Supplementary Material (ESI) for Polymer Chemistry
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Supplementary information:

Experimental section

Materials

The monomers poly (ethylene glycol) methyl ether methacrylate (PEGMEMA, M_n = 500 g mol^{-1}) and poly (ethylene glycol) diacrylate (PEGDA, M_n = 575 g mol^{-1}) were purchased from Sigma-Aldrich. 1'1 – azobis (cyclohexane-carbonitrile) (Sigma-Aldrich, 98%) was used as an initiator. The RAFT agent, 2-cyanoprop-2-yl dithiobenzoate, was synthesised according to published method [35].Butanone (HPLC grade, Aldrich), hexane (HPLC grade, Aldrich), tetrahydrofuran (THF, Aldrich) chloroform-d ($CDCl_3$ Aldrich), diethyl ether (HPLC grade, Aldrich). All reagents and solvents were used without further purification. Thiol-modified hyaluronic acid (HA-SH) was purchased from Glycosan-Hystem™ Biotime. alamarBlue®, LIVE/DEAD® viability/Cytotoxicity kit (Invitrogen), human adipose derived stem cells hADSCs (Invitrogen), MesenPRO RS™ medium and Quant-iT™ Picogreen® dsDNA Assay (Invitrogen) were used as required throughout the study.

Methods

Synthesis of PEGMEMA - PEGDA Copolymers Using RAFT polymerisation

PEGMEMA-PEGDA copolymers were synthesized by the copolymerization of PEGMEMA (M_n = 500 g mol^{-1}) and multivinyl branching monomer PEGDA (M_n = 575 g mol^{-1}) using Reverse Addition–Fragmentation chain Transfer (RAFT) polymerisation. Briefly, the copolymers were prepared in butanone at the concentration of monomer to solvent volume ratio being 1:3 in a 250 ml round bottomed flask. The initiator (1'1 – azobis (cyclohexane-carbonitrile) was added (100:0.8, monomer: initiator mol ratio) and followed by the RAFT agent at a mol ratio of 1.6:100 with respect to the monomers employed. The solution was bubbled with argon for 20-25 minutes to remove any oxygen. The reaction was conducted at 70 ˚C in an oil bath while being stirred at 700 rpm until the desired polymer molecular weight, conversion and polydispersity was acquired (monitored by Gel Permeation Chromatography - GPC). To terminate the polymerisation, the stopper was removed and exposing the reaction to oxygen and cooling the flask rapidly in water.

The resultant product was precipitated drop wise in hexane/diethyl ether (1.3:1 v/v) mixture. The precipitated product was dissolved in deionised water and purified by dialysis (spectrum dialysis membrane, molecular weight cut off 6000-8000) for 1 day to remove excess monomer. Purified

polymer samples were collected by the removal of water through freeze drying yielding a polymer which was sticky in nature.

Characterization of PEGMEMA - PEGDA copolymers

Characterization of the copolymers was carried out by ^{1}H-NMR and Gel Permeation Chromatography (GPC). Weight average molecular weight (M_w), number average molecular weight (M_n) and polydispersity (PDI, M_w/M_n) were obtained by GPC (Aligent, PL-GPC50) with RI detector. The GPC eluted THF from columns (2 x Agilent PLgel, 5µm, Mixed C 300mm x 7.5 mm) and calibration was carried out using poly (methyl methacrylate) standards. Analysis and Calibration was carried out at 40 ˚C at a flow rate of 1mL/min. ^{1}H NMR was carried out on a 300 MHz Bruker NMR with Delta NMR processing software. The chemical shifts were referenced to the lock chloroform ($CDCl_3$).

Preparation of hydrogels

The PEGMEMA–PEGDA copolymers (Entry 1, Table 2) were dissolved in 1 M phosphate buffered (PBS) at pH 7.5 to form solutions at 2%, 5% and 10% weight concentrations. These were crosslinked to form 50 µL hydrogel networks using thiol modified HA (SH-HA) with the exact weight of each gel being measured (W_0). PBS was added to the gel samples at 1:20 (hydrogel : PBS) and allowed to swell at 37 ^{o}C .To measure the swelling ratio, PBS was removed and the gels were dried on a blotting paper and weighed at regular time intervals. The swelling ratios were calculated as follows:

$$\textbf{Swelling ratio} = W_t / W_0$$

Where, W_t represents the weight of hydrogel at a certain time point and W_0 represents the original weight of the hydrogels. After measuring the weight, the hydrogel samples were rehydrated and were further incubated at 37 ^{o}C. The swelling results were the average of 6 samples for each case (n=6).

Cell viability and Proliferation assay

To determine the cytotoxicity of the copolymer, human adipose derived stem cells (hADSCs, (Invitrogen)) were used for 3D encapsulation. hADSCs were encapsulated in (Polymer-HA) hydrogel at a density of 1 x 10^6 cells per mL. hADSCs were mixed with polymer solution (4% Entry 1, table 2) just before crosslinking. Thiolated hyaluronic acid (HA-SH) at 1% was then added at equal volume. The mixture was placed at room temperature for 10 minutes to completely form a gel, followed by transferring the gels into culture medium. Hydrogels (n=6) were incubated at 37^{o}C and 5% CO_2 for 2, 5, and 7 days. hADSCs were maintained in MesenPRO RS™ Medium (includes Basal Medium and Growth Supplement) throughout the experiment with

media change every 2-3 days. alamarBlue® assay was used to analyse the effect of polymer on cell metabolic activity along with LIVE/DEAD® Viability/Cytotoxicity kit (Invitrogen).

After each timepoint, 500 uL alamarBlue® (10% in MesenPRO RS™ media) was added to assess cell metabolic activity. After 6 hours at 37°C and 5% CO_2 150 uL of alamarBlue® was removed and the absorbance at the lower wavelength filter (550 nm) was measured followed by the higher wavelength filter (595 nm) via a thermo scientific Varioskan Flash Plate Reader.

For cellular proliferation in 3D culture, the samples used in alamarBlue® assay were washed with PBS three times for 5 minutes each at 37°C and 5% CO_2 and were frozen at - 80 °C until further analysis. DNA from hydrogels (n=6) was recovered mechanically using TissueLyser LT (Qiagen). Quantification of total DNA in the hydrogel constructs was performed with Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen) following the manufacturer's instructions using a fluorescent plate reader (Varioskan Flash from Thermo Scientific) at an excitation wavelength of 485 nm and an emission wavelength of 520 nm.

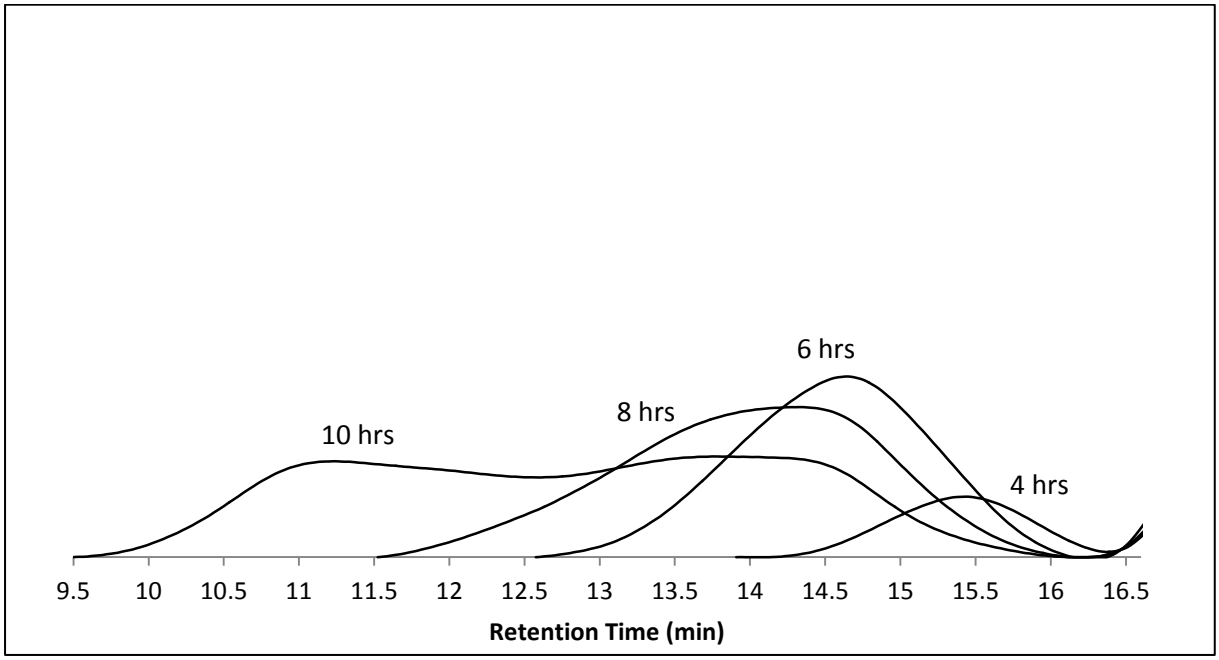

Fig. S1: GPC traces from RI detector for copolymerisation of Entry2, Table1; Final molecular weight of 376,000 g/mol and a polydispersity of 7.12 after 7.5hrs.

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Table T1: Copolymerisation of Polyethylene glycol diacrylate (PEGDA – 575 g/mol) and polyethylene methyl ether methacrylate (PEGMEMA – 500 g/mol) via RAFT polymerisation (Entry 2, Table 1).

Entry	I : RAFT Agent : M1: M2 [a]	SR (v/v) [b]	RT [c]	Monomer conversion (%) [d]	M_w (kDa) [e]	PDI [f]
1	0.8 : 1.6 : 75 : 50	1:3	4	10	9	1.22
2	0.8 : 1.6 : 75 : 50	1:3	6	28	40	1.55
3	0.8 : 1.6 : 75 : 50	1:3	8	40	59	2.19
4	0.8 : 1.6 : 75 : 50	1:3	10	53	376	7.12

[a] I - Initiator (ACHN): RAFT Agent - (2-cyanoprop-2-yl dithiobenzoate), M1:M2 - Monomer feed ratio (PEGDA (M1): PEGMEMA (M2)), [b] SR - Monomer : Solvent (Butanone) (v/v) , [c] Reaction time, [d] Monomer conversion estimated from copolymer and monomer peaks in GPC traces, [e] M_w - Weight Average molecular weight (1kDa = 1,000 g/mol), [f] Polydispersity index (M_w / M_n).

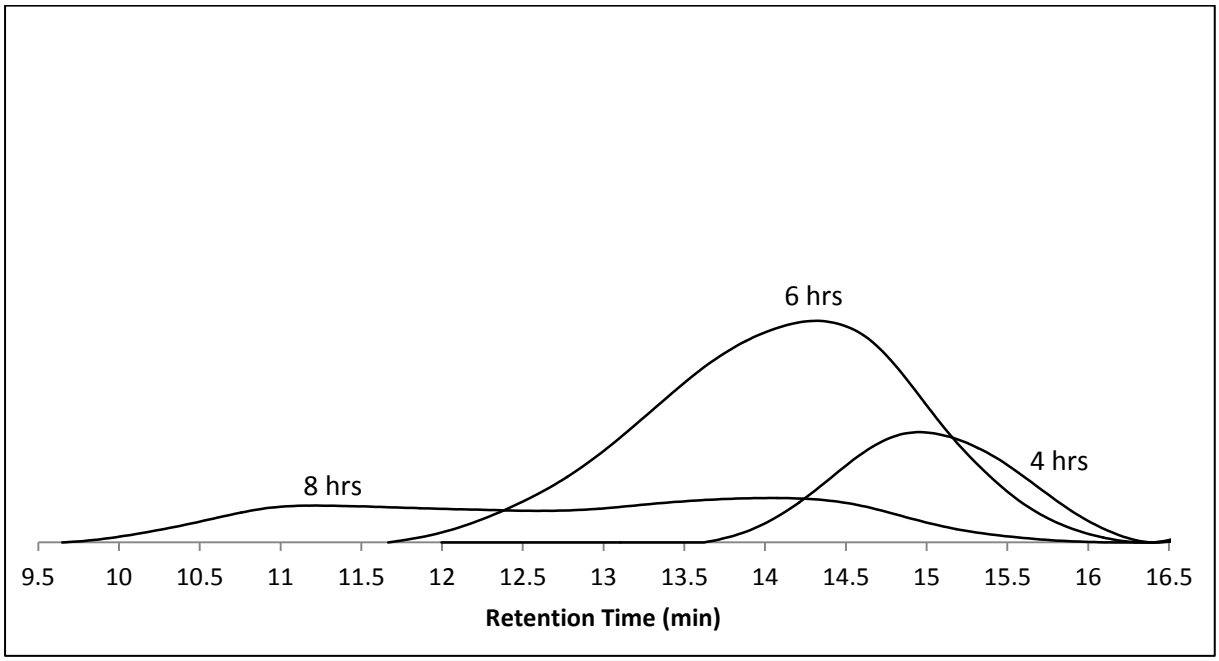

Fig. S2: GPC traces from RI detector for Entry 3, Table 2; Final molecular weight of 370,000 g/mol and a polydispersity of 7.21 after 8 hrs.

Table T2: Copolymerisation of Polyethylene glycol diacrylate (PEGDA – 575 g/mol) and polyethylene methyl ether methacrylate (PEGMEMA – 500 g/mol) via RAFT polymerisation (Entry 3, Table 2).

Entry	I : RAFT Agent : M1: M2 [a]	SR (v/v) [b]	RT [c]	Monomer conversion (%) [d]	M_w (kDa) [e]	PDI [f]
1	0.8 : 1.6 : 25 : 50	1:3	4	19	15	1.34
2	0.8 : 1.6 : 25 : 50	1:3	6	24	50	2.06
3	0.8 : 1.6 : 25 : 50	1:3	8	57	370	7.21

[a] I - Initiator (ACHN): RAFT Agent - (2-cyanoprop-2-yl dithiobenzoate), M1:M2 - Monomer feed ratio (PEGDA (M1): PEGMEMA (M2)), [b] SR - Monomer : Solvent (Butanone) (v/v) , [c] Reaction time, [d] Monomer conversion estimated from copolymer and monomer peaks in GPC traces, [e] M_w - Weight Average molecular weight (1kDa = 1,000 g/mol), [f] Polydispersity index (M_w / M_n).

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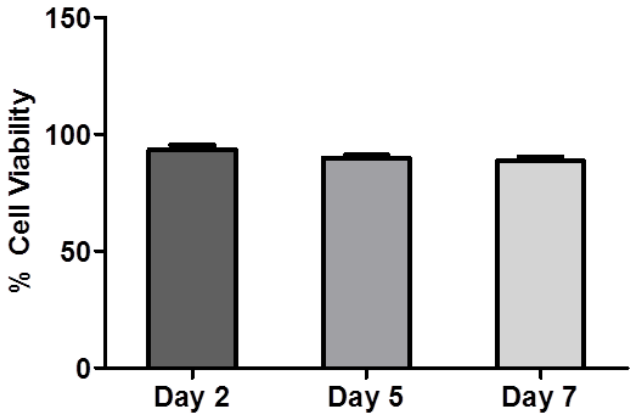

Fig.S3: The LIVE/DEAD® assay Cell Viabillity (Invitrogen) was used to visualize the distribution of living and dead cells in the hydrogel after 2, 5, and 7 days in 3D culture. Fluorescence images were taken using a Zeiss LSM 510 Axiovert Inverted Confocal Microscope.

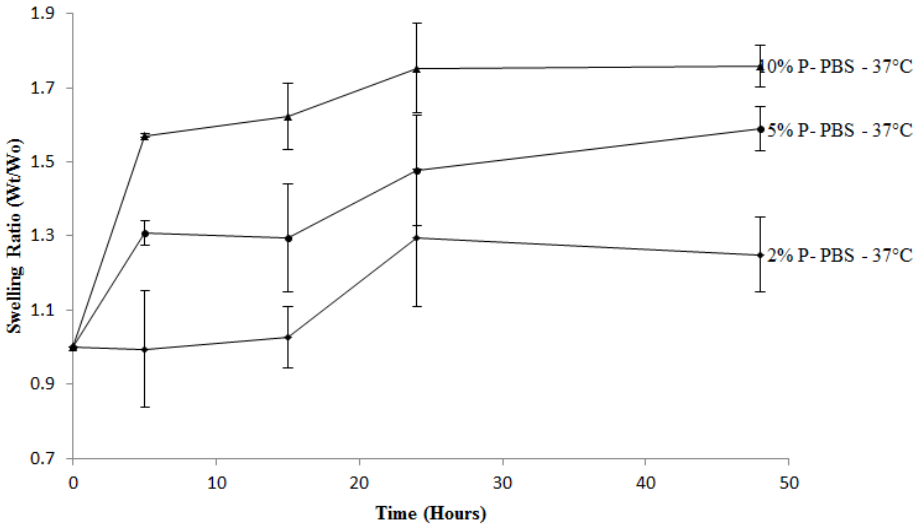

Fig. S4: Swelling studies carried out on polymer hyaluronan hydrogels in PBS (1M, pH 7.4) at 37°C during 48 hours. The higher polymer concentration in the hydrogels, the higher the swelling ratio resulted at 37 °C (i.e. 10%>5%>2%). (Mean ± SD, *n*=*6*).

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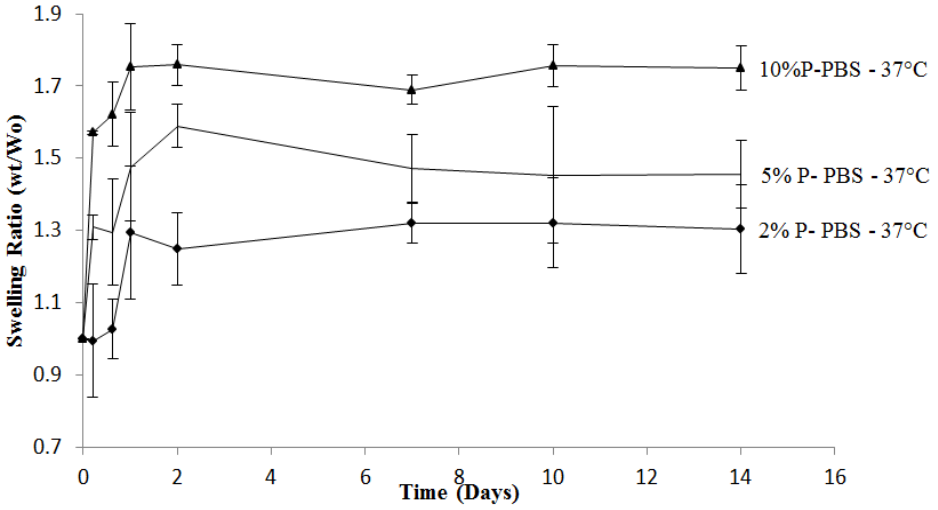

Fig. S5: Swelling studies carried out on polymer hyaluronan hydrogels in PBS (1M, pH 7.4) at 37°C during 14 days . (Mean ± SD, *n=6*).

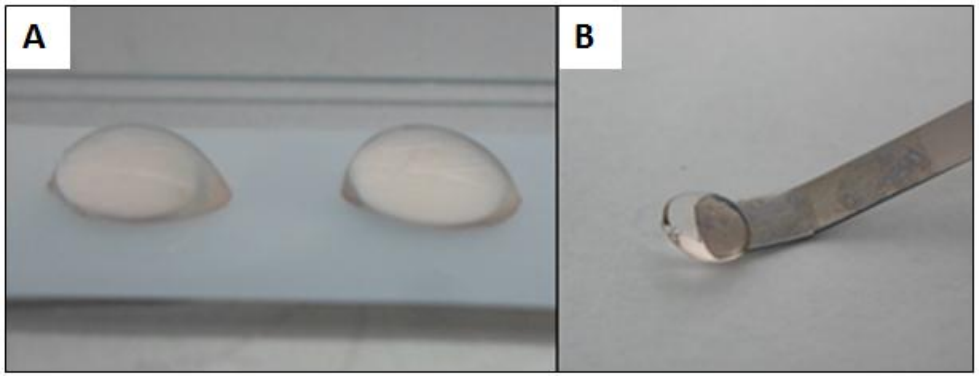

Fig. S6: (A) 40µl hydrogel formed *in situ* on Teflon Tape using (copolymer – Entry 1, table 2) **(B)** 40 µl hydrogel illustrating good workability and ease of use (easily picked up without deformation).

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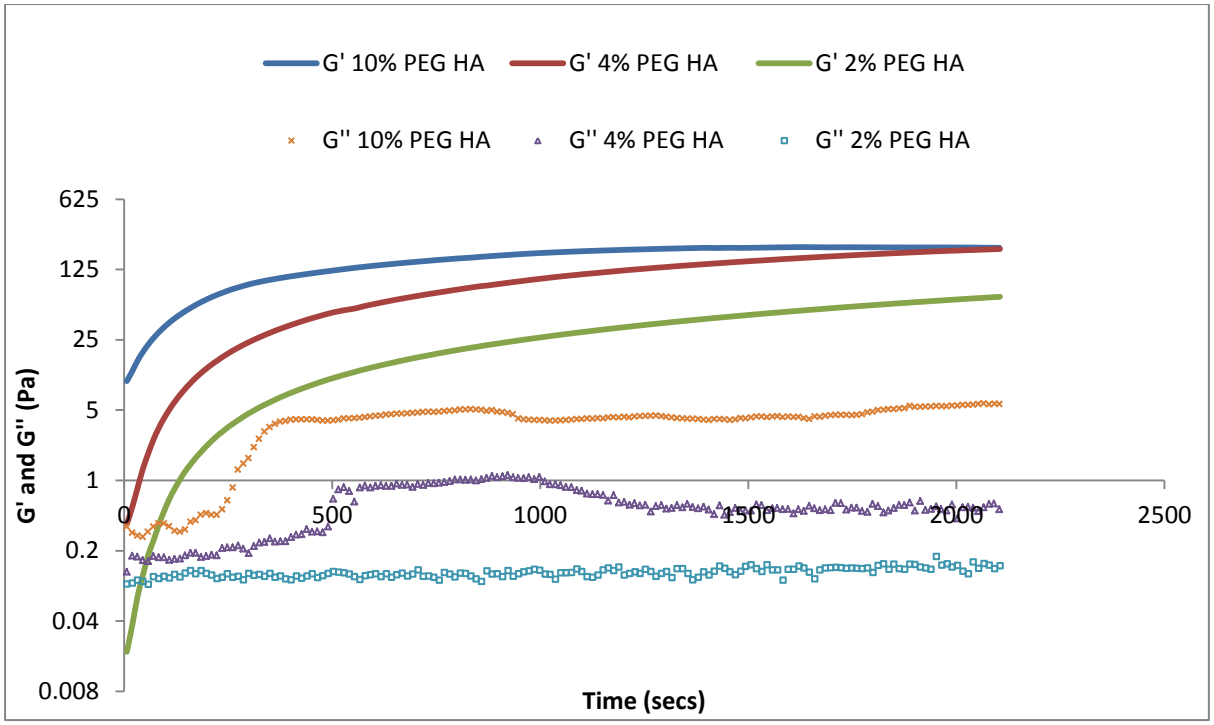

Fig.S7: A rheology study was performed obtaining the shear storage (G') and shear loss modulus (G'') to demonstrate the quick gelation of the crosslinked networks using 10%, 4% and 2% final copolymer concentrations. The chemical crosslinking system cannot be precisely determined because the mixing of components (Copolymer (Entry 1, Table 2) and HA-SH) is required before loading the samples onto the plate of the rheometer and the reaction has already begun. (Ratio of Vinyl: Thiol - 1.3:1, 3:1, 12:1 for polymer samples at the concentration of 2%, 5% and 10% w/v respectively).