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Halloysite nanotube nanocomposite hydrogels with tunable mechanical properties and drug release behavior

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ABSTRACT

Nanocomposite hydrogels with tunable mechanical properties and drug release behavior were prepared by halloysite nanotube (HNT), oligo(trimethylene carbonate)–poly(ethylene glycol)–oligo(trimethylene carbonate) diacrylate (TPT), and alginate sodium (AG). The mechanical properties of the hydrogel were greatly enhanced by the addition of small amount of HNT (2 wt%). The drug release behavior of the hydrogels was examined using bovine serum albumin (BSA) as model drug, and TPT hydrogel showed a fickcian diffusion controlled release mechanism, while the BSA transport mechanism in TPT-HNT, TPT-AG, and TPT-AG-HNT hydrogels were anomalous transport. The cytocompatibility of the hydrogel were verified by the MTT assay, and the hydrogel showed comparable cytocompatibility with the widely recognized poly(ethylene glycol) hydrogel.

1. Introduction

Nanocomposite hydrogels (NC gels), which are three-dimensional cross-linked polymer network filled with nanoparticles/nanostructures, have been applied in biomedical fields such as tissue engineering [1], drug delivery system [2], and biosensors [3]. NC gels are commonly prepared by incorporating nanofillers, such as exfoliated clay sheets and discrete inorganic nanoparticles into a hydrogel matrix [4]. The nanofiller endue the NC gels with excellent mechanical property, which in turn overcome the limitations of conventional chemically crosslinked hydrogels [5,6].

Besides the commonly used clay nanofillers such as laponite and montmorillonite [6], halloysite nanotube (HNT, $Al_2Si_2O_5(OH)_4{\cdot}nH_2O$) has been frequently as nanofiller used in the rubber industry. HNT has a characteristic hollow tubular structure with a longitude length of 1000 nm, an outer diameter of 50 nm, and a lumen of 15 nm. In aqueous solution, HNT has a positively charged inner surface and negatively charge outer surface, and can form electrostatic interaction with various kinds of positively/negatively charged molecules [7]. Due to its unique tubular structure and properties, HNT have been found great potentials as drug/protein delivery [8], anticorrosion coatings [9], and nanoreactors [10]. HNT can also been used as nanofiller for polymer nanocomposite with enhanced mechanical properties [11,12]. As for the HNT NC gels, chitosan-*g*-poly(acrylic acid) [13], polyacrylamide [14], and 2-acrylamido-2-methyl propane sulfonic acid acrylamide copolymer [15] have been explored as the polymer matrix; however, the application of such HNT NC gels as biomaterials has been retarded by the poor biodegradability and biocompatibility of the polymer matrix.

Biodegradable hydrogel from oligo(trimethylene carbonate)–poly(ethylene glycol)–oligo(trimethylene carbonate) diacrylate (TPT) precursor was reported to exhibit excellent toughness, enhanced adhesion, and spreading of stem cells on the surface of hydrogels [16]. In this work, single network NC gels were first prepared by photopolymerization of TPT in the presence of HNT. In addition, interpenetrating network (IPN) NC gels were prepared by combining photopolymerization of TPT and ionic crosslink of alginate (AG) in the presence of HNT. AG is a polysaccharide isolated from the natural brown algae, and forms hydrogel *via* ionic crosslinking with Ca^{2+} ion [17]. In AG hydrogel, the reversible ionic crosslinking may break up to dissipate energy when a stress is applied and recover or re-heal under relaxed state [18]. This unique character of AG hydrogel may be harnessed to construct IPN consist of both ionic cross-linked and chemical cross-linked moieties, resulting in improved mechanical properties. The swelling, mechanical, and drug release properties of the NC gels were examined. And the cell cytotoxicity of the NC gels was investigated in details.

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2. Experimental part

2.1. Materials

PEG with molecular weight 6000 g/mol (PEG6 K) was purchased from Shanghai Chemical Reagent Co., (China) and purified by precipitation in diethyl ether. Trimethylene carbonate (TMC) was synthesized according to literature [18], recrystallized from anhydrous ethyl acetate for three times, and vacuum-dried before use. HNT was a gift from Natural Nano Company and used after being dried overnight at 120 °C. Stannous 2-ethylhexanoate (99%, Sigma–Aldrich) was dissolved in fresh distilled anhydrous toluene. Triethylamine was purified according to standard procedure. Acryloyl chloride was re-distilled in the presence of hydroquinone. Dichloromethane (CH_2Cl_2) was refluxed over calcium cholride ($CaCl_2$) and distilled before use. All the other reagents were used as received.

2.2. Synthesis of TPT precursor

TPT was synthesized according to the literature [16]. TMC, PEG, and 0.1% (mol/mol) $Sn(Oct)_2$ was added into a silanized glass ampoule and vacuum sealed, then was immersed in an oil bath at 120 °C for 72 h. After the reaction was completed, the product was dissolved in CH_2Cl_2, precipitated in ethyl ether, filtered, and dried. Then 1 mmol of the obtained hydroxyl-terminated oligo(trimethylene carbonate)–poly(ethylene glycol)–oligo(trimethylene carbonate) (OTMC–PEG–OTMC) and 10 mmol of triethylamine was dissolved in 150 mL of CH_2Cl_2 and cooled to 0 °C in an ice bath. 10 mmol of acryloyl chloride in 15 mL of CH_2Cl_2 was added drop wise and then the reacted for 72 h at 40 °C. The mixture was concentrated and precipitated in diethyl ether. After precipitated for three times, the precipitate was collected and dialyzed against distilled water. Then the product was freeze-dried and the chemical structure of the product was confirmed by ^1H NMR. ^1H NMR ($CDCl_3$, δ ppm): 2.0 (—OCH_2—CH_2—OCH_2—, 2H), 3.6 (—O—CH_2—CH_2—, 4H), 4.2 (—O—CH_2—CH_2—CH_2—O—, 4H), 5.8–6.4 (—CH=CH_2, 3H).

2.3. Preparation of nanocomposite hydrogels

Single network NC gels were prepared by *in situ* photopolymerization. A mixture of TPT (10% w/v) and HNT (*x*% w/v; $x = 0, 1, 2, 4$) was dispersed in phosphate buffer solution (PBS, pH = 7.4) and degassed under vacuum for 6 h. Then Irgacure 2959 photoinitiator (0.05% w/v) was added and the reaction mixture was then transferred to a cylindrical mould (diameter 6 mm, height 4 mm) and exposed to 365 nm UV at 4.5 mW cm^{-2} for 40 min. The gels were named as TPT-HNT_x (*x* represents the weight percentage of halloysite in the precursor solution).

The IPN NC gels were prepared by two steps: the mixture of TPT (10% w/v), AG (1% w/v), HNT (*x*% w/v; $x = 0, 1, 2, 4$) in PBS was degassed under vacuum for 6 h. Then Irgacure 2959 (0.05% w/v) was added and the reaction mixture was then transferred to a cylindrical mould (diameter 6 mm, height 4 mm). The photopolymerization occurred by exposing the mould under 365 nm UV at 4.5 mW cm^{-2} for 40 min. After that the hydrogel was removed from the mould and immersed in aqueous solution of $CaCl_2$ (5 wt%) for 24 h. The hydrogel was named as TPT-AG-HNT_x.

2.4. Interior morphology

Fully swollen hydrogel was frozen in liquid nitrogen and then lyophilized for 48 h. The lyophilized hydrogel was fractured carefully in liquid nitrogen, sputter-coated with gold; and the interior morphology of hydrogels was examined on a scanning electron microscope (SEM, FEI-QUANTA 200, Holland).

2.5. Swelling behavior

Hydrogel was immersed in PBS and swelled to equilibrium; the sample was weighed after wiping off the water on the surface (W_s) and then dried under vacuum (W_d). Three samples for each type of hydrogel were examined, and the equilibrium swelling ratio (ESR) was calculated by the equation: $ESR = W_s/W_d$.

2.6. Compression test

Hydrogels were immersed in PBS for 48 h to reach swelling equilibrium before mechanical test. Instron 3342 Universal Testing System (Instron, Norwood, MA, USA) equipped with a Model 2519-104 force transducer was used to perform the compression tests of the hydrogel, and the data was analyzed using BlueHill® software. The maximum force load was 500 N and the compression rate was 0.5 mm min^{-1}. The compressive modulus was calculated from the slope of the initial linear region of stress-strain curve according to Hookean model. Toughness was calculated from the area integration of stress–strain curve until to fracture points. Three measurements were taken for each sample.

2.7. Drug release behavior

Bovine serum albumin (BSA) was used as the model drug to examine the drug release behaivor of the hydrogels. The hydrogel–BSA construct was fabricated by *in situ* polymerization after adding BSA (2 wt%) to precursor solution. The hydrogel–BSA construct was carefully transferred to 10 mL PBS solution in a 30 mL conical tube and incubated in a water bath at 37 °C. At predetermined time intervals, 0.5 mL of the solution was taken for analysis and the tube was replenished with 0.5 mL of PBS solution. The BSA concentration in each solution was determined using the Bio-Rad protein assay [19]. Measurements were done in triplicates. The accumulative BSA mass released at time *t* (M_t) was calculated with the following formula:

$$M_t = C_t V + \sum C_{t-1} V_s$$

where C_t is the concentration of BSA in the release solution at time *t*, *V* is the total volume of release solution (10 mL), and V_s is the sample volume (0.5 mL).

2.8. MTT assay

Swine cartilage chondrocytes (SCCs) were used to evaluate the cytocompatibility of the hydrogels. SCCs were isolated and cultured according to established protocol [20]. Chondrocyte culture medium (CCM) (Dulbecco's Modified Eagles Medium (DMEM high glucose, Hyclone) supplemented with 10 mM HEPES, 0.1 mM non-essential amino acids, 0.4 mM proline, 50 mg/L Vitamin C, 10% fetal bovine serum, and 1% penicillin–streptomycin (PenStrep, Invitrogen)) was used in the isolation, expansion, and following experiments. Hydrogels were fabricated on the bottom of the wells of 24-well plate, and sterilized in 70% ethanol for 24 h, followed by washing in PBS with 2% PenStrep for another 24 h. Passage 1 SCCs with density of 7.5×10^3 cells/well in 200 μL of CCM was seeded on top of the hydrogel and incubated at 37 °C in 5% CO_2 atmosphere for 24 h. The culture medium was removed, and 200 μL of DMEM and 30 μL of MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide, 0.5 mg mL^{-1}) in PBS solution were added into each well and incubated for 4 h. After removal of supernatant and hydrogels, 500 μL of dimethyl sulfoxide was added and