Supporting Information

Degradable and Biocompatible Hydrogels Bearing Hindered Urea Bond

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General

Materials. Thiol and amine end-capped polyethylene glycol was purchased from Layson Bio, Inc., N-t-butyl allylamine was purchased from TCI America (Portland, OR, USA). Anhydrous tetrahydrofuran (THF) was dried by a column packed with alumina. All deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received unless otherwise specified. hMSCs and MSCGM™ Mesenchymal Stem Cell Growth Medium were purchased from Lonza (Basel, Switzerland). LIVE/DEAD Viability/Cytotoxicity Kit was purchased from Invitrogen (Carlsbad, CA, USA.

Instrumentation. NMR spectra were recorded on a Varian UI400 (400 MHz), a U500 (500 MHz), or a VXR-500 (500 MHz) spectrometer. Gel permeation chromatography (GPC) experiments were performed on a system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA, USA), a DAWN HELEOS multi-angle laser light scattering detector (MALLS detector, Wyatt Technology, Santa Barbara, CA, USA) and an Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA, USA). The detection wavelength of HELEOS was set at 658 nm. Separations were performed using serially connected size exclusion columns (100 Å, 500 Å, 10^3 Å and 10^4 Å Phenogel columns, 5 μm, 300 × 7.8 mm, Phenomenex, Torrance, CA, USA) at 60 °C using DMF containing 0.1 M LiBr as the mobile phase. The mechanical characterization of hydrogels were performed on Asylum Research MFP-3D AFM. The fluorescence spectra were collected on a LS 55 spectrometer (Perkin Elma, Waltham, MA, USA). Images were obtained using the InCell 2000, (GE, Marlborough, MA, 02139)

Methods

Synthesis of urea end-capped PEG precursors: Amine di-functional PEG (PEG-DA, M_n ~ 2K, 0.200 g, 0.100 mM) was dissolved in 5 mL anhydrous THF. To the solution of m-xylene diisocyanate (0.188 g, 1.00 mM dissolved in 5 mL THF), the PEG solution was added dropwise with vigorous stirring. A reversed addition sequence was also performed as the control study (see Figure S1-2). After 20 min, *N*-tert-butyl allylamine (0.339 g, 3.00 mM) or *N*-methyl allylamine

(0.213 g, 3.00 mM) was added to quench all isocyanate groups. The solution was subject to precipitation with ethyl ether for four times to remove all small molecular byproducts. After vacuum drying, white solid was obtained as the target product (PEG-DTBU polymer: 0.212 g, yield 82%; PEG-DMU polymer: 0.197 g, yield 78%)

Hydrolysis of urea end-capped PEG precursors: In NMR tubes, two types of polymers were dissolved in D_2O (10 mg each in 550 uL D_2O). The solution was incubated at 37 ^oC, and the ^1H NMR spectra were collected at certain intervals. The amount of released amine indicated by the peak integrals was used to calculate the ratio of bond hydrolysis. Two polymers (10 mg each) were also dissolved in PBS solutions (1 mL D_2O). After 48 h incubation at 37 ^oC, the solution was dried by lyophilizer. The obtained solid was dissolved in DMF and characterized by GPC.

Synthesis of urea containing PEG hydrogels: PEG-DTBU, PEG-DMU, and PEG-4SH (M_n ~ 10K) were dissolved in PBS to make 10% solutions, respectively. To create hydrogel, PEG-DTBU (130 mg) or PEG-DMU (126 mg) and PEG-4SH (250 mg) solutions were mixed with the addition of 0.5 wt% 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (photoinitiator, dissolved in same weight of DMSO and mixed with water solution). The mixture was irradiated by UV (365 nm, 40 mW/cm^2) for 15 min, and the hydrogel was well formed through thiol-ene reaction.

Mechanical characterization of urea containing PEG hydrogels: Both the TBU gel and the MU gel were cured under UV light for 15 min in a glass mold of 1 mm thickness. The elastic moduli of both gels were measured through indentation tests by using atomic force microscope (Asylum Research MFP-3D AFM). The AFM tip (Novascan) used in the indentation measurements has a spherical polystyrene particle with a diameter of 25 μm and a cantilever with a spring constant of 0.6 N/m. The gel samples were submerged in deionized water for one hour before tests. During tests, both the samples and the tip were kept under water.

The AFM tip was first controlled to approach the surface of the gel at a relatively low speed, that is 2.5 μm/s. From experience, the contact point was set at the point when the force on the indenter reaches 0.25 nN. After the surface was detected, the AFM tip was pressed into the sample to a certain depth at a relatively high speed of 50 μm/s. After test, the tip was retracted.

The depth of indentation and the force on the indenter were recorded. From the measured retraction force, little adhesion was observed. Hertzian solution [Johnson, K. L. *Contact Mechanics*; Cambridge University Press, 1985.] was used to calculate the shear modulus of the gels by assuming Poisson's ratio equals 0.5 [Hu, Y.; Zhao, X.; Vlassak, J. J.; Suo, Z. *Appl. Phys. Lett.* **2010**, *96* (12), 121904.], that is

$$G = \frac{3F}{16h\sqrt{Rh}},$$

where F is the force on the indenter, h is the indentation depth, and R is the radius of the polystyrene particle. Figure S3 plots the experimental data and the corresponding fitting curves. Good agreement is obtained. The extracted shear modulus is 10.0 ± 0.9 kPa for the TBU gel, and 4.7 ± 0.6 kPa for the MU gel. The error bar is from 8-10 measurements at different locations on each sample.

Water degradation of urea containing PEG hydrogels: Hydrogel materials were immersed in PBS (100 mg hydrogel in 10 mL PBS) buffer, and incubated at 37 ^{o}C to start water degradation study. At different time point, samples were taken out and washed with deionized water for 3 times and weighed after drying by lyophilization. Degree of weight loss was used to characterize the degradation kinetics.

Protein release of urea containing PEG hydrogels: The synthesis of hydrogels were added additional 0.5 wt% dye modified bovine serum albumin (BSA-FITC) as the model protein. Hydrogel materials were immersed in PBS (100 mg hydrogel in 10 mL PBS) buffer, and incubated at 37 ^{o}C to start protein release studies. At different time point, 100 uL aliquots were taken out, diluted with 3 mL PBS and characterized by fluorescence spectrometer. The intensities of emission at 525 nm were used for characterization of protein release. The emission intensity of solution of 0.5 wt% BSA-FITC diluted by 30 folds (same dilution folds as hydrogels) was set as the standard for 100% release.

hMSC viability with urea containing PEG hydrogels: hMSCs were grown seeded in 24 well plates at 50k cells/well. After 24 h, different concentrations of PEG-DTBU were added to the

wells at concentrations of 0%, 5%, 10%, and 20%, and incubated for 48 h. After 48 h, XTT was added to the cells according the manufacturer's protocol, and viability was calculated as compared to the untreated sample. hMSCs were then encapsulated at 50k cells hydrogel in varying concentrations of 5%, 10%, and 20% PEG-DTBU with 0.5 wt% of photoinitiator and irradiated by UV (365 nm, 40 mW/cm^2) for 3 min and fresh hMSC media was added to each well. After 48 h, XTT was added to the cells according the manufacturer's protocol, and viability was calculated in relation to non-encapsulated cells and plated in 2D. After 48 h, the cells were stained with Live/Dead staining according to manufacturer's protocol and imaged with the InCell Analyzer 2000 at varying z stack planes.

hMSC encapsulation and release in urea containing PEG hydrogels: hMSCs were expanded until confluent. PEG-DTBU and PEG-DMU were dissolved in PBS at a concentration of 10% each and then mixed at varying ratios of DTBU:DMU at 1:0, 5:1, 2:1, 1:1, 1:2, 1:4, and 0:1 along with 0.5 wt% of photoinitiator. hMSCs were trypsinized, washed once with PBS, and then spun down at 1000 rpm for 5 min. Cells were encapsulated in the 50 uL of each PEG-HUB mixture at 50k cells per hydrogel and then added to the center of a 24-well plate. The plate was then irradiated by UV (365 nm, 40 mW/cm^2) for 3 min and fresh hMSC media was added to each well. After 5 days, cells that were released and adhered to the plates were imaged to determine if the cells were still encapsulated or adhered to the plate.

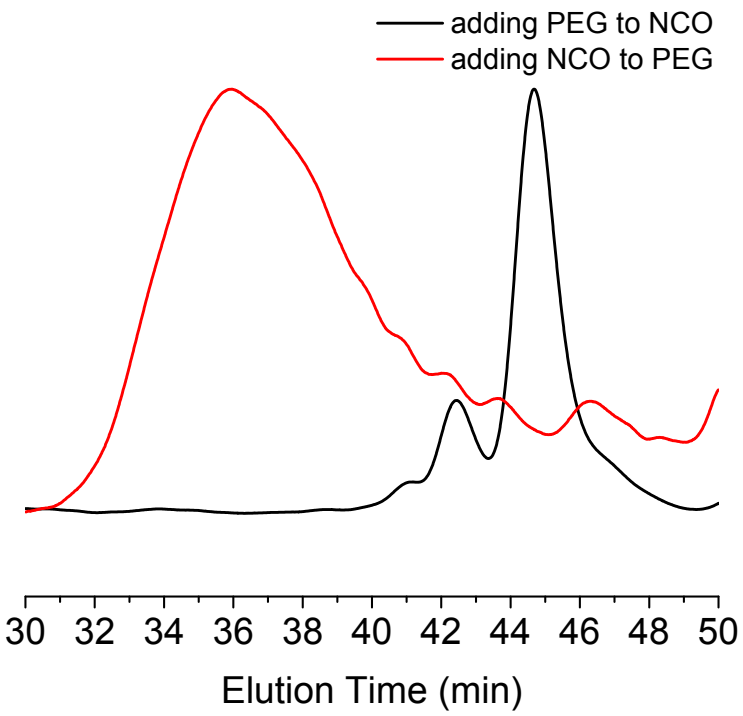

Figure S1. GPC spectra of PEG-DTBU synthesized with two different addition sequence. Red curve: diisocyanate was added dropwise to PEG; black curve: PEG was added dropwise to diisocyanate.

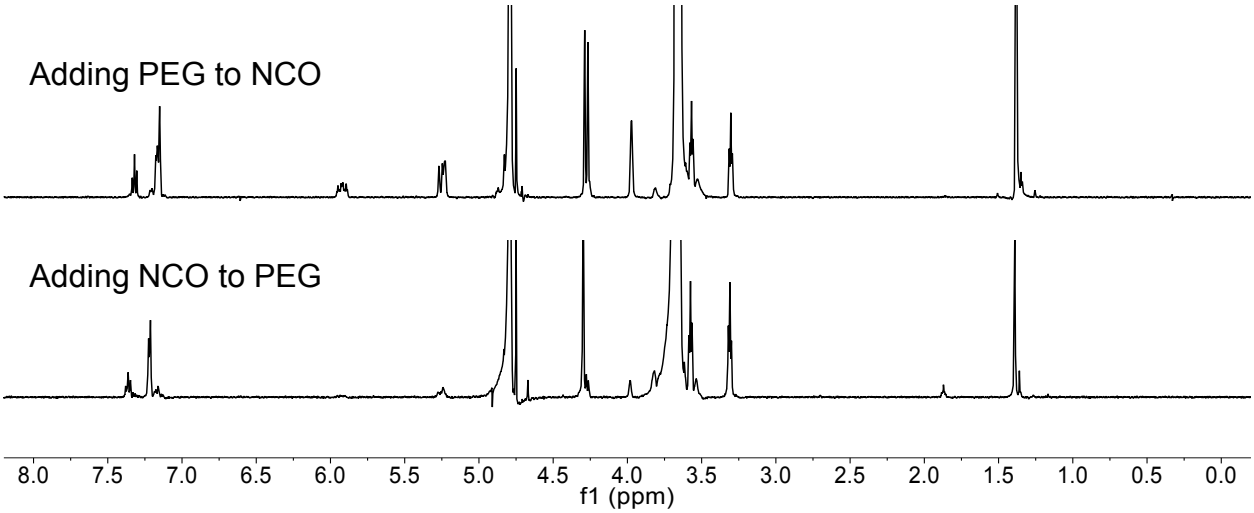

Figure S2. NMR spectra of PEG-DTBU synthesized with two different addition sequence. Upper curve: PEG was added dropwise to diisocyanate. Lower curve: diisocyanate was added dropwise to PEG.

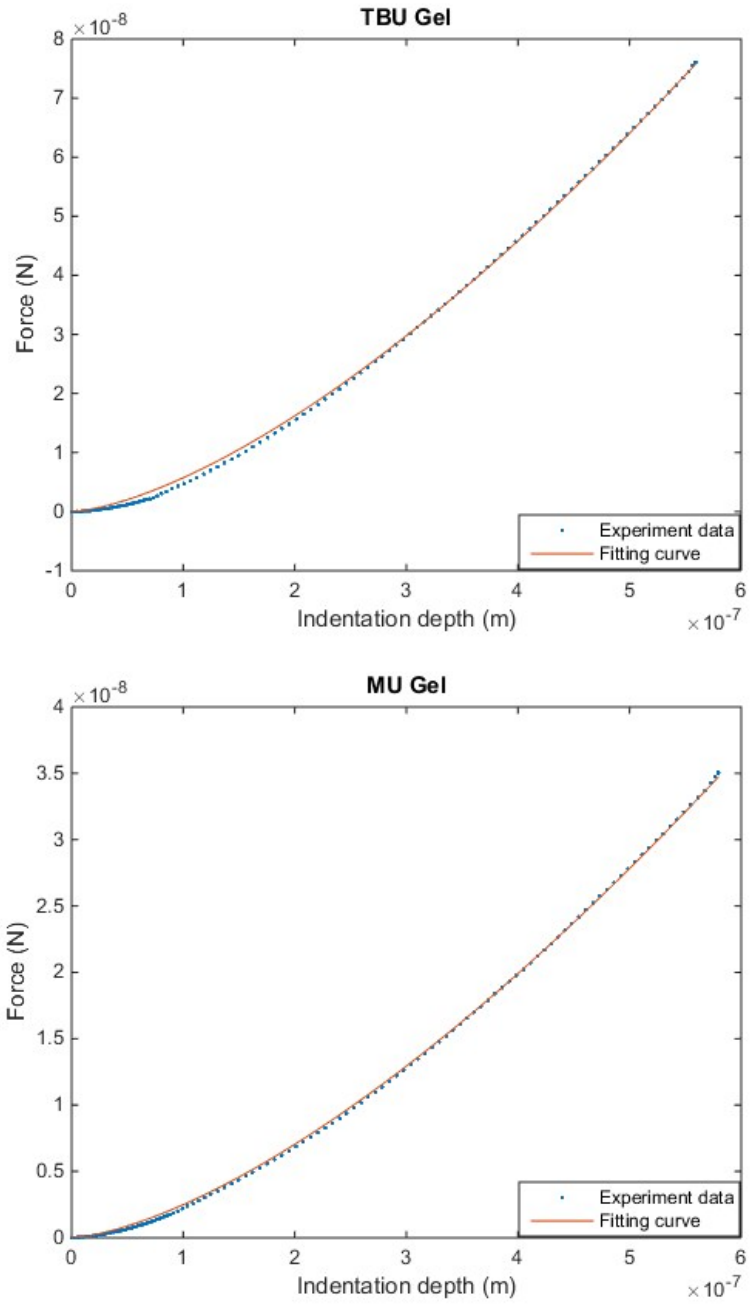

Figure S3. Indentation characterization. The force on the indenter is plotted as a function of the indentation depth. Upper plot: representative experimental data from indenting the PEG-TBU gel and the fitting curve from Hertzian solution; Lower plot: representative experimental data from indenting the PEG-MU gel and the fitting curve from Hertzian solution.