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Fabrication and characterization of a glucose-sensitive antibacterial chitosan–polyethylene oxide hydrogel

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ABSTRACT

A novel glucose-sensitive chitosan–polyethylene oxide (CS/PEO = 1:0.5–1:2.5) hydrogel with controlled release of metronidazole (MNZ) was obtained by chemical cross-linking and immobilization of glucose oxidase (GO_x). The hydrogel was characterized by Fourier-transformed infrared spectroscopy (FTIR), compressive mechanical test, rheological analysis, cytotoxicity test, and antibacterial test against *Porphyromonas gingivalis*. The study found that the CS–PEO composite hydrogel possessed significantly better mechanical properties and biocompatibility than a single-component hydrogel. This might result from the physical cross-linking and formation of semi-interpenetrating network (semi-IPN). In addition, this novel hydrogel has a self-regulating ability to release MNZ in response to the environmental glucose stimulus. Specifically, it released more drugs at higher glucose concentration, thus can lead to a greater ability to inhibit *P. gingivalis*. This study has demonstrated the glucose-sensitive antibacterial hydrogel has a great potential as a new therapeutic material for treatment or prevention of periodontitis in diabetic patients.

1. Introduction

Diabetes mellitus (DM) has become a challenging problem in today's oral therapy because it can easily cause complications of chronic periodontitis and wound inflammation [1,2]. Therefore, it is very important to effectively monitor and control oral bacterial infection in diabetic patients. Unfortunately, the conventional methods, including routine oral administration of antibiotic (e.g. amoxicillin) and wound rinse, have been proved to be inefficient in many clinical cases [3,4], because of the inflexible drug delivery formulation. Moreover, oral administration of drugs may also cause side effects to diabetic patients [5,6].

Glucose-sensitive hydrogel is considered as an ideal system for site-specific controlled drug delivery mainly because of its novel self-regulating property [7]. Theoretically, this type of materials always has a 'smart sensor' built inside, which could sense and judge the stimulus caused by the change of diabetics' glycemic concentration and further activate (swell or shrink) the special 3D structure of the hydrogel's cross-linked network to control drug release at predetermined rates and predefined time. In addition, this kind of hydrogels also plays a key role to separate the drug from ambient hostile medium before its release [8].

Although glucose-sensitive hydrogels have enormous potentials, their application has been limited largely due to some inherent drawbacks such as poor mechanical conformability, unfavorable biocompatibility, and low controlled drug release ability [9]. In this study, for the first time, we have developed a glucose-sensitive chitosan–polyethylene oxide (CS–PEO) hydrogel with controlled release of metronidazole (MNZ)-a highly effective antibiotic to treat periodontitis [10]. Chitosan (CS) was used because of its good biocompatibility and biodegradability as well as inherent

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antimicrobial properties [11–13]. In addition, polyethylene oxide (PEO), even with the relatively high molecular weight, is regarded as a safe, biocompatible material and an effective enhancement to improve the mechanical and biological properties of composite hydrogels [14–16]. In this study, the experimental hydrogel has been tested for its physicochemical and mechanical properties, biocompatibility, drug release ability in response to different glucose concentrations (GC) and antibacterial activity against *Porphyromonas gingivalis* (*P. gingivalis*), which is regarded as one of the major bacteria to be associated with human periodontitis [17].

2. Materials and methods

2.1. Materials

Chitosan (CS: Molecule weight: 100,000–300,000 Da, practical amine: 7–12%) was purchased from Polysciences, Inc., USA. polyethylene oxide (PEO, Molecule weight: 400,000 Da), glucose oxidase (GO_x, from *Aspergillus niger*, 50KU), glutaraldehyde (GA, 25% aqueous solution) and metronidazole (MNZ) were all purchased from Sigma–Aldrich, USA. Osteoblasts used in cells culture study were derived from rats. For antibacterial activity, *P. gingivalis* ATCC 33277 was grown in Trypticase soy broth (BBL™, BD Company, USA), supplemented with 0.1 g (per 100 ml) yeast extract (BD Company, USA), 0.5 mg hemin (Sigma–Aldrich, USA) and 0.1 mg vitamin K (Menadione, Sigma–Aldrich, USA).

2.2. Fabrication of hydrogel

2.2.1. Preparation of CS–PEO film

Pre-weighted CS was dissolved in 0.5 M acetic acid aqueous solution to prepare 1.5% weight/volume (w/v) CS solution. After 2 h stirring, PEO was added to form the blend with pre-determined weight ratio (CS/PEO = 1:0.5–1:2.5). Then, the mixture was stirred constantly until these two components completely dissolved and formed homogeneous solution. After that, the solution was poured onto glass surface and dried in vacuum oven at 60 °C. The CS–PEO films with thickness of 0.8 mm–1.0 mm were obtained after 24 h drying.

2.2.2. Preparation of cross-linked CS–PEO hydrogel film

The preparation of cross-linked CS–PEO hydrogel film was carried out similarly as described above. The only difference was that after obtaining the homogeneous CS–PEO blend, a certain amount of cross-linking agent (GA) was slowly dripped into this solution under constant stirring. Then, the solution was transferred onto a glass surface to form the CS–PEO hydrogel at room temperature. The resulting product was obtained by putting the hydrogel into the vacuum oven at 60 °C overnight.

A simple stability experiment was conducted to investigate the cross-linking reaction between GA and CS. The CS–PEO film and CS–PEO hydrogel film cross-linked by GA were immersed in acetic acid aqueous for 24 h, respectively. The photos at 4 different time points including 0, 1, 6, and 24 h were recorded. In addition, the possible cross-linking reaction between PEO and GA was also investigated using a similar method. First, 2 wt% PEO aqueous solution was prepared. After that, 2.5% (v/v) GA was slowly dripped into this solution under constant stirring. The photos of this blend at the beginning (0 h) and after 24 h were recorded to show if there was chemical cross-linking reaction between PEO and GA.

2.2.3. Preparation of glucose-sensitive CS–PEO hydrogel loaded with MNZ

First, the CS–PEO blend was obtained using the method mentioned in Section 2.2.1. Then, 0.5% (w/v) MNZ was added into this solution. After the drug was completely dissolved, cross-linking agent GA (0.5–2.5%, v/v) was slowly added into this mixture under constant stirring. Next, the solution was poured into a home-made cylinder mode (diameter: 25 mm, height: 20 mm) to form the hydrogel at room temperature. The hydrogel was washed three times with deionized water to remove extra GA and acetic acid. Finally, the samples were immersed in GO_x solution (phosphate buffer pH = 5) for 24 h and kept at −40 °C [18].

2.3. Characterization of hydrogel

2.3.1. Fourier-transformed infrared spectroscopy (FTIR) analysis

FTIR analysis was carried out using Thermo-Nicolet Nexus 670 spectrometer (Thermo Electron, USA) to identify the chemical transformation of our obtained CS–PEO film and cross-linked CS–PEO hydrogel film, respectively. All the specimens were recorded from 400 to 4000 cm^{-1} through 64 scans with resolution of 8 cm^{-1}.

2.3.2. Solubility test

CS–PEO hydrogel film with the CS/PEO weight ratio of 1:1.5 was prepared and dried using the method described in Section 2.2.2. After that, each dry film was adjusted to the same mass (1 g) and then all the films were divided into three groups: the first group was immersed in phosphate buffer (pH = 7.4) and then placed into a shaker with continuously shaking at 107 cycles per minute under 37 °C (type I); the second group was immersed in the same phosphate buffer and continuously agitated at 400 rpm by magnetic stirring under 37 °C (type II); the final group was immersed in 50% aqueous methanol solution (v/v) with the constant 400 rpm magnetic stirring under 60 °C (type III). After 24 h stirring, all the samples were taken out, dried in oven and measured for the dry weight. Each type of samples had three replicates.

2.3.3. Compressive mechanical test

At the beginning of this test, the cylindrical samples were prepared with the diameter 25 mm and height 20 mm (n = 8). The test was conducted using the Instron 5566 universal testing machine (Instron Co., St Paul, USA) at crosshead speed of 0.5 mm/min.

2.3.4. Rheological analysis

The dynamic rheological properties of our hydrogel blend were determined using the ARES rheometer (TA Instruments-Waters LLC, New Castle, DE). 300 μl pre-gel blend (CS, PEO and GA at pre-determined ratio listed in Table 1) was quickly dripped onto the peltier plate. Storage modulus (G′), loss modulus (G″) were

Table 1
G'_{2500}, G''_{2500}, and T_{gel} values of chitosan–PEO samples with different weight ratio or GA volume recorded by rheology analysis.

CS:PEO (w/w)	GA* (μl)	G'_{2500} (Pa)	G''_{2500} (Pa)	T_{gel} (s)
1:1.5	50	28.34 ± 2.49	6.26 ± 1.61	1927.3 ± 70.42
1:1.5	100	37.62 ± 2.71	5.43 ± 0.87	1174.7 ± 82.48
1:1.5	150	81.76 ± 6.43	8.53 ± 2.31	672 ± 50.71
1:1.5	200	120.88 ± 5.73	12.46 ± 4.38	320.7 ± 39.58
1:1.5	250	275.10 ± 22.25	29.83 ± 6.27	198.7 ± 10.26
1:0	150	32.90 ± 3.49	9.20 ± 1.20	925 ± 53.29
1:0.5	150	48.29 ± 6.73	8.07 ± 2.51	840.7 ± 68.61
1:1	150	67.89 ± 4.88	7.52 ± 1.71	739.3 ± 39.14
1:2	150	90.62 ± 7.92	11.64 ± 3.01	608.3 ± 40.79
1:2.5	150	96.74 ± 5.64	19.25 ± 4.92	579.7 ± 29.81

GA*: the volume of GA in 10 ml CS/PEO solution.
G'_{2500} and G''_{2500}: the storage modulus and loss modulus of CS/PEO gels at 2500 s.
T_{gel}: time for initiation of gelation is indicated as the time when $G' = G''$.