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Brief communication

In vitro hydroxyapatite forming ability and dissolution of tobermorite nanofibers

Kaili Lin [a,b,*], Jiang Chang [b], Rongming Cheng [a,*]

[a] *Center of Functional Nanomaterials and Devices, East China Normal University, 3663 North Zhongshan Road, Shanghai 200062, PR China*
[b] *Shanghai Institute of Ceramics, Chinese Academy of Sciences, 1295 Dingxi Road, Shanghai 200050, PR China*

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Abstract

In this paper, fiber-like and dispersible tobermorite ($Ca_5(Si_6O_{16})(OH)_2 \cdot 4H_2O$), 80–120 nm in diameter and up to tens of micrometers in length was prepared by a hydrothermal microemulsion method. In vitro bioactivity of the nanofibers were evaluated by examing the hydroxyapatite (HAp) forming ability on the surface after soaking in simulated body fluid (SBF) for various periods. After soaking in SBF, the nanofibers were completely covered by bonelike hydroxycarbonate apatite (HCA) layers, and the nanofibers after soaking still kept stability in fibrous morphology. The dissolution of the nanofibers reached about 24.5% after soaking in SBF for 14 days. The results suggested that the tobermorite nanofibers exhibited certain desirable characteristics, including bioactivity, degradability and stability in morphology, and are a potential candidate for a reinforcement material in the development of novel bioactive and degradable composites for biomedical applications.

Keywords: Tobermorite; Nanofibers; Hydroxyapatite forming ability; Dissolution

1. Introduction

One-dimensional (1D) nanoscale building blocks, such as nanofibers, nanorods and nanotubes of materials, have attracted much attention because of their importance to basic scientific research and their potential technology applications [1–3]. One of the most important characteristics is that the 1D nanoscale materials possess excellent mechanical properties, and fiber-reinforced composites exhibit high tensile, flexural and fracture strengths. Therefore, advances in 1D nanoscale reinforcement are of considerable interest in improving the mechanical properties of ceramics and polymer composites [4–6].

Preparation of calcium silicate hydrate (CSH) fibers, especially tobermorite ($Ca_5(Si_6O_{16})(OH)_2 \cdot 4H_2O$), has been attracting attention in recent years. This arises from the fact that these fibers are widely required in modern industry [7,8]. Tobermorite, as one of the most important CSH materials, possesses the highest mechanical strength of the CSH materials, and has been widely used in high quality cement [7,8]. Previous studies have suggested that various biomaterials containing the $CaO–SiO_2$ component, such as Bioglass [9], $CaSiO_3$ [10,11] and Ca_2SiO_4 ceramics [12], are bioactive and could induce the formation of a bonelike hydroxyapatite (HAp) layer in vivo and in vitro. This type of HAp layer plays an essential role in the formation of tight bone-bonding between the bioactive materials and the neighbouring bones, and has not been observed for non-bioactive materials [13–15]. Based on these results, it is proposed and confirmed that the essential requirement for materials to bond with living bones is the formation of bonelike apatite on the material's surface in the living body and that this in vivo apatite formation can be reproduced in simulated body fluid (SBF). This means that in vivo bioactivity can be predicted by examing apatite formation on a material's surface in SBF [15,16]. Therefore, we consider

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[*] Corresponding authors. Tel.: +86 21 52412810; fax: +86 21 52413903.
E-mail addresses: lklsic@mail.sic.ac.cn (K. Lin), rmcheng@phy.ecnu.edu.cn (R. Cheng).

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that tobermorite could be bioactive and that its fibers are a potential candidate for a reinforcement material.

Tobermorite fibers are usually prepared from suspensions of silica and calcium hydroxide by hydrothermal treatment [17]. However, the fiber size ranges widely, from the submicrometer range to micrometers, and the aspect ratio of the obtained fibers cannot be controlled well using the normal hydrothermal method. On the other hand, it is generally difficult to obtain long CSH fibers, and experimental results are usually affected by several factors, such as composition, crystallization, size distribution of raw materials and the weight ratio of water/solid material [18–20]. At the same time, several reports have been reported in the literature concerning hydrothermal synthesis of long CSH fibers at relatively much higher temperatures (250–350 °C) and over much longer times (72–200 h), using special instruments (continuous-type or multichamber autoclave) [21,22]. A newly developed hydrothermal microemulsion technique is used to synthesize nanopowders and nanoneedles [23,24], and this method is considered to be an effective, convenient and mild synthetic methodology. The microemulsion can serve not only as nano-reactors to control the particle size and size distribution in the processing reactions, but can also inhibit the excessive agglomeration of particles, because surfactants can absorb on the particle surfaces when the particle size approaches that of the water (or oil) pool. In addition, the surfactants in the microemulsion can also serve as a versatile "soft" template for the synthesis of 1D nanostructural materials. Furthermore, hydrothermal treatment can effectively increase the crystallinity of the product. However, no study on the preparation, bioactivity and dissolution of CSH fibers, such as tobermorite nanofibers, has been reported, until now.

In the present study, tobermorite nanofibers were synthesized by a hydrothermal microemulsion process. In vitro HAp forming ability and the dissolution of the nanofibers were evaluated by soaking in SBF.

2. Materials and methods

2.1. Synthesis and characterization of the tobermorite nanofibers

Microemulsions were prepared using cetyltrimethyl ammonium bromide (CTAB) as surfactant and *n*-pentanol as cosurfactant. First, $Ca(NO_3)_2$ solution and Na_2SiO_3 solution were obtained by dissolving 1.1335 g $Ca(NO_3)_2 \cdot 4H_2O$ and 1.3642 g $Na_2SiO_3 \cdot 9H_2O$ in 4.0 ml distilled water, respectively, and the pH of both solutions was adjusted to 10.8 by adding ammonia solution. These aqueous solutions were used as the water phase and *n*-hexane was used the oil phase. In a typical experiment, a mixture of 2.60 g of CTAB, 4.0 ml of *n*-pentanol, 65 ml of *n*-hexane and 4.0 ml of Na_2SiO_3 aqueous solution was added to a 200 ml beaker. The mixture was stirred and ultrasonicated, and optically transparent microemulsions were obtained. Then $Ca(NO_3)_2$ solution was added dropwise into the Na_2SiO_3 microemulsion solution to obtain a suspension. The suspension was transferred into a stainless steel autoclave and heated at 200 °C for 18 h, followed by cooling to room temperature naturally. After hydrothermal reaction, the obtained suspension was filtered and washed with distilled water and anhydrous ethanol three times. The resultant powders were dried at 60 °C for 48 h. The obtained powders were characterized using X-ray diffraction (XRD; Geigerflex, Rigaku Co., Japan) with monochromated Cu Kα radiation. The surface of the as-synthesized powders was sputter-coated with gold and then observed by field emission scanning electron mocroscopy (FESEM) at 10 kV accelerating voltage using a JEOL (JSM-6700F, Japan).

2.2. In vitro HAp forming ability and dissolution behavior

The bioactivity of the tobermorite nanofibers was evaluated from the formation of bonelike HAp on the nanofibers in SBF, which has ion concentrations similar to human blood plasma [15,25]. The solution was buffered to pH 7.4 using tris(hydroxymethyl) aminomethane and hydrochloric acid.

The nanofibers were soaked in the SBF at 37.0 °C in a shaking water bath for 1, 3, 7 and 14 days, respectively, at a solid/liquid ratio of 1.50 mg ml^{-1} without refreshing the soaking medium. After various soaking periods, the samples were filtered and gently rinsed twice with deionized water to remove SBF followed by drying in vacuum at 80 °C. The soaked powders were characterized by XRD and Fourier transform infrared spectroscopy (FTIR; Nicolet Co., USA). The surface of the soaked powders was sputter-coated with gold and then observed by FESEM at 10 kV accelerating voltage. The concentrations of Ca, P and Si soaked in the SBF solutions were determined by inductively coupled plasma atomic emission spectroscopy (Varian Co., USA). Changes in pH of the solutions were measured by a pH test-meter (pHS-2C, Jingke Leici Co., China).

Based on the fact that there is no Si in SBF before soaking, the dissolution ratio (*S*) of the tobermorite nanofibers at different time periods was calculated by the following equation:

$$S = (c_{\mathrm{Si}} \times v)/m_{\mathrm{Si}} \times 100\%$$

where c_{Si}, v and m_{Si} are the Si concentration in SBF (mg ml^{-1}), volume of SBF (ml) and Si content (mg) of the samples soaked in SBF, respectively.

3. Results and discussion

3.1. Characterization of the as-synthesized nanofibers

Fig. 1 shows the XRD patterns of the as-synthesized powder prepared by the hydrothermal microemulsion method. Numerous sharp peaks were observed in the