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Synthesis and mechanical properties of double cross-linked gelatin-graphene oxide hydrogels

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ABSTRACT

Gelatin is an interesting biological macromolecule for biomedical applications. Here, double cross-linked gelatin nanocomposite hydrogels with incorporation of graphene oxide (GO) were synthesized in one pot using glutaraldehyde (GTA) and GTA-grafted GO as double chemical cross-linkers. The nanocomposite hydrogels, in contrast to the neat gelatin hydrogel, exhibited significant increases in mechanical properties by up to 288% in compressive strength, 195% in compressive modulus, 267% in compressive fracture energy and 160% shear storage modulus with the optimal GO concentration. Fourier transform infrared spectroscopy, scanning electron microscopy and swelling tests were implemented to characterize the nanocomposite hydrogels.

1. Introduction

Hydrogels, which are soft and comprise a high proportion of water, have been attracting a great deal of attention in the past few decades. They have been widely studied for biomedical applications including tissue engineering and drug delivery because of their similarities to extracellular matrices, excellent biocompatibility, and inherent cellular interaction capability [1–3].

Gelatin is a natural polymer, derived from animal collagen with excellent biocompatibility, affinity to proteins, and biodegradability, as well as low cost [4]. The numerous studies on the gelatin based hydrogels have been reported for biomedical usages such as drug delivery, tissue engineering, gene therapy and biosensing [5,6]. Physical gelatin hydrogels can be obtained by cooling down pre-heated gelatin solutions to below the gelation temperature of ~25 °C (which varies subject to the type of gelatin, concentration, *etc.*) to trigger the conformational transition from coil to triple helices [7]; however, these hydrogels have poor mechanical properties and low temperature resistance [8,9]. Chemical cross-linking could improve their strength and tune biodegradation rate, but at sacrifice of ductility [5]. Further mechanical improvement is in demand to overcome the limitation for neat gelatin hydrogels to be used in load-bearing applications. There are several approaches to achieving hydrogels with high mechanical performance, for example, copolymer hydrogels, double-network hydrogels and polymer nanocomposite hydrogels [10,11]. Graphene and its derivatives have been considered as effective nanofillers for composite materials [12].

Since it was first reported in 2004, graphene has drawn substantial attention of scientists due to its intriguing properties [13,14]. Graphene oxide (GO), a graphene derivative, exhibits a large surface area, a high aspect ratio, and exceptional mechanical properties, while bearing plenty of oxygen-containing groups on their monolayer two-dimensional sheets [13]. GO can be readily exfoliated and stably dispersed as single-layer sheets in an aqueous solution owing to its hydrophilic oxygen-containing groups, which is beneficial to prepare mechanically strong nanocomposite hydrogels. These oxygen-containing functional groups enabled GO nanosheets to associate with hydrophilic polymer matrices by physical and chemical interactions to enhance the mechanical performance significantly. For example, the addition of ~5 wt% GO can dramatically increase the compressive strength of poly(N-isopropylacrylamide) (PNIPAM) hydrogels by 3-fold, owing to the interpenetrating network structure comprised of chemically cross-linked PNIPAM and connected GO sheets, as well as the strong intermolecular interaction (hydrogen-bonding) between PNIPAM chains and GO sheets [15].

Through physical and chemical interactions, gelatin (as primary components) and GO formed interesting nanocomposites and nanocomposite hydrogels [16–21]. A strong and bioactive gelatin-GO nanocomposite was reported showing 65%, 84% and 158% increases in the Young's modulus, tensile strength and fracture energy of gelatin, respectively, with the addition of 1 wt% GO [16]. Self-assembled gelatin-GO nanocomposite hydrogels [17]

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were reported possessing storage moduli of 54–115 kPa at a water content of 98.0–98.5 wt%, and gelatin-reduced graphene oxide nanocomposite hydrogels [18] exhibited storage moduli of 64–172 kPa at a water content of 98.0–98.8 wt%. These hydrogels were formed without an organic cross-linking agent, where graphene was used as a physical cross-linker in the former and a chemical cross-linker mainly in the latter. Poly(acrylic acid) (PAA)-gelatin-GO nanocomposite hydrogels were reported [19], which were synthesized by *in situ* polymerization of acrylic acid monomer in the presence of GO and gelatin. The hydrogels exhibited a 71% increase in tensile strength (150–250 kPa) when containing 90 wt% water, by the addition of 0.3 wt% GO [19]. The same group reported that PAA-gelatin-GO nanocomposite hydrogels with different compositions showed a high compressive strength (7–26 MPa) at a water content of 29–51 wt% [20]. Gelatin methacrylate-GO composite hydrogels, which exhibited a fracture strength in compression of 91–501 kPa with 94.3–94.5 wt% water, were also reported [21].

Using *N,N*-methylenebisacrylamide (BIS) as the chemical cross-linker in the presence of GO sheets, tough GO-based polyacrylamide (PAM) composite hydrogels were synthesized by *in situ* polymerization of acrylamide monomers [22]. By incorporating GO sheets, the hydrogels were double cross-linked with the predominant cross-linking contribution from BIS and the additional contribution from multifunctional cross-linking agents of GO sheets, imparting high toughness and a tensile strength of 30 kPa. Actuator materials based on PAM-GO composite hydrogels were prepared by a similar method by others [23]. As the authors suggested PAM macromolecules were grafted onto the GO nanosheets during polymerization, so the double cross-linked structure was obtained in the hydrogels which were cross-linked by BIS and GO nanosheets. The good dispersion of the GO nanosheets in the composite hydrogels, resulting from some PAM macromolecules grafted onto the GO nanosheets, endows significant improvement of their mechanical properties, *i.e.*, a 6-fold increase in the compressive strength with 1 wt% GO content in comparison to that of neat PAM hydrogel. These imply double cross-linking polymer hydrogels could be an effective strategy to develop polymer hydrogels with high mechanical properties, like in the case of double cross-linked polymer blend hydrogels [24].

In this work, we developed novel double cross-linked gelatin-GO nanocomposite hydrogels with various weight ratios prepared using cross-linking agents of glutaraldehyde (GTA) and GTA-grafted GO. The nanocomposite hydrogels were characterized with different techniques and discussed with regards to their chemical structures, morphologies, and mechanical properties in detail.

2. Experimental section

2.1. Materials

Gelatin (type B, BioReagent, bloom strength 225, number average molecular weight: 50,000), graphite powder (size < 20 μm), potassium permanganate, sodium nitrate, hydrogen peroxide (30%), concentrated sulphuric acid (98%), hydrochloric acid (35%), glutaraldehyde (50%), and glycine (1 mol L^{-1}) were all obtained from Sigma-Aldrich Corporation.

2.2. Preparation of gelatin-GO hydrogels

Graphene oxide was prepared from pristine graphite powder by a modified Hummers' method [25], and purified and freeze-dried [17]. The gelatin-GO nanocomposite hydrogels were synthesized by cross-linking gelatin using GTA in the presence of GO nanosheets. A typical synthesis of the gelatin-GO nanocomposite hydrogel is described as follows. 0.1 mL of aqueous GTA (0.056 g, 0.56 mg mL^{-1}) solution was mixed with 4.9 mL of aqueous GO (0.001 g, 0.2 mg mL^{-1}) suspension under vigorous stirring at 37 °C for 1 h. 0.999 g gelatin was added into 5 mL distilled water and then heated at 60 °C while stirring for 1 h. It was then added into the water mixture of GTA and GO under stirring for 3 min before it was cast into a cylindrical mould. The mixture was kept at room temperature (20 °C) for 24 h to complete the gelation of gelatin through chemical cross-linking. In these hydrogels, GO concentration increased from 0 to 5 mg mL^{-1}, while the weight ratio of both gelatin and GO to water was kept constant at 1:10. After gelation, the remaining aldehyde groups from GTA were blocked by immersing the bulk hydrogel into a glycine solution (100 mM) at 37 °C for 1 h, and following triple wash in distilled water. The hydrogels were named as GHn, in which n denoted ten times of the concentration (mg mL^{-1}) of GO in the final hydrogel.

2.3. Structure characterization

Gelatin, GO and gelatin-GO hydrogels were analyzed by Fourier transform infrared spectroscopy (FT-IR) (Perkin Elmer Spectrum 100, a resolution of 4.0 cm^{-1}). An aqueous GO suspension and the nanocomposite hydrogels were frozen at −20 °C, and then dried under vacuum at −10 °C for two days by using a FreeZone Triad Freeze Dry System (Labconco Corporation). As a control sample to study the interactions between GO and GTA, the GTA-modified GO was also investigated by FT-IR. This sample was prepared using the same procedure as for nanocomposite hydrogel, namely mixing the same amount of GO suspension and GTA solution at 37 °C for 1 h, followed by dialysis for 3 days to remove the unreacted GTA and then air-dry at room temperature for 3 days. A neat GO suspension was also air-dried and characterized as a control. Morphologies of the gelatin-GO hydrogels were studied by using scanning electron microscopy (SEM). The lyophilized samples were fractured and then fixed on aluminium stubs. Samples were gold coated by using an Emscope SC500A sputter coater before the morphological images were taken under an FEI Inspect F scanning electron microscope. The average pore sizes were calculated (at least 100 pores) by using ImageJ software.

2.4. Compression tests

Uniaxial compression testing was performed using a mechanical testing system (Model TA500, Lloyd Instruments) equipped with a control and analysis software of NEXYGEN. The hydrogel rods (20 mm high and 10 mm in diameter) were compressed at a speed rate of 1 mm min^{-1} using a 50 N load cell. Measurements were performed on 5 replicate samples in each group.

2.5. Rheological measurements

The rheological properties were measured by using a MCR 301 rheometer (Anton Paar). The shear moduli were recorded against angular frequency with a fixed strain of 0.1% (within the linear viscoelastic region) at room temperature. Parallel-plate (diameter 25 mm) geometry was used and the gap distance was fixed (2.0 mm) between the parallel plates.

2.6. Swelling tests

Hydrogel discs ($10 \times 10 \times 2\ mm^3$) were air-dried for one week at room temperature and then submerged in distilled water at room temperature (23 ± 1 °C) for swelling test. The samples were weighed after a week when the weights became constant. Three