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Hydrothermal fabrication, characterization, and biological activity of cellulose/$CaCO_3$ bionanocomposites

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ABSTRACT

Bionanocomposites with the combination of natural polymers and inorganic nanoparticles may induce unique properties and exhibit promising functions for different applications. Herein, we report a hydrothermal route to the preparation of cellulose/$CaCO_3$ bionanocomposites using the cellulose solution, $Ca(NO_3)_2 \cdot 4H_2O$ and $Na_2SiO_3 \cdot 9H_2O$. The cellulose solution was previously prepared by the dissolution of microcrystalline cellulose in NaOH–urea aqueous solution. The urea also acts as the CO_3^{2-} source for the synthesis of $CaCO_3$. The influences of several reaction parameters, such as the heating time, the heating temperature, and the types of additives on the products were investigated by X-ray powder diffraction, Fourier transform infrared spectrometry, scanning electron microscopy, thermogravimetric analysis, and differential thermal analysis. The experimental results demonstrated that the hydrothermal conditions had an effect on the morphology of the bionanocomposites. Cytotoxicity experiments indicated that the cellulose/$CaCO_3$ bionanocomposites had good biocompatibility, so that the bionanocomposites could be ideal candidate for practical biomedical applications.

1. Introduction

Bionanocomposites that consist of natural polymers and inorganic nanoparticles have gained more interest as environmentally friendly and biofunctional materials due to its interesting properties such as biocompatibility, biodegradability and potential applications in optical, electrochemical, magnetic, catalytic, and biomedical fields (Alcantara, Aranda, Darder, & Ruiz-Hitzky, 2010; Darder, Aranda, & Ruiz-Hitzky, 2007; Luckarift, Dickerson, Sandhage, & Spain, 2006; Ruiz-Hitzky, Darder, Aranda, & Ariga, 2010; Ruiz-Hitzky, Darder, Aranda, del Burgo, & del Real, 2009). Until now, several successful strategies including drop-casting process (Biswas et al., 2010), ultraviolet irradiation reduction method (Shameli et al., 2010), grafting from approach (Habibi et al., 2008), casting/evaporation technique (Habibi & Dufresne, 2008), and solvent casting/particle leaching (Rezwan, Chen, Blaker, & Boccaccini, 2006) have been reported to prepare bionanocomposites such as chitosan/clay (Shchipunov, Ivanova, & Silant'ev, 2009), xanthan gum/sepiolite (Ruiz-Hitzky et al., 2009), and vanadium oxide/gelatin (Carn et al., 2010). Among of these bionanocomposites, the synthesis of cellulose-based bionanocomposites has attracted particular attention (Chen, Liu, Chang, Cao, & Anderson, 2009; Habibi & Dufresne, 2008; Rhim & Ng, 2007) because cellulose is one of the most abundant natural polymers and renewable resources on the earth (Eichhorn et al., 2010).

As the main inorganic component of human bone, teeth, and shells, hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HA] and calcium carbonate ($CaCO_3$) are known to have biological activities of protein-adhesive properties, cell compatibility, and hard tissue compatibility (Hanein, Sabanay, Addadi, & Geiger, 1993; Hott, Noel, Bernache-Assolant, Rey, & Marie, 1997; Luo & Andrade, 1998; Ohgushi et al., 1992). Cellulose/HA bionanocomposites have been extensively explored (Hong et al., 2006; Wan et al., 2006; Zhang et al., 2009). It is possible that the composites of bioactive $CaCO_3$ with cellulose induce unique functional properties. However, there have been only few literatures reporting on the synthesis of cellulose/$CaCO_3$ bionanocomposites (Dalas, Klepetsanis, & Koutsoukos, 2000; Fimbel & Siffert, 1986; Shen, Song, Qian, & Yang, 2010; Subramanian, Maloney, & Paulapuro, 2005; Vilela et al., 2010). The research of the interaction of $CaCO_3$ (calcite) with cellulose fibers in aqueous medium was first reported in 1986 (Fimbel & Siffert, 1986). Then, Dalas et al. (2000) investigated the kinetics process of the calcium carbonate deposition on cellulose substrate. After that, the coprecipitation of calcium carbonate as filler in papermaking was reported (Subramanian et al., 2005). More recently, Vilela et al. (2010) prepared cellulose/$CaCO_3$

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bionanocomposite by the controlled reaction of $CaCl_2$ with dimethylcarbonate ($(CH_3)_2CO_3$) in alkaline medium in the presence of cellulose fibers. To date, however, there has been no report on special discussion of cellulose/$CaCO_3$ bionanocomposites prepared by hydrothermal method, which provides unique temperature–pressure environments and has an advantage of controlling morphology of the products by adjusting the reaction parameters. In previous studies, we synthesized cellulose-carbonated HA bionanocomposites using cellulose solution, $CaCl_2$, and NaH_2PO_4 in aqueous solution by hydrothermal method at 180 °C for 24 h (Jia, Li, Ma, Sun, & Zhu, 2010).

Herein, we report the synthesis of the cellulose/$CaCO_3$ bionanocomposites by hydrothermal method using the cellulose solution, $Ca(NO_3)_2 \cdot 4H_2O$ solution, and $Na_2SiO_3 \cdot 9H_2O$ solution. The urea acts as both the additive for the dissolution of cellulose and the ${CO_3}^{2-}$ source for the synthesis of $CaCO_3$. The biological activity of cellulose/$CaCO_3$ bionanocomposites was also studied by cytotoxcity experiments.

2. Experimental

2.1. Preparation of cellulose/$CaCO_3$ bionanocomposites

All chemicals were of analytical grade and used as received without further purification. All experiments were conducted under air atmosphere. The preparation of cellulose solution followed our previous report (Jia et al., 2010). In a typical synthesis, 7.00 g of NaOH and 12.00 g of urea were added into 81 mL of distilled water under vigorous stirring to form NaOH–urea aqueous solution. Then, 3.24 g of microcrystalline cellulose was added into the above solution under vigorous stirring. The above solution was cooled to −12 °C for 12 h. The obtained cellulose solution was used for the preparation of cellulose/$CaCO_3$ bionanocomposites.

For the synthesis of cellulose/$CaCO_3$ bionanocomposites, 5 mL of $Ca(NO_3)_2 \cdot 4H_2O$ solution (0.40 mol/L) and 5 mL of $Na_2SiO_3 \cdot 9H_2O$ solution (0.40 mol/L) were added into the above obtained cellulose solution (10 mL) under vigorous stir. The mixture solution was transferred into a 25-mL Teflon-lined stainless steel autoclave. The autoclave was maintained at a certain temperature for a certain time. The product was separated from the solution by centrifugation, washed by water and ethanol several times and dried at 60 °C for further characterization.

2.2. Characterization

X-ray powder diffraction (XRD) patterns were obtained in 2θ range from 10° to 70° on a X'Pert PRO MPD diffractometer operating at 40 kV with Cu Kα ($\lambda = 1.5405$ Å) radiation. Fourier transform infrared (FT-IR) spectroscopy was carried out on an FT-IR spectrophotometer (Nicolet 510), using the KBr disk method. Scanning electron microscopy (SEM) images were recorded with a Hitachi 3400N scanning electron microscopy. All samples were Au coated prior to examination by SEM. Thermal behavior of the samples was tested using thermogravimetric analysis (TGA) and differential thermal analysis (DTA) on a simultaneous thermal analyzer (DTG-60, Shimadzu) at a heating rate of 10 °C min^{-1} in flowing air.

2.3. Cell cytotoxicity

The human gastric carcinoma cells (MGC-803) that were cultured in an RPMI-1640 medium supplemented with 10% FBS (fetal bovine serum) and 1% penicillin–streptomycin at 37 °C for 48 h, were used for cell viability test. Then, the cells were seeded in 96-well flat-bottom microassay plates at a concentration of 1×10^4 cells/mL and cultured for 24 h. The sterilized samples (the cellulose/$CaCO_3$ bionanocomposites) were added into wells at the concentration from 10, 20, 30, 50 to 100 μg/mL, and were co-cultured with cells for 48 h. The sample free tissue culture plate was used as a control. Cell viability was quantified by MTT (3-(4.5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Data are representative as mean value of five parallel experiments.

3. Results and discussion

3.1. The phase, microstructure, and morphologies of the cellulose/$CaCO_3$ bionanocomposites

The phase of the cellulose/$CaCO_3$ bionanocomposites was characterized by XRD, as shown in Fig. 1. The pattern of the sample prepared by hydrothermal method at 160 °C for 24 h indicated the existence of the mixed phases of cellulose and $CaCO_3$: cellulose ($2\theta = 20.0°$ and 21.9°) and calcite (marked with C in Fig. 1e). This result shows the successfully preparation of cellulose/$CaCO_3$ bionanocomposites. The solubility product (K_{sp}) of $CaCO_3$ is 2.8×10^{-9}, which is smaller than that of $CaSiO_3$ (2.5×10^{-8}). As we all know, the product is first obtained with low K_{sp} value under normal condition. Therefore, the obtained products are not the cellulose/$CaSiO_3$ bionanocomposites, but cellulose/$CaCO_3$ bionanocomposites. There is still existence of a small amount of aragonite. As we know, aragonite is the metastable phase of $CaCO_3$, while calcite is the thermodynamically stable phase of $CaCO_3$. This phenomenon might be explained by the part formation of aragonite in the bionanocomposites, and then the aragonite was converted to the calcite after long time reaction. The phases of the samples synthesized at 160 °C for 2 h, 4 h, 6 h, and 12 h were also investigated (Fig. 1a–d). One can see that all of the samples have similar XRD patterns. However, the peak intensity of aragonite decreased with increasing heating time, further implying the conversion from the metastable aragonite to the thermodynamically stable calcite. Some groups (Hosoda, Sugawara, & Kato, 2003; Lakshminarayanan, Valiyaveettil, & Loy, 2003; Ogomi, Serizawa, & Akashi, 2005; Serizawa, Tateishi, & Akashi, 2003) have found the similar phenomenon on the synthesis of poly(vinyl alcohol)/$CaCO_3$ composite and thought that poly(vinyl alcohol) might affect the conversion of aragonite. Herein, we used cellulose as matrix, which

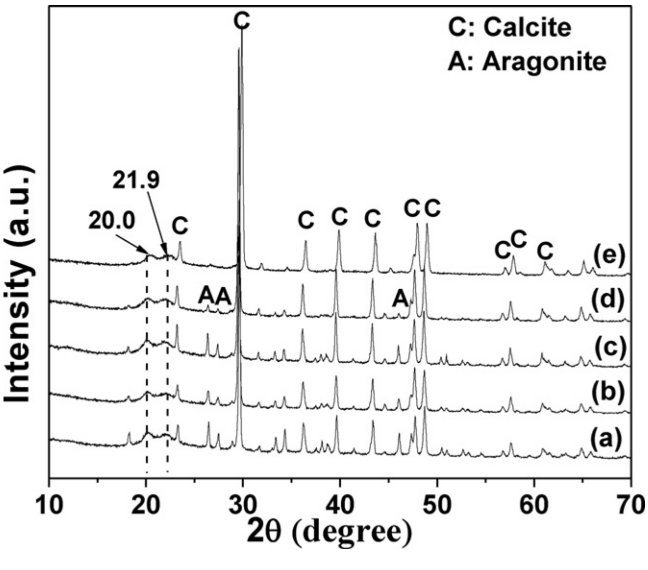

Fig. 1. XRD patterns of the cellulose/$CaCO_3$ bionanocomposites prepared by hydrothermal method at 160 °C for different times: (a) 2 h; (b) 4 h; (c) 6 h; (d) 12 h; and (e) 24 h.