Materials Chemistry and Physics 173 (2016) 262–267

Contents lists available at ScienceDirect

Materials Chemistry and Physics

journal homepage: www.elsevier.com/locate/matchemphys

One-step exfoliation and surface modification of lamellar hydroxyapatite by intercalation of glucosamine

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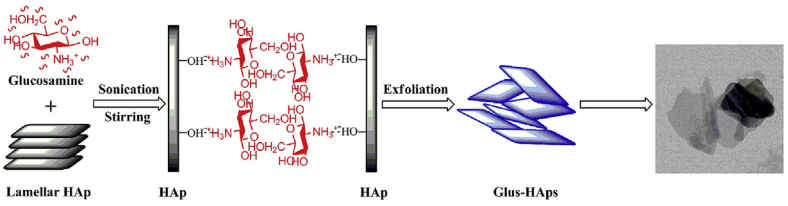
HIGHLIGHTS

- Glucosamine was used as intercalation agent to exfoliate lamellar hydroxyapatite.
- Glucosamine was grafted onto the as-exfoliated nanoplate-like hydroxyapatite.
- Exfoliation and surface grafting were accomplished in one step.
- Glucosamine-grafted HAp showed improved biocompatibility over nongrafted one.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 August 2015
Received in revised form
28 January 2016
Accepted 2 February 2016
Available online 11 February 2016

Keywords:
Biomaterials
Nanostructures
Surfaces
Interfaces

ABSTRACT

Effective exfoliation is crucial to the application of layered materials in many fields. Herein, we report a novel effective, scalable, and ecofriendly method for the exfoliation of lamellar HAp by glucosamine intercalation such that individual HAp nanoplates can be obtained. The as-exfoliated HAp nanoplates were characterized by Fourier transform infrared (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM), and thermogravimetric (TG) analysis. It is found that the glucosamine intercalation not only results in complete exfoliation of lamellar HAp but also introduces the glucosamine molecules onto the surface of individual HAp nanoplates, thus obtaining separated glucosamine-grafted HAp nanoplates (Glu-HAps). Results from MTT assay demonstrate that glucosamine grafting on HAp nanoplates greatly improves the cell growth and proliferation as compared to nongrafted HAp counterparts.

1. Introduction

Exfoliation of lamellar materials has been believed to be crucial to their applications in many fields [1]. For instance, Coleman and co-workers exfoliated layered materials into individual layers, which were used to prepare composites and excellent properties have been demonstrated [1]. Unlike other lamellar materials such as WS_2 and MoS_2, lamellar hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$)

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http://dx.doi.org/10.1016/j.matchemphys.2016.02.012
0254-0584/

is of special importance in biomedical fields since it is a major inorganic material and an essential component of bones and teeth and has been widely utilized as implant materials for many years due to its excellent biocompatibility, bioactivity, osteoconductivity, biodegradability. In our previous studies, a template-assisted self-assembly method was developed to prepare lamellar HAp with highly ordered structure and a large *d* spacing of 3.1 nm [2,3]. This lamellar structure exhibits advantages as a carrier for DNA [2,4] and drugs or as the reinforcement in dense polymer composites [5]. In order to prepare HAp-based composites with well dispersed HAp nanoplates, exfoliation of the lamellar HAp is necessary to obtain separated individual HAp nanoplates. Although the HAp nanoplates are more favorable than HAp nanoparticles from the biomimetic point of view because natural apatite in human bones is plate shaped rather than particle [6,7], the dispersion of these HAp nanoplates in polymers is more difficult due to their high aspect-ratio. Therefore, surface modification of these HAp nanoplates is necessary.

To date, many substances have been used to alter the surface of HAp including acids, alcohols, bases, polymers, proteins, silane coupling agents, peptide sequences, and natural polysaccharides [8]. For instance, Wilson et al. found that chitosan functionalized HAp exhibited enhanced colloid stability for processing of chitosan/HAp nanocomposites [9]. Chitosan is a typical natural polysaccharide, exhibiting nontoxicity, biodegradability, biocompatibility, and antibacterial properties [10–12]. In vivo, chitosan is hydrolyzed into cell-activating oligomers and monomers, namely glucosamine and N-acetylglucosamine [13]. Glucosamine is an amino monosaccharide, one of the most abundant monosaccharides, and is produced commercially by the hydrolysis of chitin [14,15]. In clinic, glucosamine is widely used for osteoarthritis patients to relieve pain and slow down the rate of joint space narrowing [16]. A recent study demonstrated the anti-cancer activity of glucosamine [17]. Other studies demonstrated that glucosamine could promotes osteogenic differentiation of dental pulp stem cells through modulating the level of the transforming growth factor-beta type I receptor [18]. Interestingly, glucosamine can also be used as the material for surface modification of biomaterials. For instance, a previous study by Russo et al. demonstrated that glucosamine-functionalized poly(e-caprolactone) showed enhanced cell density and spreading over the non-functionalized samples [19]. In another study, Wang et al. declared that glucosamine-modified poly(ethylene glycol) hydrogels were favorable for cell proliferation [20]. It is therefore expected that the modification of HAp with glucosamine should result in improved biocompatibility. On the other hand, it was reported that the exfoliation of layered rectorite could be reached when D-glucosamine hydrochloride was intercalated into the gallery of layered rectorite [21]. It would be desirable if glucosamine can be intercalated into the gallery of lamellar HAp and simultaneously results in the exfoliation of lamellar HAp and surface modification of the as-exfoliated HAp nanoplates. However, neither exfoliation of lamellar HAp via glucosamine intercalation nor surface modification of HAp nanoplates using glucosamine is reported in literature.

Herein, we report, for the first time, a scalable and ecofriendly method for the exfoliation of lamellar HAp using glucosamine as the intercalation agent. The solution intercalation method was employed and the exfoliation of lamellar HAp and glucosamine grafting of HAp nanoplates was accomplished in one step. The main goal of this study was to prepare glucosamine-grafted HAp nanoplates (abbreviated as Glu-HAps hereinafter) and preliminarily evaluate their biocompatibility. The as-prepared Glu-HAps were characterized by X-ray diffraction (XRD), Fourier-transform infrared (FTIR), transmission electron microscopy (TEM), and thermogravimetric (TG) analysis. In addition, preliminary biocompatibility was assessed by the 3-(4,5-dimethylthiazoyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method using a human osteoblast-like cell line (MG-63). The studies on the extensive in vitro and in vivo biocompatibility and their dispersibility in polymers are underway and will be presented in due course.

2. Experimental

2.1. Synthesis of lamellar HAp

The template-assisted synthesis procedures for the preparation of lamellar HAp were described in our previous work [4]. In a typical process, 0.5 g of sodium dodecyl sulphonate (SDS, $C_{12}H_{25}SO_3Na$) was added into a mixture of 15 mL of deionized water and 30 mL of ethanol under magnetic stirring (2000 rpm). The mixture was then heated to 60 °C at which 15 mL of 3.3 M $Ca(NO_3)_2{\cdot}4H_2O$ solution was added dropwise. Immediately afterward, 30 mL of 1 M $(NH_4)_2HPO_4$ and 30 mL of ethanol were added, followed by the addition of 20 mL of 2.5 M NaOH solution and 20 mL of ethanol. The mixture was refluxed at 83 °C for 14 h. The precipitate was deposited at room temperature for 12 days, then centrifuged and immersed in deionized water for 3 days. Finally, the precipitate was centrifuged again and dried at 60 °C to obtain lamellar HAp powder.

2.2. Intercalation of glucosamine

The exfoliation of lamellar HAp was carried out via the solution intercalation method. Typically, 0.1 g of glucosamine (Beijing Sinopharm Chemical Reagent Co., Ltd.) was dissolved in 100 mL deionized water under magnetic stirring at room temperature. Then 0.5 g of the lamellar HAp was added into the above glucosamine solution and the mixture was drastically sonicated for 2 h to get a homogenous dispersion followed by constant stirring for 3 days at room temperature. Finally, the resultant product was centrifuged and dried at 60 °C for 24 h, and the sample named as Glu-HAps-1 was obtained. When 0.3, 0.5, or 0.7 g was dissolved in 100 mL deionized water separately, the resultant samples were named as Glu-HAps-3, Glu-HAps-5, and Glu-HAps-7, respectively.

2.3. Characterizations

XRD analysis was performed using a Rigaku D/Max 2500 v/pc diffractometer (Rigaku, Japan) with Cu Kα radiation generated at 40 kV and 200 mA. The scanning rate was 4°/min over a range of 10–60° for wide-angle diffraction and 1°/min over a range of 1–10° for small-angle measurement. FTIR analysis was conducted by a Bio-Rad FTS 6000 FTIR spectrometer using the KBr pellet technique at a wavelength range of 450–4000 cm^{-1}. The sample morphology was investigated by TEM (Tecnai F30 G2, FEI). The grafting ratios of Glu-HAps were determined using a TGA analyzer (TA Instruments, SDT 2960) from room temperature to 800 °C at a heating rate of 10 °C/min under nitrogen flow.

2.4. Proliferation assay

For cell studies, film-like samples were fabricated by vacuum filtration of the dispersions of Glu-HAps and non-grafted HAp nanoplates (abbreviated as nGlu-HAps, prepared by calcination of Glu-HAps at 800 °C for 2 h) through a micropore filter membrane. The samples attached to the filter were washed several times with deionized water, then completely dried, and finally peeled off from the filter. The cell proliferation was evaluated by MTT assay following the similar procedures reported in our previous studies [22] except that MG-63 cells were used. Cells were cultured in