



Design and characterization of hybrid hydrogels composed of imogolite fibrous nanotubular clay and hyaluronic acid



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ABSTRACT

Hybrid hydrogels composed of imogolite fibrous nanotube (IG) and hyaluronic acid (HA) were prepared. Hexamethylenediamine (HMDA) was used as a non-toxic chemical cross-linker. The homogeneous dispersion of IG in the HA solution was accomplished by careful pH control, and subsequent chemical cross-linking provided mechanically stable self-standing hybrid hydrogels. The IG/HA hybrid hydrogels were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), swelling test, compression test, oscillatory rheological analysis, and enzymatic degradation test. The IG/HA hybrid hydrogels show better mechanical properties and lower degradation rate than native HA-based hydrogels without IG hybridization because the cross-linking density increases by incorporation of additional IG network into chemically cross-linked HA network.

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1. Introduction

Imogolite is an aluminosilicate fibrous nanotubular clay mineral which has an outer diameter of ca. 2 nm and an inner of ca. 1 nm, and a length of 60 nm to 2 μm, therefore it has a high aspect ratio and specific surface area. Its outer and inner surfaces are composed of aluminol groups (Al-OH) and silanol groups (Si-OH), respectively [1–7]. Both the chemical structures are intrinsically hydrophilic to make imogolite water-dispersible hydrophilic clay nano-tube. Various applications of the imogolite fibrous nanotube (IG) such as nano-building blocks for organic-inorganic hybrid materials [8–12], catalyst [13,14], water absorption [15], and gas storage [16–18] have been proposed. One dimensional nanotubes such as carbon nanotubes, and aluminosilicate halloysite and imogolite nanotubes have been reported to show good biocompatibility, cell adhesion, proliferation, migration and differentiation that is feasible for nanocomposite scaffolds for bone and cartilage tissue engineering [19–25]. However, biomedical applications of the IG-based polymer composites as a drug carrier and tissue engineering scaffolds for regeneration and restoration of damaged tissue are

hardly reported.

Green polymer hybrids that consists of bio-polymers and natural fillers have been proposed for use in reinforced polymer composites and bio-medical application [26–33]. Hyaluronic acid (HA) based hydrogels are good candidate for biomedical applications because hyaluronic acid is a vital component of the extracellular matrix (ECM) of native tissues of human body. HA has a high capacity of water absorption and retention, shock absorbance, wound healing and cell motility [34,35]. But it has too poor mechanical properties and short degradation time in the soft tissues to build the entire function of the cells in the tissues [36,37]. Preparation of HA-based hydrogels with improved mechanical strength and delayed degradation rate has been tackled [38,39]. Especially, carbon nanotube-HA hybrid hydrogels showed profound increased mechanical strength without sacrificing the swelling properties [40]. IG can be a good candidate for replacing the carbon nanotube-HA hybrid hydrogels because the hydrophilicity would provide the well dispersion in aqueous solution. In addition, the biocompatibility is also expected to be an advantage of the natural clay-based hybrid materials. However, HA-based hybrid hydrogels incorporated with inorganic hydrophilic IG have never been reported. Because of the weak interaction of hyaluronic acid and IG, the gelation has never been accomplished without chemical cross-linking. In this paper, a preparation method for the chemically

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cross-linked hybrid hydrogels composed of HA and IG was proposed, and the physicochemical properties including swelling ratio, morphology, mechanical properties, and degradation rate were studied.

2. Experimental

2.1. Materials

IG was synthesized according to the methods reported by Farmer et al. [41] HA ($M_w = 1.6 \times 10^6$ D) was purchased from Sigma-Aldrich, USA. Hexamethylenediamine (HMDA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxybenzotriazole (HOBt), 2-morpholinoethanesulfonic acid (MES), bovine testes hyaluronidase were purchased from Tokyo Chemical Industry, Japan. Sodium hydroxide (NaOH), hydrochloric acid (HCl; 1 N), phosphate buffered saline solution (PBS; 0.01 M, pH 7.2–7.4) were purchased from Wako Pure Chemicals, Japan. All chemicals were used as received.

2.2. Preparation of hydrogels

2.2.1. Preparation of HA-HMDA hydrogels (IG-0)

HA hydrogels were prepared according to a procedure reported by Yeom et al. with slight modification [42]. HMDA was dissolved in 0.01 M MES buffer solution (pH 6.7) containing 0.154 M NaCl. HA powder was dissolved in the HMDA solution, and the concentration was adjusted to 2.0% (w/v). Molar ratio of HMDA to carboxyl groups in HA was 1/1. EDC (4 molar equivalent to HA) and HOBt (2 molar equivalent to HA) were dissolved in deionized water, and added to the HA solution. The solution pH was adjusted to 6.7 by the addition of 1 M HCl, and the solution was stirred vigorously for 10 min. The precursor solution was incubated at 37 °C for 1 h to complete the cross-linking reaction. The as-prepared self-standing hydrogels were used as samples for rheology test and compression mechanical test. The self-standing hydrogels (denoted as IG-0) were dialyzed with PBS solution for 24 h at 37 °C in incubator at a rotation speed of 100 rpm to remove the unreacted reagents, and the purified gels were used for swelling test and enzymatic degradation test.

2.2.2. Preparation of IG/HA-HMDA hybrid hydrogels (IG-1, IG-5, IG-10)

IG was finely dispersed in MES buffer solution at pH 6.7. MES buffer solution with IG contents of 0.02, 0.1, and 0.2% (w/v) were prepared. HA was dissolved in the HMDA dissolved MES buffer solution containing IG, and the HA concentration was adjusted to 2.0% (w/v) to make IG to HA content 1, 5, 10% (w/w). Subsequent cross-linking was performed by the same procedure as hydrogel preparation described in the above section. Resulting cross-linked hybrid hydrogels were denoted as IG-1, IG-5, IG-10 with respect to IG contents.

2.3. Characterization of hydrogels

2.3.1. Measurements

Fourier transform infrared (FTIR) spectra were measured by Spectrum 100 spectrometer (Perkin Elmer Inc., Waltham, MA, USA) using KBr pellets method. IR spectra were recorded by averaging 64 scans between 4000 and 500 cm^{-1} with the resolution of 4 cm^{-1} . Scanning electron microscopy (SEM) observations were conducted by Real Surface View VE7800 (Keyence Co., Ltd, Osaka, Japan) with applied voltage of 5 kV. The cross-section of hydrogels was obtained by splitting freeze-dried hydrogels immediately after freezing by liquid nitrogen. The cross-sectioned samples were

sputter-coated with an osmium layer using HPC-1SW Hollow Cathode Plasma CVD (Shinkuu Device Co., Ltd, Ibaraki, Japan). Compressive mechanical test was carried out by a tensile tester (EZ-Graph, Shimadzu Co., Ltd, Kyoto, Japan) with 50 N load cell at the loading rate of 1 mm/min. The dimension of the samples was cylinder shape with a diameter of 12 mm and a height of 12 mm. Compression tests were performed 6 times for each case of sample at room temperature until the rupture of hydrogels was recorded. The strain was measured from the displacement of the crosshead. Dynamic viscoelasticity was measured by oscillatory rheometer (Physica MCR 101, Anton Paar, Graz, Austria). The dimension of the samples was cylinder shape with a diameter of 16 mm and a height of 2 mm. Parallel plate with a diameter of 20 mm was used and temperature was controlled to 25 °C. Amplitude sweep and frequency sweep tests were performed three times for each case of sample.

2.3.2. Swelling test

Freeze-dried hydrogels were immersed in PBS buffer solution (0.01 M, pH 7.4), and incubated at 37 °C at the rotation speed of 100 rpm to set in vivo environment. Hydrogels were picked up at pre-determined time points and weighed after the removal of excessive surface water. The equilibrium swelling ratio was calculated according to the following equation (1)

$$\text{Swelling Ratio} = (W_s - W_d)/W_s \quad (1)$$

where W_s and W_d are the weights of the hydrogels in swollen and dry state, respectively.

2.3.3. Enzymatic degradation test

Freeze-dried hydrogels were immersed in PBS buffer (pH 7.4) containing hyaluronidase from bovine testes with the concentration of 4 Unit/ml, and incubated at the rotation speed of 100 rpm at 37 °C. At pre-determined time point, hydrogels were picked up from the enzymatic solution, and washed three times to remove the salts and enzyme on the surface of hydrogels. The hydrogels were immersed in ethanol for 2 h and dried for 1 day at room temperature [43]. The percentage of the weight loss was calculated according to equation (2).

$$\text{Weight Loss (\%)} = [(W_i - W_d)/W_i] \times 100\% \quad (2)$$

where W_i is the weight of the initial freeze dried hydrogel and W_d is the weight of the remained dried hydrogel. Enzymatic degradation tests were performed three times for each sample and the weight loss data was summarized as averaged value.

3. Results and discussion

3.1. Fabrication of hybrid hydrogels

IG has an interaction with anionic charged polymers in acidic environment because its isoelectric point is around pH 7 [44]. Cross-linking reaction of HA with HMDA is very sensitive to pH because ester intermediate coupling for amide bond formation is generated around neutral pH and the intermediate is necessary to accomplish stable cross-linking reaction of carboxyl groups [38]. Also, buffer solutions without carboxylic groups have to be chosen for stabilization of pH because carboxylic groups in buffer solution are also involved in the carbodiimide-mediated amidation reaction. To balance the stable dispersion and interaction potential of IG with HA and the efficient carbodiimide-mediated cross-linking reaction, pH of the MES buffer solution was adjusted to 6.7. Surface charge of IG is almost neutral at that pH, whereas HA is net negative.

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