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Bioactivity evaluation of novel nanocomposite scaffolds for bone tissue engineering: The impact of hydroxyapatite

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ABSTRACT

The objective of this study was to prepare scaffolds based on cellulose-graft-polyacrylamide composed of different contents of nano-hydroxyapatite (n-HAp). To this end, polyacrylamide was grafted onto cellulose in the presence of n-HAp through free radical polymerization. Then, the scaffolds of the dispersed grafted polymer nanocomposite powder were fabricated by the freeze-drying method. The grafted polymer nanocomposite scaffolds were tested and characterized using tensile test instrument, Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD) analysis. Finally, bioactivity and apatite formation on the surface after immersion in a simulated body fluid (SBF) were determined by XRD and SEM analysis. According to the results, as the n-HAp content in the scaffold structure increased, the porosity, elastic modulus and compressive strength were increased. In addition, apatite was deposited very well on the interconnected irregular pores on the surface of the scaffolds after incubation in SBF, while the size of precipitated apatite was reduced by increasing the soaking time. The results indicated that the prepared grafted polymer nanocomposite scaffolds have a great potential as biocompatible materials for use in bone tissue engineering.

1. Introduction

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is a biocompatible material resembling the mineral component of bone and teeth [1]. Hydroxyapatite (HAp) belongs to a group of calcium phosphates with a Ca/P molar ratio of 1.67 and is an interesting biomaterial with potential orthopedic, dental, and maxillofacial applications [2,3]. Because of its excellent biocompatibility, bioactivity, and osteoconductivity, it has been considered the ideal material to construct bone-tissue engineering scaffold [4]. Bone-tissue engineering is a complex and dynamic process that begins with the migration and recruitment of osteoprogenitor cells followed by their proliferation, differentiation, and matrix formation, along with remodeling of the bone [5,6]. Successful bone-tissue scaffolds should possess the desired criteria, such as strong, flexible, and porous, which are significant to their role as biological substitutes [7]. This is because the scaffold should mimic the extracellular matrix (ECM) of the body. The ECM is a network of connective tissue that supports and anchors body cells, similar to the role of scaffolds. Therefore, the selection of the most appropriate material to produce a scaffold with these qualities is a critical step.

Scaffolds composed of HAp have shown that despite achieving supporting cell attachment and biointegration, they have failed to demonstrate suitable mechanical properties (i.e., strength and elasticity). To solve this problem, varieties of natural and synthetic polymers as flexible reinforcing agents have been combined with HAp to reduce the scaffold brittleness. HAp-reinforced degradable natural polymers, such as chitosan [8], collagen [9], cellulose [10], and chitin [11], as well as synthetic polymers, such as poly-hydroxybutyrate [12], poly(lactide-co-glycolide) [13] a block copolymer of poly(ethylene glycol) and poly(butylene terephthalate) [14] and polyacrylamide [15], have been used in different areas of tissue engineering. Polyacrylamide is a biocompatible synthetic polymer, which has been clinically proved non-toxic, non-immunogenic, and stable in human [15,16]. Although the toxicity of the acrylamide monomer has been documented, polyacrylamide is widely used in biomedical applications [17], such as a cartilage substitute [18], a skin substitute [19] and a drug delivery system [20,21]. Polyacrylamide with a very low elastic modulus of

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30 ± 10 KPa compare with 18 ± 5 GPa of HAp can decrease the elasticity of scaffolds containing only HAp [22,23].

In this study, an efficient scaffold of n-HAp embedded in the network of cellulose-graft-polyacrylamide was synthesized. 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 [24].

Cellulose, a neutral biocompatible polysaccharide, is a promising natural material for bone, cartilage tissue engineering, and tissue engineering in post-injury brain tissue [25]. It has been employed in drug delivery systems and for constructing three-dimensional scaffolds for growing functional cells due to its non-toxicity, renewability, biocompatibility, and readily modified properties [26]. Despite its very useful properties and the vast area of its applications, a limitation of cellulose is that the polymer forms a dense mesh that can limit cell infiltration [27]. Therefore, graft polymerization of cellulose with hydrophilic polymers such as polyacrylamide can decrease the elasticity of cellulose. Acrylamide with vinyl groups in its structure can easily participate in free radical polymerization. The strong interpenetrating polymeric network of cellulose-graft-polyacrylamide can imprint n-HAp molecules via ionic electrostatic interactions.

The objective of this study is the synthesis of scaffolds based on a cellulose-graft-polyacrylamide/n-HAp nanocomposite. In the previous work, the effect of polyacrylamide in the properties of the cellulose-graft-polyacrylamide/n-HAp nanocomposite scaffolds was investigated [28]. In this study, the scaffolds with different contents of n-HAp were prepared by applying an efficient solvent. The prepared scaffolds were characterized using FTIR, XRD, and SEM analysis. In addition, the mechanical properties of the scaffolds were investigated. Finally, their bioactivity and the apatite formation of samples after immersing in the SBF for 7, 14 and 28 days were determined using XRD and SEM analysis.

2. Experimental procedures

2.1. Materials and equipments

The synthesis of the nanocomposite scaffold was achieved using microcrystalline cellulose (Aldrich) with a viscosity of 20 mPa s obtained from cotton linters and potassium persulfate (BDH Chemicals, UK) as an initiator. In addition, the cellulose was dissolved in a mixture of N,N-dimethylformamide (Merck) and lithium chloride (Aldrich). Acrylamide (Merck) and N,N′-methylene-bis-acrylamide (MBA) from Aldrich as the monomer and crosslinker, respectively, were applied without further purification. HAp powder with 99.9% crystallinity and an average diameter of 122 nm was purchased from CAM implants, the Netherlands. Finally, sodium hydrogen carbonate (Aldrich), sodium chloride (Merck), potassium chloride (Merck), calcium chloride (Merck), sodium sulfate (Merck), di-potassium hydrogen phosphate (Merck), magnesium chloride (Aldrich), hydrochloric acid (Aldrich), tris(hydroxymethyl) aminomethane (Aldrich), were used as received for the preparation of used simulated body fluid (SBF) solution.

2.2. Synthesis of grafted polymer nanocomposite powder

Polyacrylamide was grafted onto cellulose backbone via the free radical polymerization method. For this purpose, cellulose (2% w/v) was dissolved in a lithium chloride/dimethyl formamide solution (8% w/v). Then, potassium persulfate (3 wt%) was added to the cellulose solution in a two-neck round bottom flask fitted with an argon gas inlet with vigorous stirring at 65 °C. Different amounts of acrylamide (0.2, 0.5 and 1 g) with MBA (4 wt% of acrylamide) as a crosslinker was added to the initiated cellulose solution. After that, n-HAp powder (~50% wt. of cellulose) was added slowly, and the solution was stirred for 3 h. Finally, the resulting suspension was vacuum-filtered, washed with a large amount of distilled water and dried in an oven at 50 °C overnight.

2.3. Preparation of scaffolds

For fabrication of the nanocomposite scaffold, 2 g of synthesized grafted polymer nanocomposite was dispersed in water/ethanol with a powder/liquid ratio of 1:10 and was casted in a cylindrical aluminum mold ($1.5 \times 10\ cm^2$). The samples in the mold were frozen at −20 °C for 24 h and then placed inside of a freeze dryer at −60 °C and 0.5 Torr. After 48 h, the samples were removed from the freeze dryer, and finally, the grafted polymer nanocomposite scaffolds were prepared. Using this method, three different nanocomposite scaffolds containing different amounts of cellulose/n-HAp ratio (1:0.1, 1:0.25, 1:0.5), namely S1, S2 and S3 were synthesized while the amounts of acrylamide were maintained constant.

2.4. Characterization of grafted polymer nanocomposite scaffolds

To verify the synthesis of the nanocomposite scaffolds, FTIR spectra of the samples were obtained (Thermo Nicolet, USA, Nexus 670 Fourier transform infrared spectrophotometer) in the range of 500 to 4000 cm^{-1}. To observe the major phase of the scaffold components, a phase analysis was performed using an X-ray diffractometer (Equinox 300) with Cu-Kα radiation (1.45 Å) over a 2θ range of 10° to 60° with voltage and current settings of 40 kV and 30 mA, respectively. The morphology of the gold-coated scaffolds was observed using SEM (Philips, XL30) operated at the acceleration voltage of 25 kV. The pore size of the prepared scaffolds was measured using ImageJ software [29,30]. First, ten different cross-sectional SEM micrographs representing different regions of the scaffold were selected. Then, the dimensions of more than 30 pores were measured and averaged to obtain a mean pore size. Finally, the mechanical properties of the prepared nanocomposite scaffolds determined using an SANTAM (STM-20, Iran) compression test apparatus with 0.5 mm/min. The cylindrical scaffold dimensions were approximately 5 mm in diameter and approximately 10 mm in length. The elastic modulus was calculated as the slope of the initial linear portion of the stress–strain curve. The compressive strength was determined as the maximum point of the stress–strain curve. At least three specimens were tested for each sample.

2.5. Porosity measurements

The porosity of synthesized scaffolds was determined by using a liquid displacement method [31]. For this purpose, the dried scaffold was immersed in a graduated cylinder containing a known volume (V_1) of water. The new volume of cylinder called V_2, which is containing water and scaffold. The scaffold was kept under water overnight and allowed the water to penetrate and fill the pores until no air bubbles were seen emerging from the scaffold. Then, the scaffold was removed from the cylinder. The volume of the cylinder after removing the scaffold was recorded as (V_3). Therefore, the volume of the scaffold was calculated by a summation of the volume of water held by the scaffold ($V_1 - V_3$), which was identified as the void volume and the volume of the polymer-grafted nanocomposite ($V_2 - V_1$). The porosity (%) of the prepared scaffolds were calculated using the following equation [28]: