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SUPPORTING INFORMATION

Versatile Hyperbranched Poly(β-Hydrazide Ester) Macromers as Injectable Antioxidative Hydrogels

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Materials

Poly(ethylene glycol) diacrylates (PEGDA, Mn = 575, and 700 Da), were purchased from Sigma-Aldrich. Dimethyl sulfoxide (DMSO, HPLC grade), d6-DMSO (99.8%), ethyl alcohol (99.5%), diethyl ether (ACS reagent grade), dimethylformamide (DMF, HPLC grade) were purchased from Fisher and used as received. 3,3'-Dithiodipropionic acid, sulfuric acid (98%), and hydrazine hydrate were used to synthesize 3,3'-dithiobis(butanoic hydrazide) (DTP). HA-SH (R&D grade) was purchased from Blafar Ltd. Phosphate buffered saline Tablets (PBS) and H_2O_2 were purchased from Sigma-Aldrich and dissolved with ultra-pure water. 2,4,6-Trinitrobenzenesulfonic acid solution (TNBS, 5% (w/v) in H_2O) and Sodium tetraborate were purchased from Sigma-Aldrich.
Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), and penicillin / streptomycin were purchased from Invitrogen. 3T3 cells were purchased from ATCC. Adipose-derived stem cells (ADSCs) were extracted from SD rats previously and stored in liquid nitrogen. AlamarBlue reagent was purchased from Sigma-Aldrich. Live/Dead Viability Kit was purchased from Bio Science.

Supplementary Methods

Preparation of DTP

DTP was synthesized according to a published paper.[1] Briefly, 3,3'-Dithiodipropionic acid (10 g) was dissolved in ethyl alcohol (100 mL) and one drop of sulfuric acid was added into the flask. The solution was heated to reflux for 5 h. The solvent was evaporated by a rotavapor. Diethyl ether (150 mL) was added to dissolve the mixture. The solution was washed by H_2O (3×100 mL) then the organic layer was dried over Na_2SO_4. Diethyl ether was removed by rotavapor. The crude diester (12.1 g, 87.5% yield) resembled colorless oil. Diester (10 g) and hydrazine hydrate (8 equiv.) were dissolved into ethyl alcohol (25 mL), respectively. The solution of diester was added into the solution of hydrazine hydrate dropwise. The solution was heated to 50 °C and stirred for 2 h, then it was transferred to a beaker and left to cool to room temperature. DTP was precipitated then filtered and washed with cool hexane to afford the white crystal. The final product was dried with vacuum oven for 2 days to fully remove the hydrazine hydrate (7.9 g, 84.6% yield). ^{1}H-NMR (400 MHz, DMSO-d_6): 9.07 (s, 2H), 4.24 (s, 4H), 2.89 (t, 4H), 2.41 (t, 4H).

Preparation of SDH

SDH was synthesized via a similar method of DTP synthesis. Briefly, suberic acid (10 g) was dissolved in ethyl alcohol (100 mL) and one drop of sulfuric acid to act as a catalyst was added into the flask. The solution was heated to reflux for 6 h. The solvent

was evaporated by a rotavapor. Diethyl ether (150 mL) was added to dissolve the mixture. The solution was washed by H_2O (3×100 mL), then the organic layer was dried over Na_2SO_4. Diethyl ether was removed by rotavapor. The crude diester (11.7 g, 82.6% yield) resembled colorless oil. Diester (10 g) and hydrazine hydrate (8 equiv.) were dissolved into ethyl alcohol (25 mL), respectively. The solution of diester was added into the solution of hydrazine hydrate dropwise. The solution was heated to 50 °C and stirred for 2 h, then transferred to a beaker and allowed to cool to room temperature. SDH was precipitated, then filtered and washed with chilled hexane to achieve the white crystal. The final product was dried with vacuum oven for 2 days to fully remove the hydrazine hydrate (7.4 g, 83.3% yield). ^{1}H-NMR (400 MHz, DMSO-d_6): 8.90 (s, 2H), 4.15 (s, 4H), 1.98 (t, 4H), 1.46 (m, 4H), 1.20 (m, 4H).

Table S1.

List of the macromers.

Monomer	PEGDA 575	PEGDA 700
DTP	575-DTP	700-DTP
SDH	575-SDH	700-SDH

Table S2.

Profiles of the obtained macromers monitored by GPC.

	Mn (Da)	Mw (Da)	PDI	Conversion (%)
575-DTP	4488	10009	2.23	77
700-DTP	6097	11249	1.85	73
575-SDH	5551	11660	2.10	71
700-SDH	6222	12326	1.98	72

Scheme S1.

Synthesis of HB-PBHEs by SDH and PEGDA *via* "A2+B4" Michael addition approach in DMSO at 90 ℃.

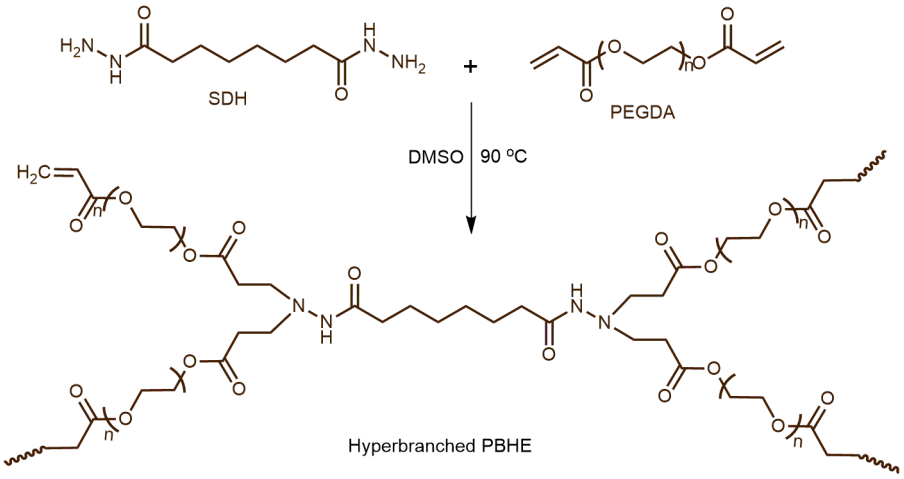


Figure S1.

GPC monitoring the synthesis of 575-DTP and 700-DTP macromers. (A) and (C) are GPC traces; (B) and (D) are Mw versus PDI with time.

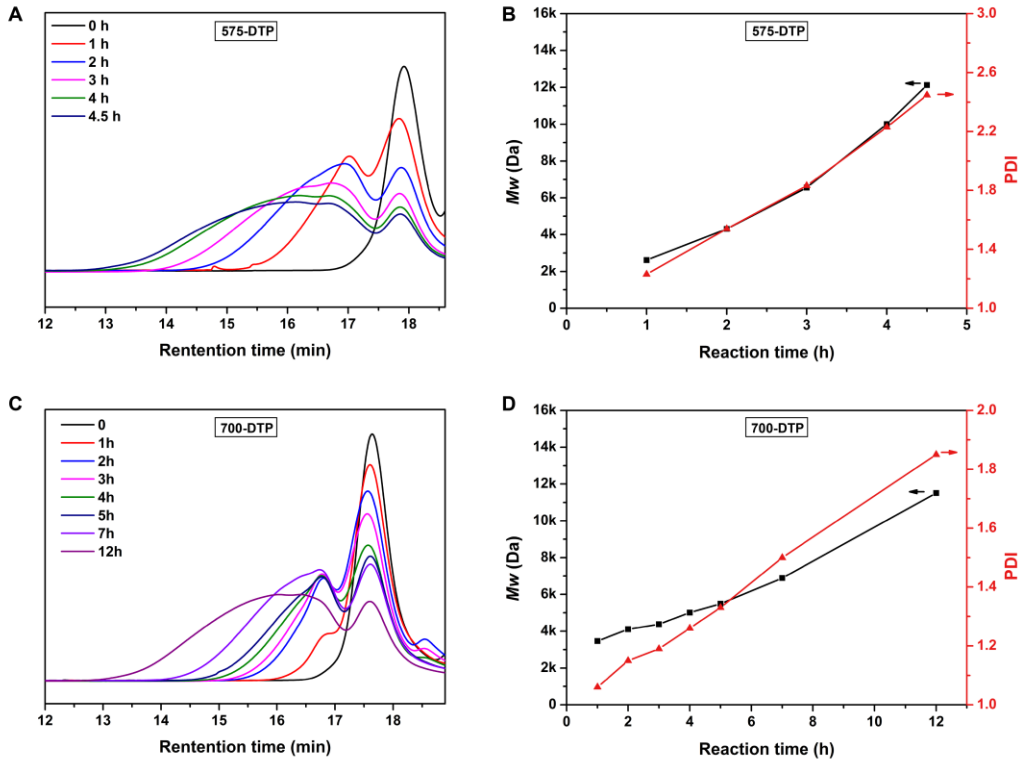


Figure S2.

GPC monitoring the synthesis of 575-SDH and 700-SDH macromers. (A) and (C) are GPC traces; (B) and (D) are Mw versus PDI with time.

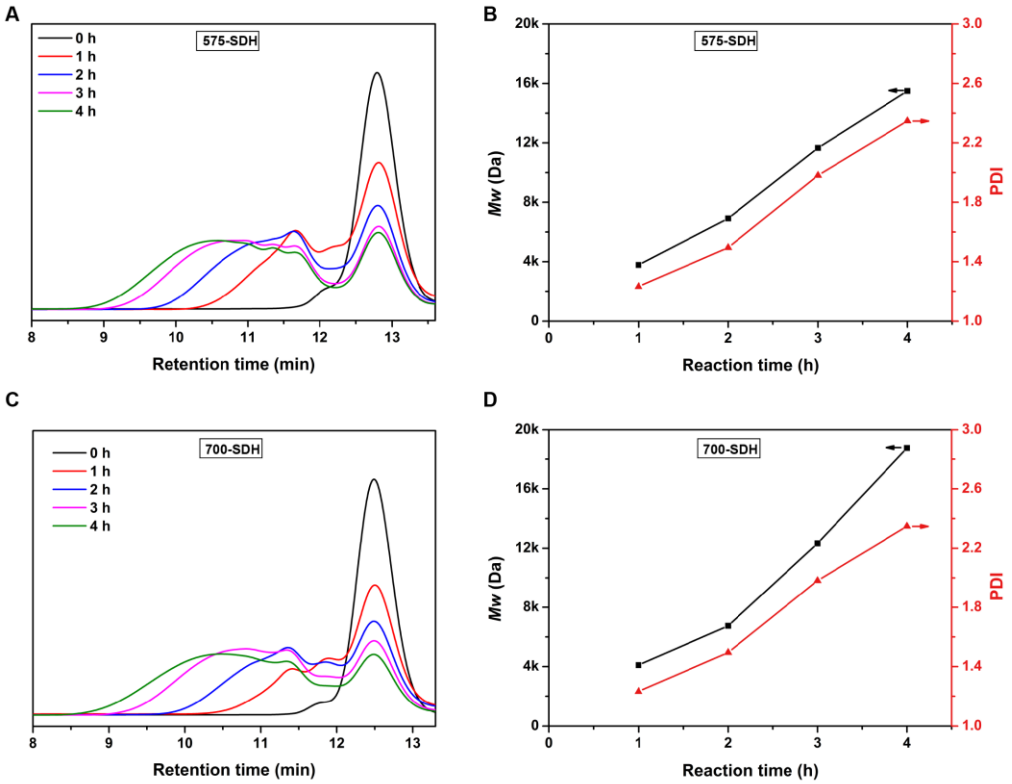


Figure S3.

^{1}H-NMR and ^{13}C-NMR spectra of 700-DTP macromer.

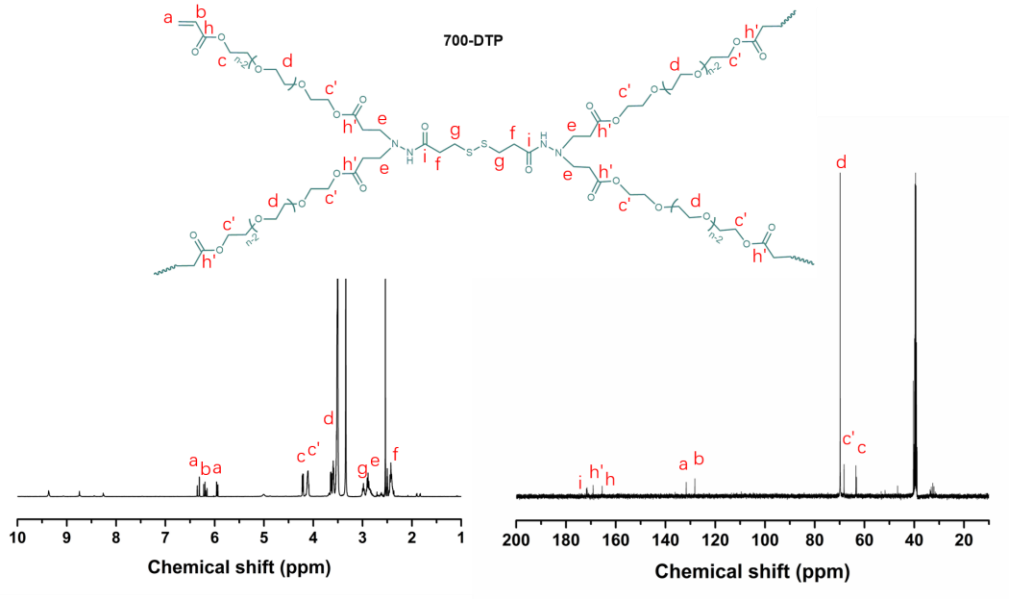


Figure S4.

^{1}H-NMR and ^{13}C-NMR spectra of 575-SDH macromer.

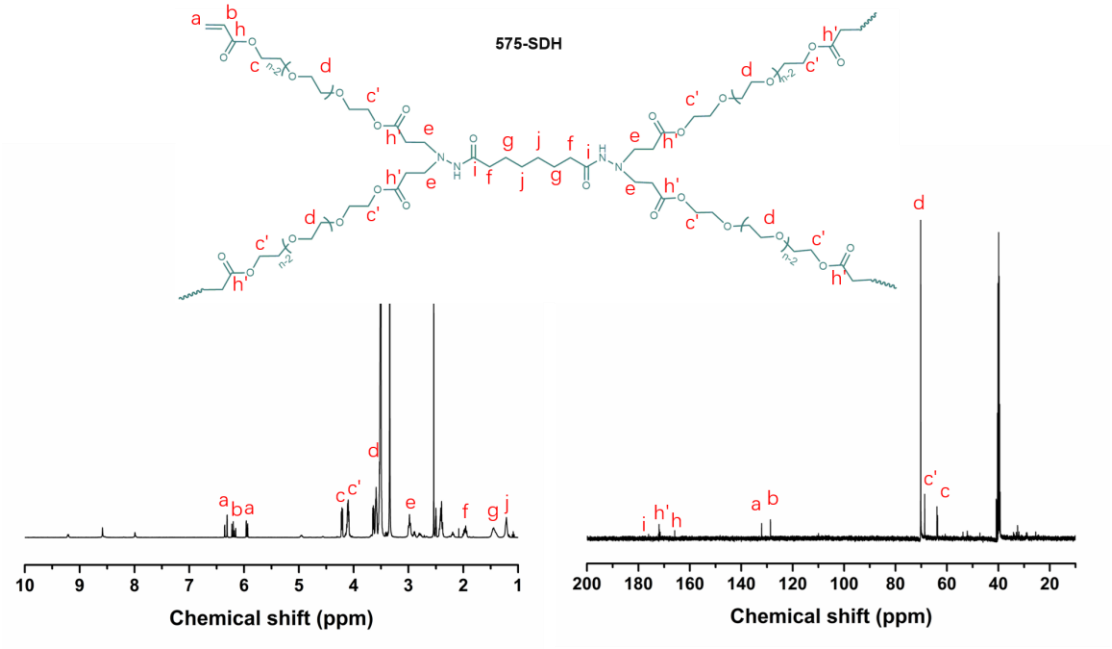


Figure S5.

^{1}H-NMR and ^{13}C-NMR spectra of 700-SDH macromer.

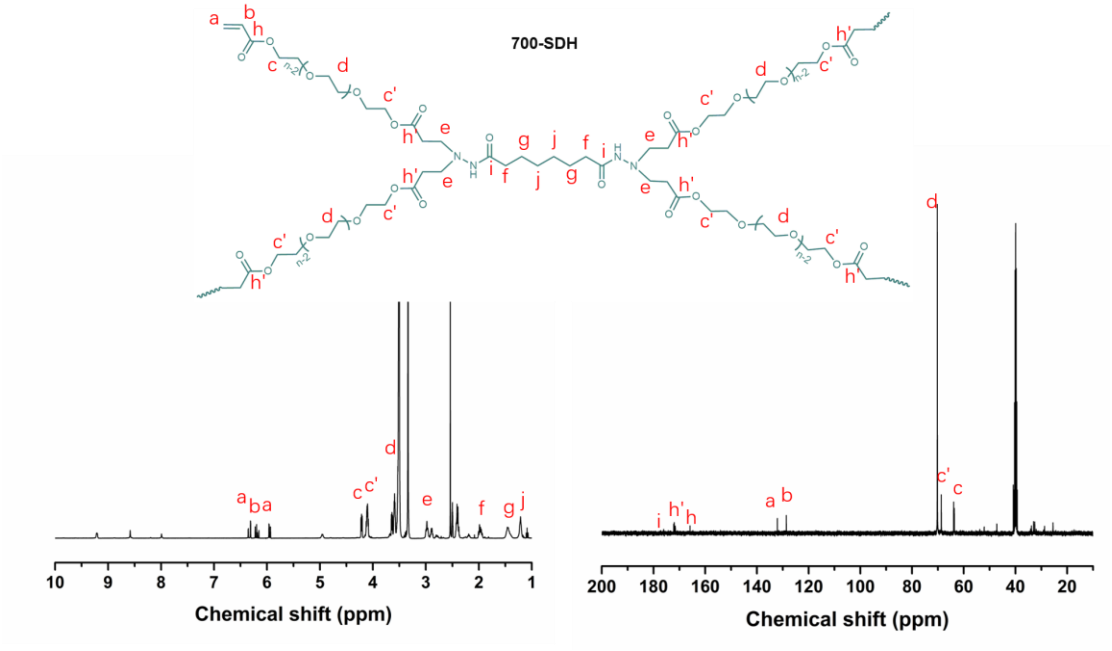


Figure S6.

Vinyl content of all four macromers.

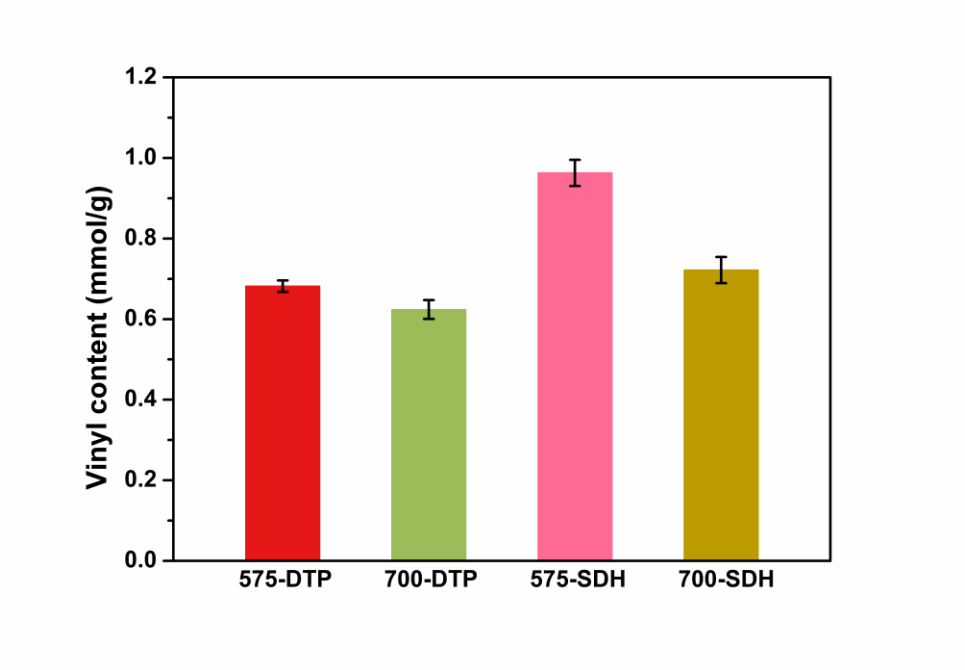


Figure S7.

Hydrazide content of all four macromers.

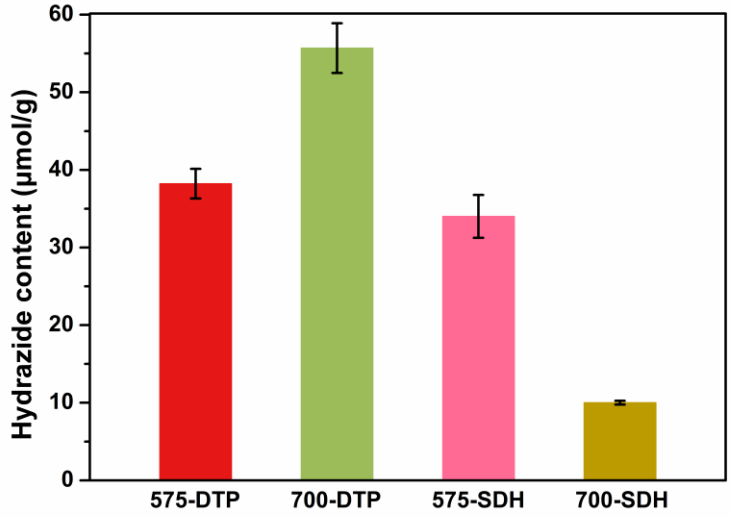


Figure S8.

Gelation process and rheological assay of SDH based injectable hydrogels (different concentrations of HP-PBHEs with 1% w/v HA-SH) by rheometer. (A) and (B) storage and loss moduli with time sweep. (C) and (D) storage and loss moduli with strain sweep.

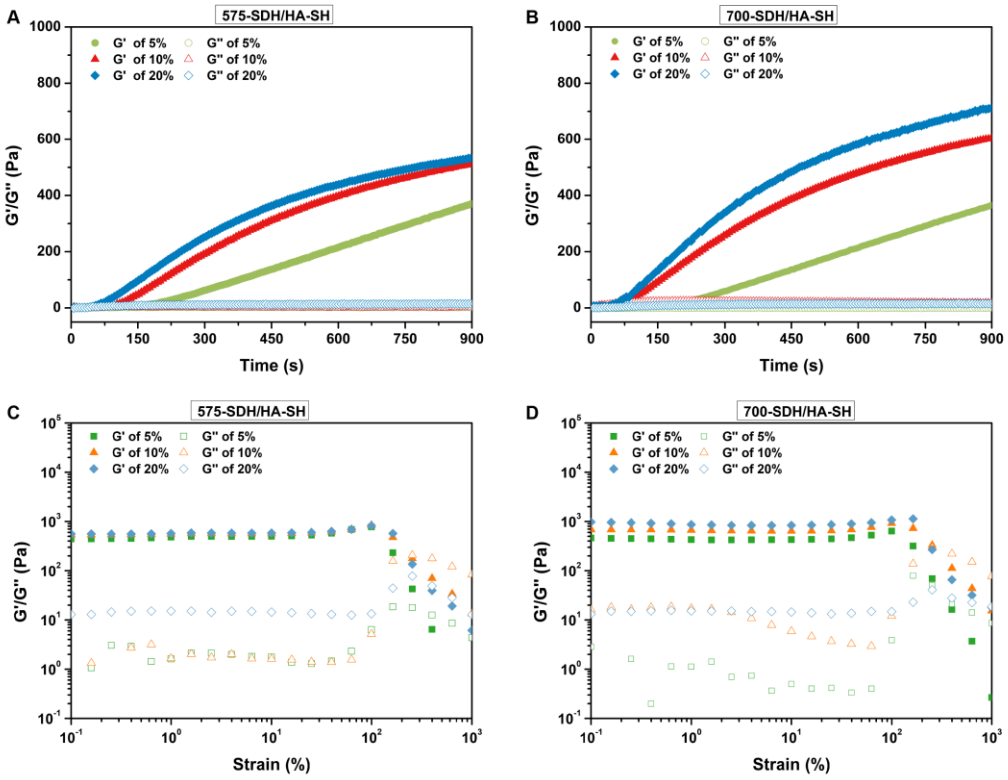


Figure S9.

Gelation process and rheological assessments of SDH-based UV crosslinked hydrogels by rheometer. UV irradiation was applied from 60 s to 180 s. (A) and (B) storage and loss moduli with time sweep. (C) and (D) storage and loss moduli with strain sweep.

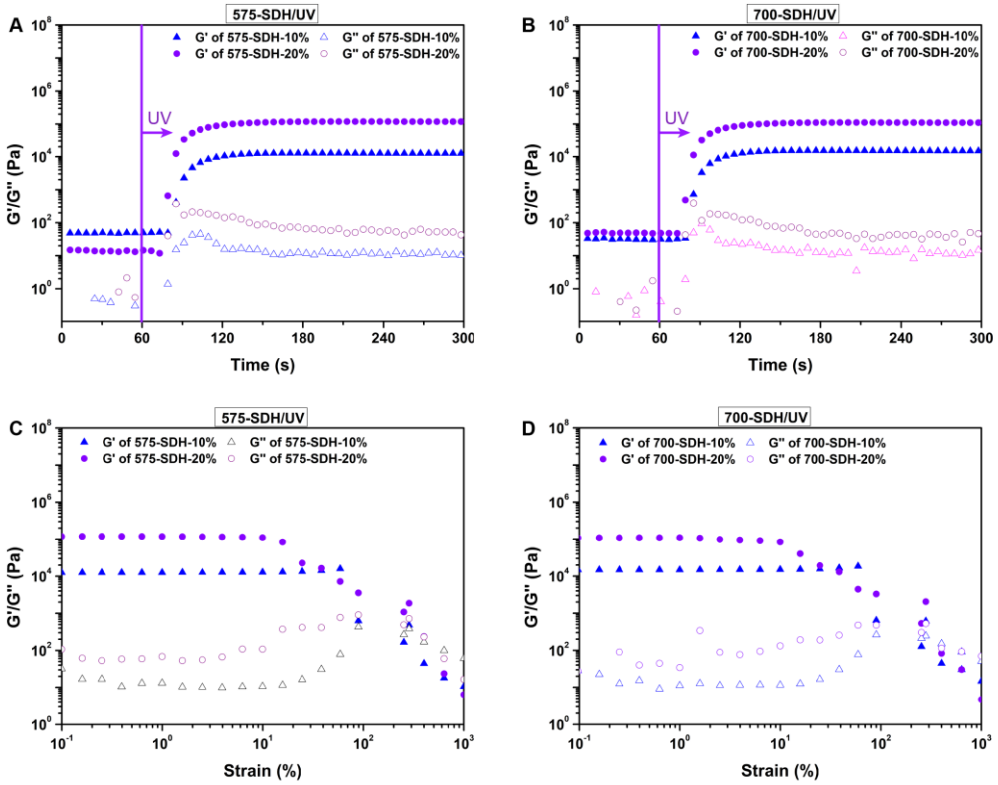


Figure S10.

575-DTP and 700-DTP macromers' biocompatibility test using an alamarBlue assay for 3T3s (A) and ADSCs (B).

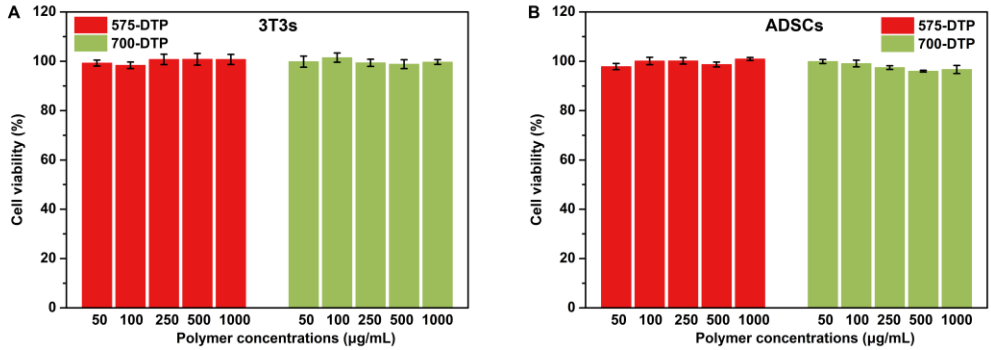
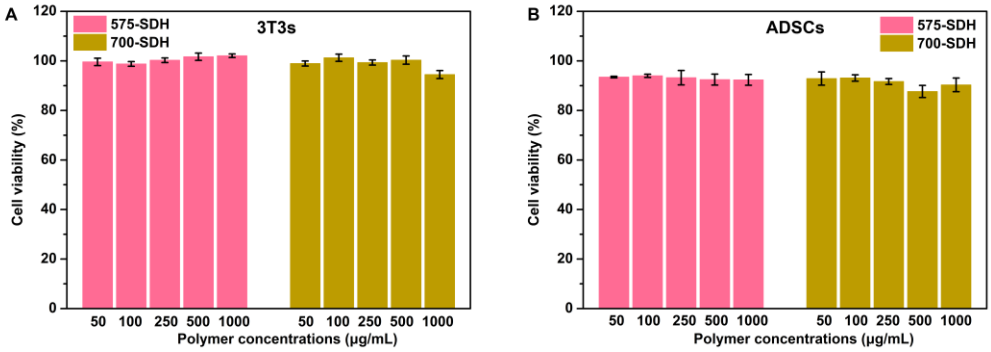


Figure S11.

575-SDH and 700-SDH macromers' biocompatibility test using an alamarBlue assay for 3T3s (A) and ADSCs (B).



Supplementary Reference

(1) Shu, X. Z.; Liu, Y.; Luo, Y.; Roberts, M. C.; Prestwich, G. D. Disulfide Cross-Linked Hyaluronan Hydrogels. *Biomacromolecules* **2002**, *3* (6), 1304–1311.