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The role of titanium dioxide on the morphology, microstructure, and bioactivity of grafted cellulose/hydroxyapatite nanocomposites for a potential application in bone repair

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ABSTRACT

Fabrication and characterization of a novel 3D nanocomposite scaffold have been reported in this research. The purpose of developing this new 3D nanocomposite scaffolds is introducing an alternative treatment for bone tissue replacement. Outcomes confirmed that the morphology, microstructure and mechanical properties of synthesized 3D nanocomposite scaffolds closely mimics the properties of real bone tissue. The 3D nanocomposite scaffolds compose of nanoparticle hydroxyapatite which is embedded in the semi-IPN of polyacrylamide-grafted cellulose by free radical polymerization. TiO_2 nanoparticles utilized as an auxiliary component. According to the SEM images the 3D nanocomposite were highly porous with maximum porosity of 87% inter connected with a pore size of around 70–130 μm. The FTIR spectrum and XRD pattern confirmed the graft polymerization process and the presence of TiO_2 in the structure of 3D nanocomposite structures. A tensile test instrument measured elastic modulus and compressive strength of the samples. Comparing to the trabecular bone tissue, the nanocomposite scaffold with the highest content of TiO_2 revealed the adequate compressive strength of 4.1 MPa. The in vitro swelling behavior of the scaffolds was determined in simulated body fluid for 72 h. Results suggested that increasing the amount of TiO_2 decreases the swelling behavior of the nanocomposite scaffolds. The cytotoxicity of the scaffolds was determined by MTT assays on L929 cells. The results of cell culture experiments showed that the scaffold extracts do not have cytotoxicity in any concentration. Our results suggested that the introduced 3D nanocomposite scaffolds have a great potential as a bone tissue substitute.

1. Introduction

Biopolymer-based composites play an important role in application in nanoscience and nanotechnology [1–6]. Recently there is a significant interest in developing novel bioactive 3D scaffolds for tissue engineering or enriching the abilities of existing bioactive scaffolds for many tissue engineering applications [7,8]. In the case of bone tissue engineering, bone repair is a major issue in maxillofacial orthopedic surgery because of various bone diseases, such as bone infections, bone tumors, skeletal abnormalities, and bone loss by trauma [9]. The traditional treatments for bone defects include autografts, allografts, and xenografts with some drawbacks such as donor site morbidity, difficulty in constructing a graft of the desired shape, and pathogen transfer or immune reaction [10–12]. In order to overcome such limits the novel tissue engineering aims at developing 3D biocompatible scaffolds. The purpose of the 3D bone scaffold is to provide optimal conditions for tissue regeneration and allow bone cell attachment onto its surface as well as provide sufficient space for cell proliferation and migration and not obstructed tissue ingrowth and having adequate mechanical properties [13,14]. To this end, the 3D scaffold is required to have a well-interconnected pore network with large pore volume and an average pore connection size exceeding 100 micrometers [15–17] and average pore size larger than 300 micrometers [18] for viable bone formation in a non-resorbable scaffold structure [19].

Choosing the appropriate materials for a bone substitute scaffold is a crucial step, because the properties of these materials will determine the final characteristics of the scaffold. Cellulose is a promising material for tissue engineering, because of its biocompatibility, non-toxicity, and biodegradability [20–22]. However, only a few studies have been conducted on the application of cellulose as a scaffold for cell culturing because of the low mechan-

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ical properties [23]. As an interesting biomaterial with potential orthopedic applications hydroxyapatite has good mechanical properties [24]. Hydroxyapatite with the chemical composition of $Ca_{10}(PO_4)_6(OH)_2$ is a biocompatible material resembling the mineral component of bone and teeth, and has good bioactivity and osteoconductivity [25–29]. However, hydroxyapatite is not biodegradable and can be removed and remodeled in the host. If implanted directly, it may dislocate within the tissue [30]. To eliminate such an undesired mobility, hydroxyapatite is usually mixed with natural or synthetic polymers [31–35]. The addition of a polymer phase can have extra functions since the biodegradable polymer can act as vehicle for biomolecules, growth factors and antibiotics, hence improving the overall performance of the scaffold [36]. Polyacrylamide is a synthesized polymer used also in tissue engineering [37–40] because of its non-toxic and biologically inertness, capacity for preserving its shape and mechanical strength, and convenient adjustability of mechanical, chemical, and biophysical properties [41,42]. Recent studies also show that TiO_2 could be a potential material for bone repair applications [43,44]. TiO_2 scaffolds have been suggested as cell carrier material whose properties, such as good permeability and high biocompatibility, serve to enhance cell vitality [19,45]. However, the main drawbacks of porous TiO_2 scaffolds are low strength and fracture toughness [46]. This work is based on the successes of cellulose, hydroxyapatite, polyacrylamide and TiO_2 that they have shown in bone tissue engineering.

The aim of this study was incorporation of TiO_2 into the cellulose-graft-polyacryliamide/hydroxyapatite/TiO_2 scaffolds for improving mechanical properties (elastic modulus and compressive strength) and biological properties (bioactivity) for bone tissue engineering applications. To this end, cellulose-graft-polyacrylamide/hydroxyapatite/TiO_2 nanocomposite scaffolds were synthesized by freeze-drying method. The morphology and structure properties were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD) and energy-dispersive X-ray spectroscopy (EDS). The mechanical properties of the scaffolds were measured by a tensile test instrument. The swelling behavior was examined in phosphate buffer saline (PBS) solution. The biocompatibility of the scaffolds was tested by culturing L929 cells in vitro.

2. Materials and methods

2.1. Materials

Cotton linters with the particle size of 20 μm and the bulk density of 0.5 g L^{-1}, were extracted to fabricate microcrystalline cellulose which was utilized as an essence of composite construction. The purity of microcrystalline cellulose powder was 95% The cotton linters were purchased from Aldrich and their characteristics is reported according to the manufacturer data sheet. For dissolving of cellulose, a 5% w/v mixture of dimethylformamide and lithium chloride (TAT, Iran) was used. Nano-particle hydroxyl apatite (*n*-HA provided from CAM implants, Netherland), initiator of potassium persulfate (BDH Chemicals, UK), and a crosslinker of *N*,*N*″ – methylene-bis-acrylamide (Aldrich) were applied. Acrylamide (Merck) as a monomer and TiO_2 with <25 nm particle size and 45–55 m^2/g surface area, Merck) as an auxiliary component were used. For preparing PBS solution with pH of 7.4, four components including sodium chloride, potassium chloride, sodium hydrogen phosphate, and di-potassium hydrogen were used as received from TAT, Iran.

2.2. 3D nanocomposite scaffolds synthesis

To prepare the nanocomposite scaffolds, 0.8 g of cellulose was dissolved in a solution of 3 g lithium chloride and 60 mL of dimethylformamide. The solving process was carried out in flask placed on argon gas at 70 °C. Afterwards, *n*-HA was added to the solution and stirred gently. 0.06 g of potassium persulfate as an initiator was added to the solution. For the next step, 1.2 g of acrylamide and 0.03 g of its crosslinker, *N*,*N*″-methylene-bis-acrylamide were added to the solution gently. After 5 min, different amounts of TiO_2 were added to the solution. The mixture was exposed to the argon gas and was kept at 70 °C. The graft polymerization process of acrylamide and cellulose was completed after 3 h. The unwanted reagent, monomers and oligomers were completely removed from the as-grown polymer by dispersing in distilled water. Finally, the powders were filtrated and dried at 50 °C overnight. To prepare the scaffolds, nanocomposite powders were dispersed in a diluted acetic acid solution (4%) and stirred at room temperature for 72 h. After sonication for 90 s, the solutions were placed into plastic molds and kept in a freezer at −20 °C for 24 h. Finally, the solidified mixtures were transferred into a lyophilizer (FD-10, Pishtaz Engineering Co., IRAN) and then freezed up to −56 °C (vacuum 6.2 mbar) for 8 h and then warmed to −5 °C (1.4 mbar) for 6 h and to 24 °C (0.98 mbar) for 2 days in the main drying phase. Using this method, five different samples containing 0, 1, 2, 3 and 4 g of TiO_2, namely S0, S1, S2, S3 and S4, respectively were synthesized.

2.3. 3D nanocomposite scaffolds characterization

SEM instrument was employed to investigate the morphological properties of the samples. Also, the chemical composition of the nanocomposite scaffolds was studied by SEM-EDS analyses. FTIR spectrometer (Perkin-Elmer 8700, Japan) was used to verify the synthesis of cellulose-graft-polyacryliamide/hydroxyapatite/TiO_2 nanocomposite powder. The FTIR experiment carried out in the range of 500–4000 cm^{-1}. To analyze the scaffolds major phases, an X-rays diffractometer (EQuiox 300, France) was run by using Cu-kα radiation (1.54 Å) with 2θ range of 0–70°. The voltage set at 40 kV and the applied current was 30 mA. To study the mechanical property of the samples, a SANTAM compression test instrument (ASTM D5024-95, Iran) was used. The loading rate was 0.5 mm/min. Scaffolds were prepared as cylinders with 5 mm of diameter and 10 mm of height. In addition, the elastic modulus and compressive strength of the 3D scaffolds were calculated from their stress-strain curves.

2.4. 3D nanocomposite scaffolds porosity determination

To determine the porosity percentage of the nanocomposite scaffolds, a liquid displacement method was used [22]. The first step of this method was soaking 0.5 g of a sample in a graduated cylinder of water. After loading of all sample pores, the scaffold was removed from the water. The following equation was used to calculate the porosity percentage of the scaffold:

$$\text{Porosity}(\%) = \frac{V1 - V3}{V2 - V3} \times 100 \quad (1)$$

Where V_1 is the volume of water before sample replacement, V_2 is the water volume after sample immersion, and V_3 is the volume of water after removing the water loaded sample from the graduated liquid cylinder.

To calculate the pore size of the samples, the cross-sectional images of the nanocomposites were investigated., By using 10 different SEM images from various regions of nanocomposite scaffold the dimension of more than 50 pores were determined and the normalized to the average.

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