths smaller than 0.25 μm might have serious carcinogenic potential. Various fibrous materials such as SiC [5], carbon [6], Si_3N_4 [7], $K_2Ti_6O_{13}$ [8], Al_2O_3 [9], ZrO_2 [10], and even superconductors [11] have been reported. Even though they may pass the Stanton–Pott criterion on their morphology, the chemical species constituting these materials (Ti, Zr, Si, C, Al, Y etc.) might be bioinert but they are not biocompatible. In other words, the requirement on the morphologies may be satisfied but those on the chemical composition are not satisfied in existing whiskers.

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ (HAp), is the main mineral constituent of the hard tissues (bones and teeth) in the human body, and thus it is one of the most biocompatible of materials [12, 13, 14]. The chemical species constituting HAp crystals (Ca, P, O and H) are expected to have no toxicity. Although needle-shaped HAp crystals or fibres have been prepared by Mortier et al. [15], Kinoshita et al. [16] and Christiansen and Riman [17], they have a relatively low crystallinity, are not uniform and are not a pure HAp phase. We have, therefore, tried to develop a preparation – in which HAp whiskers have high crystallinities, are of uniform shape and possess a single phase – by a hydrothermal method from Ca, P and some chelating agents such as EDTA, citric acid and lactic acid. HAp can be synthesized by a variety of methods including both conventional routes such as solid-state reactions [18] and wet chemical routes based on precipitation at low temperature [19]. These conventional methods, however, mostly prepare irregular forms of powders. In contrast, hydrothermal methods using high temperature and high-pressure aqueous solutions [20] allow growth of HAp crystals of a certain shape. In fact, we succeeded in obtaining fine HAp single crystals of high crystallinity in homogeneous rod shapes by the hydrothermal treatment of wet-chemically-prepared HAp precursors of low crystallinity and irregular morphology [21, 22]. More recently, it has been reported that even *c*-axis-elongated HAp single crystals can be successfully synthesized by the hydrothermal treatment of precipitated HAp precursors in various solutions containing additives such as K_3PO_4, KOH or EDTA [23]. In this paper,

we report the success of the hydrothermal synthesis of HAp whiskers of controlled morphology and size using citric acid and beta-tricalcium phosphate (beta-TCP).

2. Materials and methods

HAp whiskers were obtained by the hydrothermal treatment of a 500 ml solution containing 1 g of beta-TCP and 5 g of citric acid at a temperature of 200 °C and a pressure of 2 MPa for 1 h. Before the hydrothermal treatment, the solution was virtually clear and colourless without any precipitation, but it turned a faint yellow after the treatment, suggesting the decomposition of the Ca chelate. The pH value of the solution was 2.9 and 3.5 before and after the treatment, respectively. Higher concentrations of beta-TCP and/or citric acid, lower temperatures and a shorter treating period would yield monetite ($CaHPO_4$) crystals instead of HAp whisker [24].

3. Results and discussion

The scanning electron micrography (SEM) photomicrograph in Fig. 1 shows that the HAp whiskers prepared under this hydrothermal condition have a length of 20–30 μm, a width of 0.1–1 μm and an aspect ratio (length/width) of about 30. It is of particular importance that most of the whiskers have the same morphology and size. Moreover, it is also possible to obtain HAp whiskers with a desired shape and size by selecting the synthetic conditions [24]. An X-ray diffraction (XRD) pattern for HAp whiskers is shown in Fig. 2. The pattern can be indexed on the basis of a hexagonal unit cell with $a = 0.9424 \pm 0.0002$ nm and $c = 0.6879 \pm 0.0004$ nm which are typical values for hydroxyapatite. The infrared (i.r.) spectrum of the HAp whiskers (Fig. 3) indicates that there are characteristic peaks in hydroxyapatite, corresponding to PO_4^{3-} (560–600 cm^{-1}, 1030–1090 cm^{-1}) and OH^- (630 cm^{-1}, 3570 cm^{-1}) groups and that these HAp whiskers contain a trace of CO_3^{2-} (1450 cm^{-1}) in their

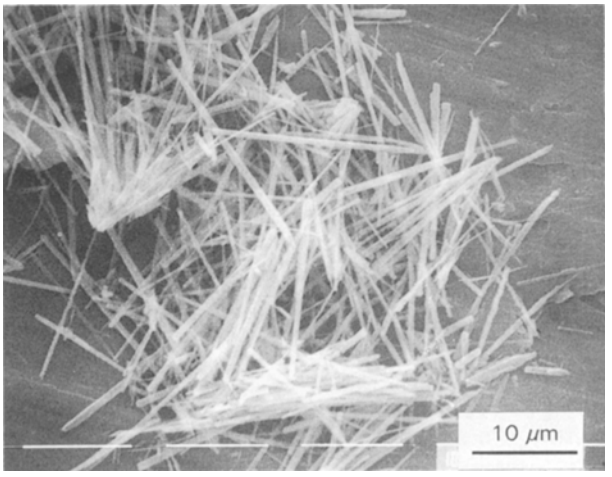

Figure 1 Scanning electron micrograph of HAp whiskers prepared by the hydrothermal treatment of beta-TCP with citric acid at 200 °C at 2 MPa for 1 h.

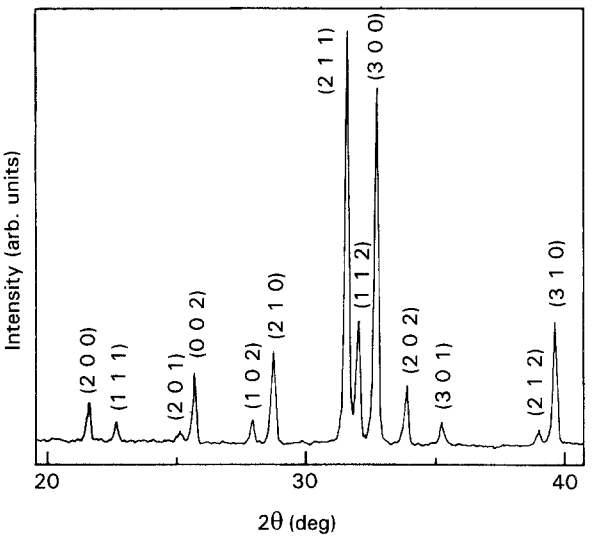

Figure 2 An XRD (CuK_α) pattern of HAp whiskers showing their high crystallinity and the non-existence of phases other than hydroxyapatite. The numbers in parenthesis indicate the plane indices, based on the hexagonal space group.

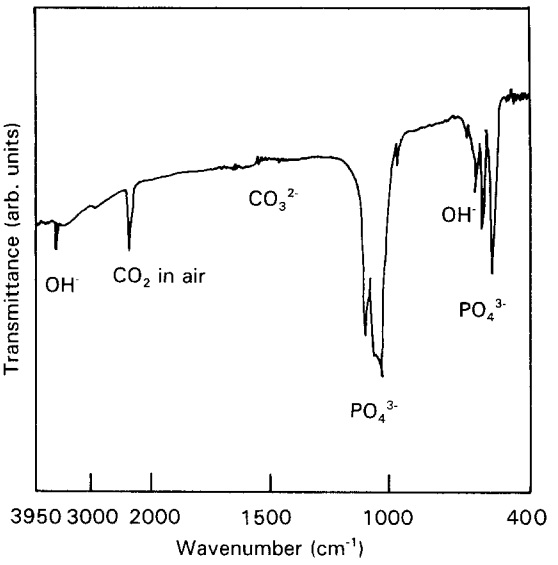

Figure 3 Infrared spectra of HAp whiskers indicating some internal vibrational peaks characteristic of hydroxyapatite.

structure. This hydrothermal method can also easily make HAp with a chemical composition which is analogous to that in the human body (for example, HAp in tooth enamel contains 3–4 wt% of CO_3^{2-}), this changes the physico-chemical properties such as the solubility and the biocompatibility of apatite as well [12–14]. This means that more biocompatible HAp whiskers can be prepared by this method. A chemical analysis of our HAp whiskers revealed a slightly lower Ca/P molar ratio (1.63) than in stoichiometric HAp (Ca/P = 1.67). It is generally known that Ca-deficient HAp tends to precipitate beta-TCP on heating at 900 °C, depending upon the deficiency of Ca [25]. From the amount of beta-TCP formed after calcining the whiskers at 900 °C for 1 h, we estimated the Ca/P ratio as around 1.63, which is in good agreement with the chemical analysis [26]. A transmission electron microscopy (TEM) photomicrograph

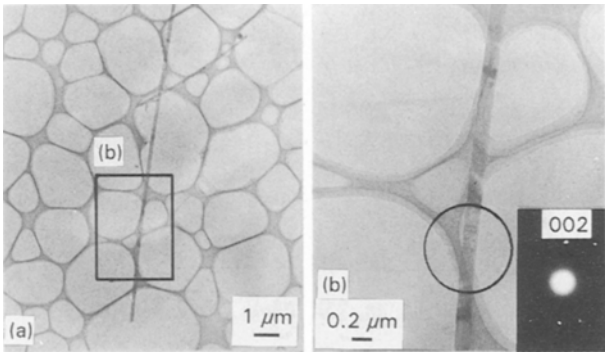

Figure 4 Transmission electron micrographs of: (a) a HAp whisker; and (b) an enlargement of (a) and a selected-area diffraction pattern from the area indicated by the circle, showing that the HAp whisker is a single crystal elongated along the *c*-axis.

of one of the HAp whiskers is shown in Fig. 4a, Fig. 4b is a four fold expansion of a section of Fig. 4a. The inset in Fig. 4b is a selected-area diffraction pattern of the area circled. These results reveal that the HAp whisker is a single crystal elongated along the crystallographic *c*-axis.

Elongation of HAp crystals is an urgent requirement in their applications as reinforcing materials, insulating agents, etc. HAp is also important as a packing media for column chromatography which is used to separate protein, etc. Since each crystal face adsorbs different types of proteins [27], using elongated HAp crystals would enable separation of some proteins more efficiently. In these applications, the morphology of HAp crystals plays an important role, however there have been few reports, so far, on the morphology control of HAp crystals.

Needle-shaped HAp (Ca/P = 1.50) crystals have been prepared by Mortier *et al.* [15] by boiling solutions containing brushite ($CaHPO_4 \cdot 2H_2O$) and urea below 100 °C. Kinoshita *et al.* [16] have also reported fibrous HAp in a similar procedure starting from a solution containing Ca^{2+}, PO_4^{3-} and urea. HAp formation from solutions containing monetite ($CaHPO_4$) and EDTA has been reported by Simpson [28] – slow release of Ca^{2+} into the phosphorous-ion-containing solution resulted in the HAp precipitaion. Christiansen and Riman [17] also prepared rod-like HAp single crystals by a hydrothermal treatment of a solution containing PO_4^{3-} and Ca-EDTA chelates. Their crystals, however, were not uniform and they were often contaminated with smaller powders or with an unknown amorphous phase. In contrast, the HAp whiskers we obtained were uniform and not contaminated by other powders.

Clarification of the formation mechanism of HAp whiskers requires further study, however, it is worthwhile noting that the hydrothermal decomposition of Ca-chelates is responsible for the precipitation of HAp. In fact, chromatograms of solutions before and after hydrothermal treatment indicated that the citric acid which had existed before the treatment diminished gradually, but itaconic acid, which was one of the derivatives from the decomposition of Ca-chelates, appeared during the treatment.

HAp whiskers can also be obtained from solutions containing Ca^{2+}, PO_4^{3-} and citric acid or lactic acid under particular hydrothermal conditions. The formation properties of HAp whiskers have also been investigated in more detail in [24].

Attention must be paid to whether whiskers or fibres are biohazardous or not when they are used. This biohazard problem is delicate, not only because of the scientific, medical and environmental problems but also for political and economical reasons. For example, in 1988, the Environmental Protection Agency (EPA) [29] postulated a law which would prohibit the use of asbestos, because of its health hazard, although it has been estimated that it will cost as much as $53 billion to remove the asbestos used in buildings [29]. Stanton and Pott's criterion [3, 4] is widely used to judge whether fibrous material is biohazardous or not. However, it might be true that the carcinogenic potential of fibres or whiskers depends not only on their morphology but also on their biocompatibility. Even though the HAp whiskers we obtained are potentially dangerous on morphology considerations alone, they are not expected to cause diseases since they can dissolve gradually in a human body to produce non-toxic species such as Ca, P or OH. Therefore, these biocompatible HAp whiskers must be a promising material for applications as a reinforcing filler for composites, as insulating agents, as packing media for column chromatography or in the other biomedical areas such as bone-bonding biomaterials.

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