Supplementary Material (ESI) for Soft Matter

Electronic Supplementary Information

for

A novel single precursor-based biodegradable hydrogel with enhanced mechanical properties

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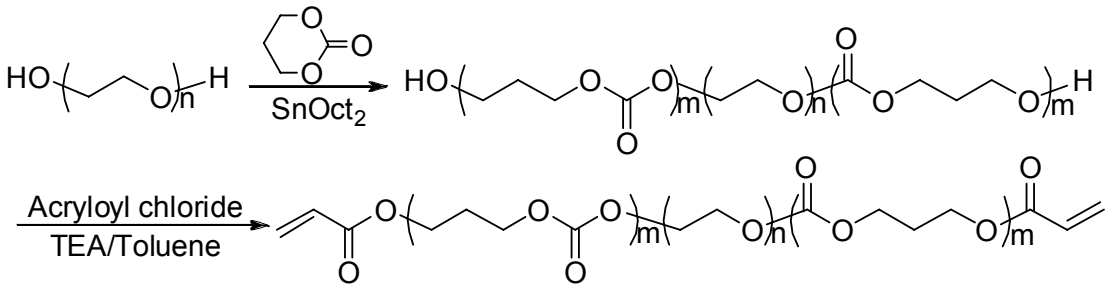
Materials

1, 3-propane diol, triethylamine (TEA), ethyl chloroformate, and tetrahydrofuran (THF) were purchased from Shanghai Chemical Reagent Co., Ltd. Poly(ethylene glycol) M_n 20,000 g/mol (PEG20K), poly(ethylene glycol) M_n 3,400 g/mol (PEG3.4K), toluene, ethyl acetate, anhydrous diethyl ether, and Sephadex® G-25 were purchased from Aldrich. TEA and THF were purified according to previously reported methods.[1] Acryloyl chloride was purchased from Acros and distilled over anhydrous calcium chloride before use. Photoinitiator Irgacure 2959 was obtained from Ciba Specialty Chemicals and dissolved in 70% ethanol to make a 10% (w/v) solution. Trimethylene carbonate was synthesized according to previously illustrated methods,[2] and recrystallized from ethyl acetate five times prior to use.

Human bone marrow derived stem cells (hMSCs) were purchased from Cambrex Bioscience (East Rutherford, NJ, USA) and grown in hMSC growth medium containing 10% FBS (Premium Select grade, Atlanta Biologicals, Inc., Lawrenceville, GA, USA) in addition to high-glucose DMEM (Invitrogen

Corporation, Carlsbad, CA, USA) supplemented with 40 mg/mL of proline and 100 mg/mL of sodium pyruvate. hMSCs (after passaging four times) were used for two-dimensional culture and three-dimensional encapsulation in this study.

Synthesis of OTMC-PEG-OTMC diacrylate



Scheme S1. Synthesis of the OTMC-PEG-OTMC diacrylate.

OTMC-PEG-OTMC tri-block copolymer was synthesized by the ring-opening polymerization of trimethylene carbonate in the presence of PEG. Briefly, PEG20K (8.19 gram, 0.41 mmol) and trimethylene carbonate (0.5022 gram, 4.92 mmol) were charged to a silanized glass ampoule; the glass ampoule was degassed at 5.0 Pa for one hour. Then $SnOct_2$ (2.175 mg) in anhydrous THF (58 μL) was added and the ampoule was degassed at 5.0 Pa for two hours and sealed. The reaction was carried out in an oil bath at 90 ^{o}C for 24 hours and then quenched in an ice bath; the crude product was dissolved in chloroform and precipitated in anhydrous diethyl ether. The white precipitate was collected and dried in a vacuum oven at 40 ^{o}C for 24 hours.

The acrylation of the OTMC-PEG-OTMC tri-block copolymer was performed according to literature with slight modification.[3] The OTMC-PEG-OTMC tri-block copolymer (8.13 gram, 0.39 mmol) was dissolved in toluene (200 mL) and refluxed in a round bottom flask at 160 ^{o}C for 8 hours, followed by an azeotropical distillation to

remove trace water in the solution. The flask was placed in an ice bath and cooled down for 30 minutes; Upon cooling of the solution to room temperature, triethylamine (0.1522 gram, 1.5 mmol) was added to the solution under vigorous stirring. Then the flask was moved to an ice bath and stirred for 30 minutes. Acryloyl chloride (0.1365 gram, 1.5 mmol) in anhydrous tetrahydrofuran (15 mL) was then added to the reaction mixture dropwise in 30 minutes. After the reaction was performed in the ice bath for another 30 minutes, the flask was heated to 45 ^{o}C for 12 hours before further steps. The reaction mixture was cooled to room temperature and filtered through Celite® 545 filter aid (2 cm thick) on a Büchner funnel to remove the quaternary ammonium salt; the filtrate was condensed on a rotary evaporator and then precipitated in cold methanol. The white precipitate was collected by filtration and vacuum dried at room temperature for 24 hours. The OTMC-PEG-OTMC diacrylate was further purified by column chromatography on Sephadex® G-25 gel. This precursor was named TMC20. PEG20K diacrylate and all other OTMC-PEG-OTMC diacrylates with varied OTMC segment length were synthesized similarly.

Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy

^{1}H NMR spectrum of the precursors was recorded on a Varian Mercury 400 MHz spectrometer. Deuterated water (D_2O) was used as the solvent. Degree of polymerization of the TMC was calculated based on the relative integral between the methylene protons of TMC repeating units and the ethylene glycol repeating units. Degree of substitution of the acryloyl group on both ends of the oligomer was also

calculated from the relative integral between the acrylate protons and the ethylene glycol repeating units. ^{1}H NMR (400MHz, D_2O, δ): 1.89 (-O-CH_2-CH_2-CH_2-O-CO-), 3.35-3.75 (CH_2-CH_2-O-), 4.10 (CO-O-CH_2-CH_2-CH_2-O-CO-), 5.80-6.30 (CH_2=CH-CO-). The NMR spectrum (**Fig**. **1**) showed both OTMC segments and acryloyl groups were successfully incorporated into the PEG main chain. The M_n calculated from ^{1}H NMR was 20,650 g/mol and 3,970 g/mol for TMC20 and TMC3.4 precursors, respectively, which means there were about three TMC repeating units on both ends of the TMC20 (precisely 3.2) and TMC3.4 (precisely 2.8) precursors; the degree of substitution (DS) of the acryloyl group was 39.9% and 60 % for TMC20 and TMC3.4, repsectively. The DS of the acryloyl group for PEG3.4 and PEG20 was 74.5% and 24.3%, respectively.

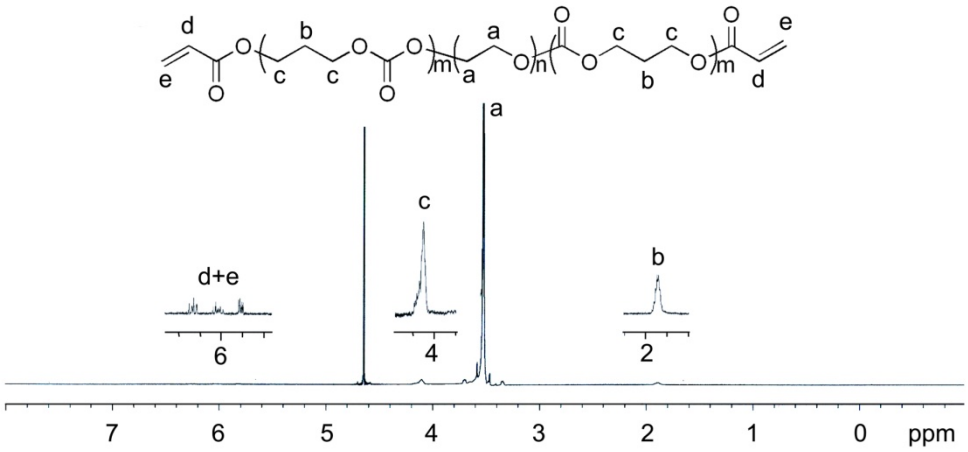


Fig. **S1** ^{1}H NMR Spectrum of TMC20 precursor.

Synthesis of hydrogel

The hydrogel was synthesized by photopolymerization under 365 nm UV light. Typically, a solution of 10% (w/v) of the precursor (TMC20, PEG20, TMC3.4 or

PEG3.4) and 0.05% (w/v) photoinitiator were prepared in phosphate-buffered saline (PBS); the solution was then transferred to a mold and subjected to 365 nm UV at 42 $\mu W/cm^2$ (Blak-Ray Model XX-15L UV lamp) for five minutes. After that, the hydrogel was removed from the mold before further steps.

Swelling Ratio

Hydrogels were synthesized as described above with a sample size of 80 μL, followed by swelling in PBS at 37 ^oC for 48 hours (which is roughly double the time required) to reach swelling equllibrium; the wet weight of the hydrogel was measured (W_w). The gel was then washed in deionized water for 24 hours with frequent changing of deionized water and dried in vacuum until a constant dry weight (W_d) was achieved. Swelling ratio (SR) as the average of three samples was calculated as below:[4]

$$SR= W_w/ W_d$$

Mechanical test

The hydrogels used for compression tests were synthesized according to above procedures and immersed in PBS for 24 hours to reach swelling equilibrium before testing. The compression tests were performed on an Instron 3342 Universal Testing System (Instron, Norwood, MA, USA) equipped with a Model 2519-104 force transducer. Hydrogels with height of 7 mm and diameter of 8 mm were subjected to the compression test. The maximum force load was set to 250 N, and the compression

rate was 10 mm/min. The data acquisition and processing were performed with BlueHill® software. The initial compressive modulus of the hydrogel was determined by the slope of a linear range. The ultimate stress and strain was determined from the peak of the stress-strain curve. All experiments were conducted in triplicate. Average and standard deviation were calculated.

Hydrolytic and enzymatic degradation

After the photopolymerization, the synthesized hydrogels were washed in 50 mL of deionized water immediately for 24 hours and the deionized water was changed every 6 hours. Then the hydrogels were dried in vacuum at 40 ^{o}C until a constant dried weight (W_{d0}) was reached for each gel. The dried gels were immersed in 1 mL of PBS (pH 7.4) in a 2 mL vial at 37 ^{o}C, and the PBS was changed every 7 days. Three samples were taken out on 3, 7, 14, 21, and 28 days. The dried weight was measured after the samples were washed by deionized water for 48 hours and dried in vacuum (W_{dt}). The weight loss of the hydrogel after hydrolytic degradation was calculated as:

$$\text{Weight loss}(\%) = 100\times (1-W_{dt}/W_{d0})$$

Enzymatic degradation was conducted similarly; 5000 IU/mL enzyme (lipase from *thermomyces lanuginosus*, E. C. 3.1.1.3.) solution in deionized water was used and 0.1% (w/v) of sodium azide was added to the enzyme solution as anti-bacterial reagent. All other procedures were the same as that of hydrolytic degradation.

Hydrolytic and enzymatic degradation of the hydroegls were also carried out for longer time periods, 8 and 12 weeks.

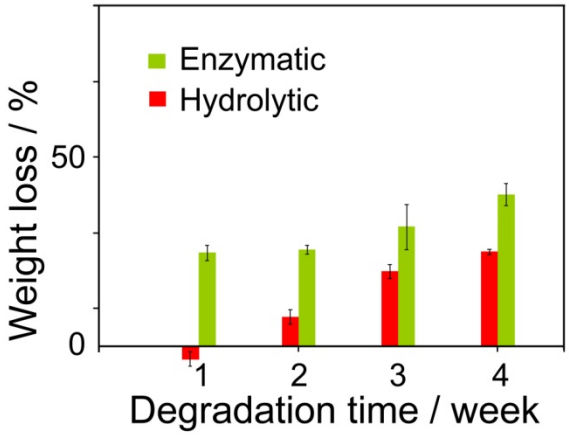


Fig. **S2** Hydrolytic and enzymatic degradation of TMC20 hydrogels

Two-dimensional (2D) culture of human mesenchymal stem cells (hMSCs)

TMC20 hydrogel sheet was synthesized in a mold made of two glass slides; spacers with thickness of 1 mm were used to control the thickness of the hydrogel sheet. The TMC20 oligomer solution (10%, w/v) containing irgacure 2959 (0.05%, w/v) was injected into the mold, then the mold was exposed to 365 nm UV light at intensity of 42 μW/cm^2 for five minutes. After the photopolymerization was finished, the hydrogel sheet was removed from the mold and soaked in PBS for 48 hours, then the hdyrogel was cut into 1×1 cm pieces. The 1×1 cm pieces of hydrogels were sterilized in 70% ethanol for 48 hours, after which they were washed in PBS with 2% PenStrep for another 48 hours. The hydrogels were then immersed in hMSC growth medium for 24 hours and moved to a 24-well plate before the cell seeding. P4 hMSCs suspensions were seeded on top of the hydrogels at a density of 10000 cells/cm^2. The hydrogel sheets with P4 hMSCs were incubated at 37 ^{o}C in 5% CO_2 atmosphere for 3

hours to allow cell adhesion, after which 2 mL of hMSC growth medium was added to each well. Pictures were taken under microscope at 3 hours and 24 hours to evaluate the cell viability and cell adhesion.

Three-dimensional photoencapsulation of hMSCs

TMC20 oligomer solution (10% w/v) was prepared in PBS; Irgacure 2959 in 70% ethanol was added to the TMC20 solution and mixed thoroughly to make a final concentration of 0.05% (w/v). Immediately before photo-encapsulation, P4 hMSCs were re-suspended in the TMC20 solution. 60 μL of the hMSC/TMC20 suspension containing 6×10^5 cells was transferred to a cylindrical mold and exposed to 365 nm UV light with intensity of 42 $\mu W/cm^2$ for 5 min to form a hydrogel. hMSC/TMC20 hydrogels were then transferred to a 24-well plate and incubated up to 21 days with hMSC growth medium. hMSC-laden PEG20 hydrogels were prepared following the same procedures.

Cell viability

Live/Dead assay was conducted to evaluate the cell viability after 24 hours of 3D photo-encapsulation according to reported protocol.[5] Thin slices (thickness<1 mm) of the construct was incubated for 30 minutes in 200 μL of the Live/Dead assay dye solution, which was composed of 0.5 μl of Calcein AM and 2.0 μl of Ethidium homodimer-1 in 1 mL of high glucose DMEM. After the incubation, the hydrogel slices were rinsed with PBS and observed under fluorescence microscope (Zeiss

Axio Observer A1 Inverted Microscope with X-Cite® 120 Fluoresence Illumination Unit) .

References

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