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Green synthesis of degradable conductive thermosensitive oligopyrrole/chitosan hydrogel intended for cartilage tissue engineering

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ABSTRACT

Electroactive scaffolds containing conductive polymers can promote tissue repair and regeneration. However, these polymers are non-degradable and cannot be removed from body. To overcome this limitation of conductive polymers, we developed a novel injectable electroactive hydrogel containing pyrrole oligomers which possessed the unique properties of being both electrically conductive and biodegradable. First, pyrrole oligomers were synthesized via chemical polymerization and were found to be amorphous with a non-globular morphology. Then, three different compositions of injectable chitosan/beta glycerophosphate hydrogels containing different concentrations of pyrrole oligomers were synthesized and characterized for chemical structure, morphology, conductivity, swelling ratio, *In vitro* biodegradation and gelation time. An increase in oligopyrrole content resulted in decreased pore size, and increased gelation time, swelling ratio, conductivity and degradation time. Among all the hydrogel compositions, the sample with pyrrole oligomer:chitosan ratio of 0.1 (w/w) showed the most prominent biodegradability, biocompatibility, electro-activity, swelling ratio and pore size values and was chosen as the optimal electroactive hydrogel composition in this work.

1. Introduction

In the last decades, tissue engineering has gained a great attention as a promising approach for tissue repair and regeneration. Three important constituents of cells, biodegradable scaffolds and bioactive factors are needed to be combined together to provide an effective tissue engineering platform. Scaffolds must resemble a mimicking structure of extracellular matrix, indeed. In other word, they should be biocompatible, biodegradable and three-dimensional substrates which support cell adhesion, proliferation and differentiation. In recent years, a multitude of different scaffolds such as hydrogels, non-woven meshes, nanofibers and decellularized matrix scaffolds have been developed for tissue engineering [1–3]. Hydrogel scaffolds are among ideal extracellular matrix substitutes for tissue regeneration, because of their porous structure with interconnected pores which provides nutrient and metabolite exchange, cell migration, and encapsulation of cells or growth factors. Among different hydrogel scaffolds, *in-situ* injectable hydrogelsor thermosensitive hydrogels are promising [4] because they can easily be administered and match any shape of injured tissue and promote tissue repair by a minimal invasive method [2,3]. These hydrogels are flowable aqueous solutions before administration, but after injection, they rapidly transform into gel under physiological condition, and do not need any extra chemical treatment. Therefore, they are especially attractive in biomedical applications [5,6].

Chitosan (CS) is a hydrogel polymer which has good biodegradability, biocompatibility, immunological and antibacterial properties. CS has been widely used for controlled drug delivery, gene delivery, cell culture and tissue engineering [7,8].

Along the same lines, electrical stimulation affects a wide range of cellular activities and tissue regeneration [9–12]. Electroac-

Abbreviations: CS, chitosan; BGP, β-glycerophosphate; OP, oligopyyrole; CS-BGP, composite hydrogel of chitosan/β-glycerophosphate; CS-BGP-OP, composite hydrogel of chitosan/β-glycerophosphate/oligopyrrole.

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tive hydrogels are polymeric blends or co-networks that combine electroactive polymers with highly hydrated hydrogels. These polymeric materials indicate the combination of the perpetual properties of their elements [13]. Thus, electroactive polymers, such as polypyrrole have been used in preparation of numerus tissue engineering scaffolds [1,12,14–16]; however, their inability todegrade is one of their major limitations for tissue engineering applications. To overcome this drawback, some solutions have put forwarded. One method, as we applied in this work, is using the Oligomers instead of polymers. They have well-defined structures, good solubility, high flexibility during either synthesis or processing, and an electroactivity similar to that of their corresponding conducting polymers. In addition, the oligomers would be consumed by macrophages, and subsequently cleared by the kidney which eliminate the need for surgical removal of scaffold [1]. Therefore, here we would attain degradable and electroactive hydrogels by adding pyrrole oligomers to CS. Basically, degradation of the hydrogels would be consumed by macrophages and reducing the long-term adverse response. CS-polypyyrole scaffolds have been synthesized before [17,18] but there is no report on preparation of oligopyrrole-CS scaffold. Therefore, in the present study we aimed to develop a novel biodegradable, electroactive, and injectable composite hydrogel intended for cartilage tissue regeneration by CS and oligopyrrole. The novel and linear oligomers were prepared via a simple procedure using Ionic liquid as solving medium.The properties of pyrrole oligomer, formation process, electrical conductivity, swelling behavior, biocompatibility and biodegradability of the hydrogels were investigated in this paper.

2. Material and methods

2.1. Materials

Pyrrole monomer (98% pure) was purchased from Merck; Medium molecular weight chitosan powder (75–85% deacetylated, 200–800 cP, 1 wt.% in 1% acetic acid) and β-glycerophosphate were obtained from Sigma Aldrich. All other reagents were of analytical grade and used as received.

2.2. Synthesis of pyrrole oligomers

The oligopyrrole was prepared via chemical polymerization method. First, Pyrrole solution (1 M) in distilled water was prepared, then imidazolium based ionic liquid, synthesized by Kowsari et al. [19], was added to the solution. The solution was stirred till became transparent, then $NaClO_4$ (10% W/W) were added to the solution. Ammonium peroxy disulfatewas used as oxidant at the ratio of 1:0.065 (monomer: oxidant). Both solution were cooled down to 10 °C. Then oxidant solution was added dropwise to pyrrole solution under stirring and the reaction was continued for five minutes. In order to stop the polymerization reaction, the dark green solution of oligopyrrole was frozen for 24 h. Finally, the oligomer powder was washed with distilled water several times to remove any impurities and was dried for 2 days at room temperature [20,21].

2.3. Preparation of electroactive thermosensitive chitosan-based hydrogels

Chitosan powder was first sterilized by autoclaving at 126 °C for 20 min. Then chitosan solution (1.5% w/vin acetic acid)and β-glycerophosphate solution (10% w/v) were prepared and chilled in an ice bath for 15 min β-glycerophosphate solution were sterilized using a 0.2 μm filter. Then, four different compositions of chitosan (CS) and oligopyrrole (OP) were prepared as presented in Table 1. Finally, the β-glycerophosphate solution was added dropwise to the CS-OP solutions under stirring and the resulting mixtures were stirred for another 10 min.

Table 1
Oligopyrrole content of different electroactive hydrogels.

samples	oligopyrrole:chitosan ratio (w/w)
CS-BGP	0
CS-BGP-OP1	0.05
CS-BGP-OP2	0.1
CS-BGP-OP3	0.15

2.4. Characterization of pyrrole oligomers

2.4.1. Structural characterization

Infrared spectroscopy and Ultraviolet-visible spectroscopy (UV–vis) were performed using an Nicolet nexuses 670 equipment and a Carry 100 conc spectrophotometer [20]. The oligomer was characterized by XRD using EQuniox 3000.The XRD patterns were recorded between $2\theta = 10^\circ$ to 40° The X-ray diffractometer used CuK α radiation of λ = 1.541874 Å generated at 40 kV/30 mA.

2.4.2. Morphological observation

Surface morphology of oligopyrrole powder was observed using a AIS2300C scanning electron microscope (SEM).

2.4.3. Conductivity measurement

The conductivity was measured via two probe KEITHLEY method in the voltage range of 0–12 V.

2.4.4. Solubility

Solubility of the oligopyrrole in different organic solvents including: *N*,*N*-dimethylformamide (DMF), tetrahydrofuran (THF), DMSO, Chloroform, PBS, DMSO, Water, Hydrochloric acid (1% N), and Acetic acid (1% v/v), were examined. The solutions were obtained after 5 min of ultrasonic treatment of 50 mg of oligomer powder in 1 mL of the organic solvent.

2.5. Characterization methods of hydrogels

2.5.1. pH measurement

The pH of ice cold CS-BGP-OP solutions and extraction medium were measured using an electronic pH meter (Vantakool digital pH meter).

2.5.2. Morphology observation

Surface morphology of the freeze-dried hydrogels was monitored using scanning electron microscopy (AIS2300c, Korean). Before examination, the surface of the hydrogels was coated with a conductive gold layer [3].

2.5.3. Structural characterization

The structural characteristics of freeze dried samples were determined using XRD and FTIR analyses.X-ray diffraction pattern of sample powder were obtained using a EQuniox 3000 instrument, CuKα radiation of λ = 1.541874 Å generated at 40 kV/30 mA. The XRD patterns were recorded between $2\theta = 5^\circ$–118°. The FTIR spectra were obtained using a Nicolet nexuses 670, in the 4000–400 cm^{-1} spectral range with a resolution of 4 cm^{-1} [22].

2.5.4. Conductivity measurement

Before performing the conductivity test, the hydrogels were swollen in deionized water and the excess water on the hydrogel surface was ridden using filter paper. Then, the conductivity of the hydrogels was measured by two probes KEITHLEY [3].

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