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Synthesis of cellulose–nanohydroxyapatite composite in 1-*n*-butyl-3-methylimidazolium chloride

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Abstract

In this study cellulose–nanohydroxyapatite composite was fabricated for bone tissue engineering applications. In this composite a natural biopolymer was reinforced with bioactive nanohydroxyapatite for replacement or healing of bone. The ionic liquid 1-*n*-butyl-3-methylimidazolium chloride (BmimCl) was used for dissolution of cellulose.

Nanohydroxyapatite (n-HAp) powder was characterized by X-ray diffractometry (XRD), scanning electron microscopy (SEM) and FT-infrared (FTIR) spectroscopy. Thermogravimetric analysis (TGA) as well as MTT assay of nanocomposite was conducted to assess the thermal stability and cytotoxicity of the samples.

The results of XRD showed the formation of hydroxyapatite crystals and also SEM images indicated that n-HAp powder was nanocrystalline. The composite was thermally less stable than native cellulose. Also native cellulose had higher decomposition rate and mass loss. Results of biological test showed that the samples were biocompatible with no toxicity. Also, SEM observations demonstrated that human osteoblast cells can attach to the surface of the nanocomposite samples and this composite can be used as bone tissue engineering.

Keywords: Cellulose; Nanohydroxyapatite; Ionic liquid

1. Introduction

A variety of materials have been used for replacement and repair of damaged or traumatized bone tissues [1–3]. These materials include metals, ceramics, polymers (natural and synthetic) and their combinations. Metals and ceramics have two major disadvantages for tissue engineering applications: they are lack of degradability in a biological environment, and their processability is very limited [4]. In contrast, polymers have great design flexibility because the composition and structure can be tailored to specific needs. They are therefore attractive candidate. Biodegradability can be imparted into polymers through molecular design [5]. However, polymers have lower modulus and strength as compared to metals and ceramics [6]. For the mechanical reinforcement of polymers or for certain applications such as bone regeneration, composite materials of biopolymers and bioceramics have been produced for bone tissue engineering [7]. The most frequently used bioceramic material is hydroxyapatite (HAp). It is bioactive, osteoconductive, non-toxic and non-immunogenic [8]. Many efforts have been made to modify HA by polymers since the natural bone is a composite mainly consisted of nano-sized, needle-like HAp crystals (accounts for about 65 wt% of bone) and collagen fibers [9,10].

Natural biodegradable polymers have been used in tissue engineering [11,12]. One important category of natural biopolymers is polysaccharides. Cellulose is an abundant renewable polysaccharide consisting of a linear chain of (1,4) linked β-D-glucopyranose units aggregated to form a highly ordered structure due to its chemical constitution and spatial conformation [13] (Fig. 1).

The individual cellulose chains are joined by a network of inter-and intra-molecular hydrogen bonding and van der waals forces. The crystalline structure of cellulose, due to hydrogen bonds, in combination with the high molecular weight, gives it unique properties like chemical stability and mechanical strength. In the biomedical field, cellulose and its derivatives have been extensively used for decades [14].

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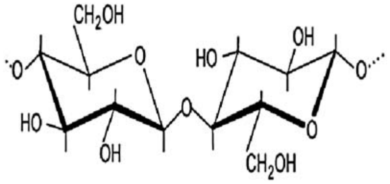

Fig. 1. The cellulose molecule – $(C_6H_{10}O_5)_n$ – in its chair configuration [13].

The biocompatibility of several cellulosics is well established. Cellulose has a long background in medical applications, essentially due to its lack of toxicity, water solubility or high swelling ability and stability to temperature and pH variations [15].

However, processing and derivatization of cellulose are difficult in general, because this natural polymer is neither meltable nor soluble in conventional solvents due to its hydrogen bonded and partially crystalline structure. Over the past decades, several solvent systems have been developed for manufacturing cellulose materials. Typical examples for these solvents include LiCl/N,N-dimethylacetamide (DMAc), LiCl/N-methyl-2-pyrrolidine (NMP), dimethyl sulfoxide (DMSO)/paraformaldehyde, and N-methyl morpholine-N-oxide (NMMO) [16,17]. However, the above solvent systems are limited to their dissolving capability, toxicity, high cost, solvents recovery, uncontrollable side reaction, and instability during cellulose processing and/or derivatization [16].

Recently, ionic liquids (ILs) were found to dissolve native cellulose. ILs are organic salts with polar character and low melting points (<100 °C) [18]. They are thermally and chemically stable and have a practically non-detectable vapor pressure. Their non-volatile nature allows for easy recycling after usage and, thus, places them high in the realm of green chemistry [19]. Quite few ionic liquids are known and have been used to dissolve cellulose. The most frequently used are the hydrophilic ILs 1-*n*-butyl-3-methylimidazolium chloride (BmimCl) and 1-allyl-3-methylimidazolium chloride (AmimCl).

The dissolution mechanism of cellulose in ionic liquids involves the oxygen and hydrogen atoms of cellulose-OH in the formation of electron donor–electron acceptor complexes which interact with the ionic liquid (Fig. 2).

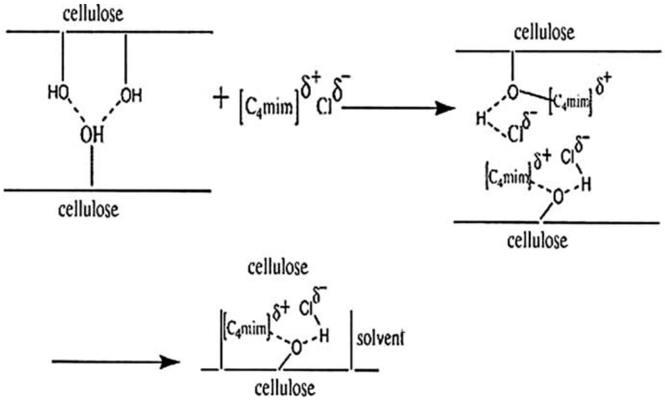

Fig. 2. Dissolution of cellulose in ionic liquids [19].

For their interaction, the cellulose atoms serve as electron pair donor and hydrogen atoms act as an electron acceptor [19].

In the present study, cellulose and cellulose–hydroxyapatite composites were successfully prepared and then were characterized.

2. Experimental

2.1. Materials and methods

Microcrystalline cellulose (MCC), calcium hydroxide ($Ca(OH)_2$) and orthophosphoric acid (H_3PO_4) were purchased from Merck and1-*n*-butyl-3-methylimidazolium chloride (BmimCl) and other chemical materials were received from Sigma–Aldrich.

2.2. Preparation of nanoHAp (n-HAp)

n-HAp with Ca/P ratios of 1.67 was synthesized by a wet chemical precipitation reaction between $Ca(OH)_2$ and H_3PO_4 [20]. 0.5 M $Ca(OH)_2$ powder was added slowly into deionized water at 25 °C and vigorously agitated for 1 h. To this solution a 0.3 M H_3PO_4 solution was added dropwise with ratio of 6 ml/min approximately to produce a white precipitate. The pH was controlled to a minimum of 8.0 ± 1.0 during the addition of acid to the $Ca(OH)_2$ suspension using a 1.0 M NH_4OH solution. The solution rested at room temperature for 24 h. After decantation, the precipitates were washed for three consecutive times with deionized water and dried at 75 °C for 12 h. Then, the powders were milled and passed through sieve set of 200 mesh.

$$10Ca(OH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O \qquad (1)$$

For characterizing the crystal phases of n-HAp, XRD (JDX-8030 JEOL, Cu Kα) and FTIR (SHIMADZU, 8400S) were performed on the powder. The morphology of particles was analyzed on SEM (Philips, XL300). From the full width at half maximum (FWHM), the average crystalline size calculated with the (0 0 2) diffraction peaks in the peak in the XRD pattern according to the Scherrer formula:

$$d = \frac{0.9\lambda}{\beta \cos\alpha} \qquad (2)$$

where d is the crystallite size (nm), λ is the wavelength of the X-ray ($\lambda_{Cu} = 0.154056$ nm), α is the peak diffraction angle (°) and β is the experimental full width at half maximum [21].

2.3. Preparation of cellulose solution in BmimCl

The solution of cellulose in BmimCl (3%, w/w) was obtained by continuous stirring of the starting materials at 95 °C for 5 h. After complete dissolution of cellulose, an appropriate mass of nanohydroxyapatite (n-HAp) particles were added in order to obtain composites with n-HAp load of 0.16 (16% n-HAp and 84% cellulose, w/w) and stirred for 15 min. Sonication was then applied four times for 5 min each, a total of 20 min. After preparation, the solution was poured in a