Supporting Information

Rapid Bioorthogonal Chemistry Enables *in Situ* Modulation of Stem Cell Behavior in 3D without External Triggers

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Keywords: Michael addition; tetrazine ligation; hydrogel; synthetic extracellular matrices; mesenchymal stem cells.

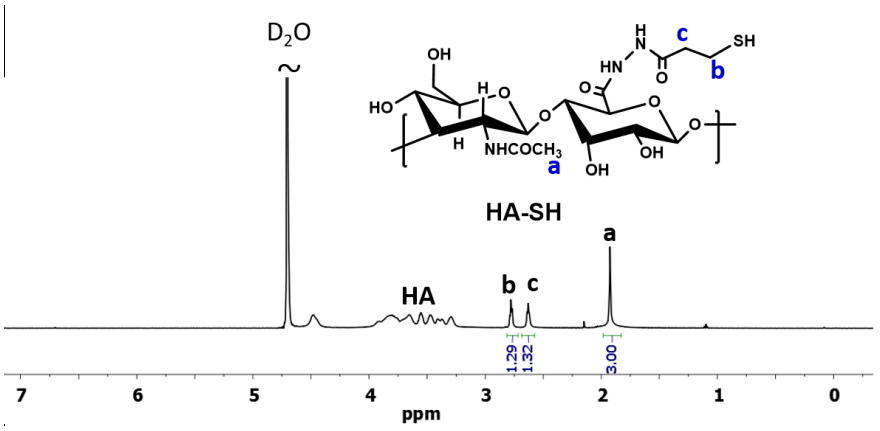
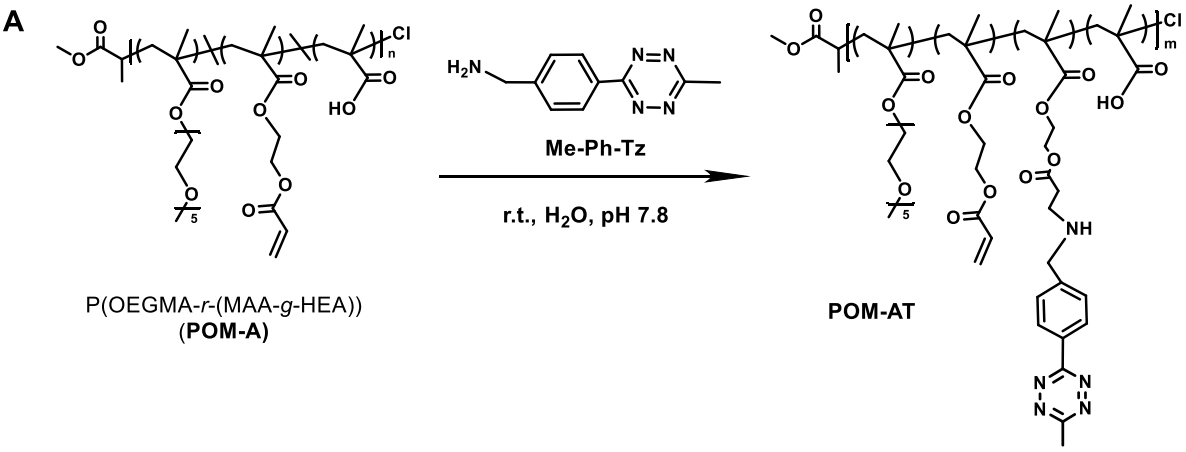
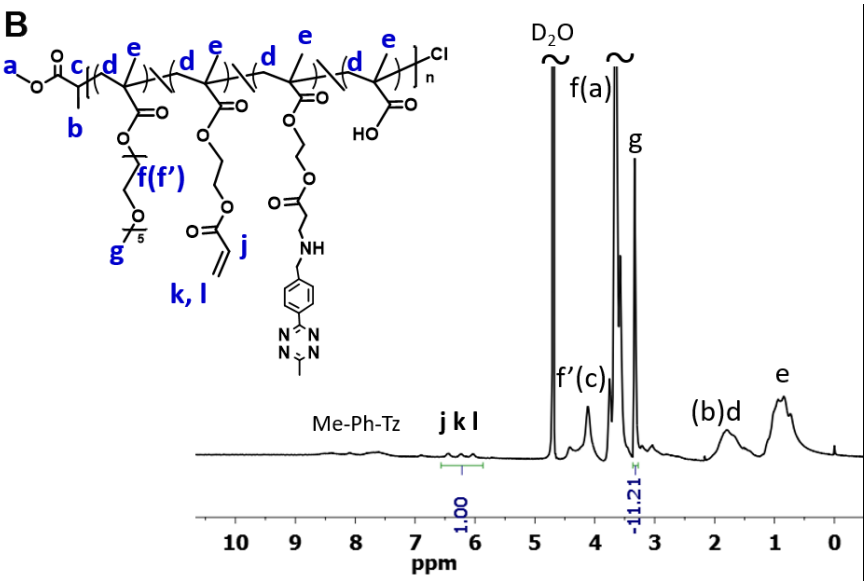


Figure S1: Structure and ^{1}H NMR spectrum of HA-SH in D_2O.





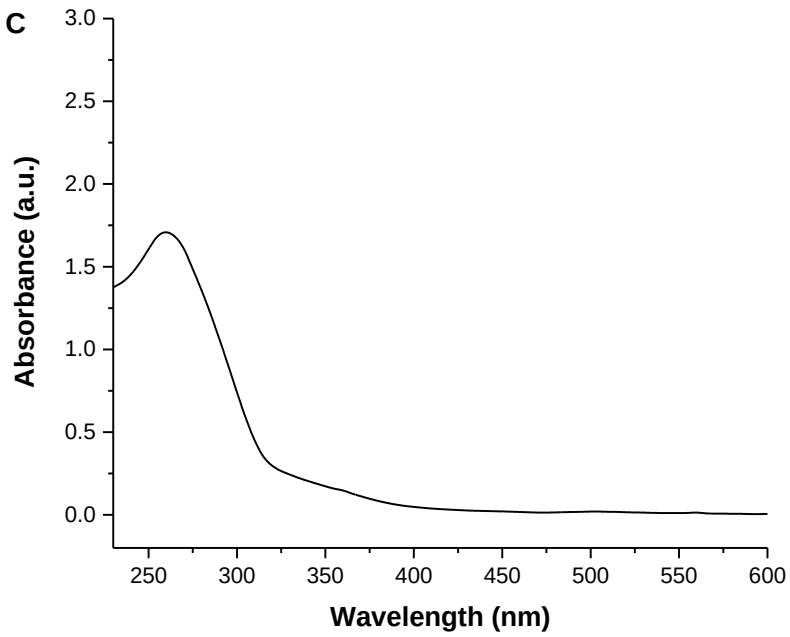
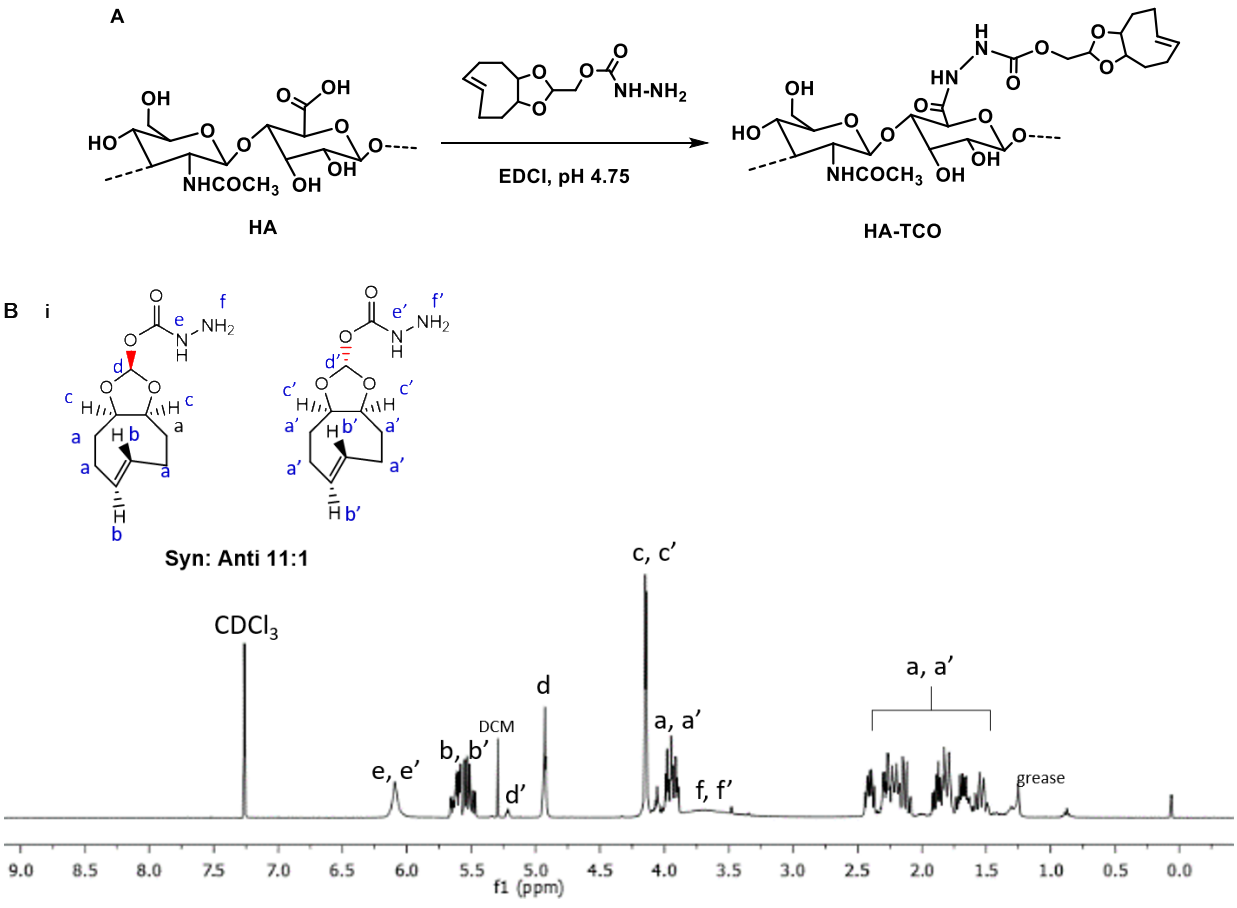


Figure S2: (A): Synthesis of POM-AT. (B): ^{1}H NMR spectrum of POM-AT in D_2O. (C): UV-vis spectrum of POM-AT in H_2O (λ_{max} 260 nm).



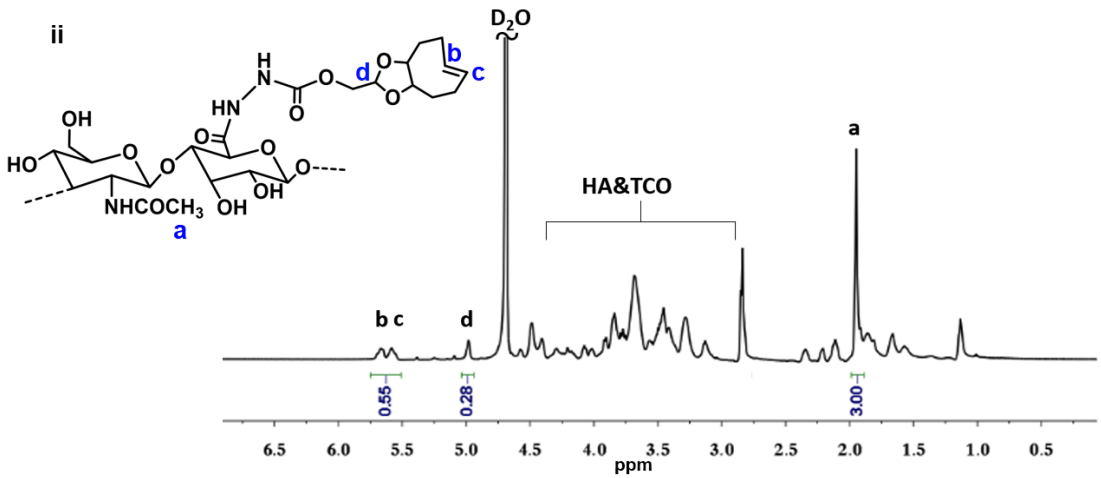
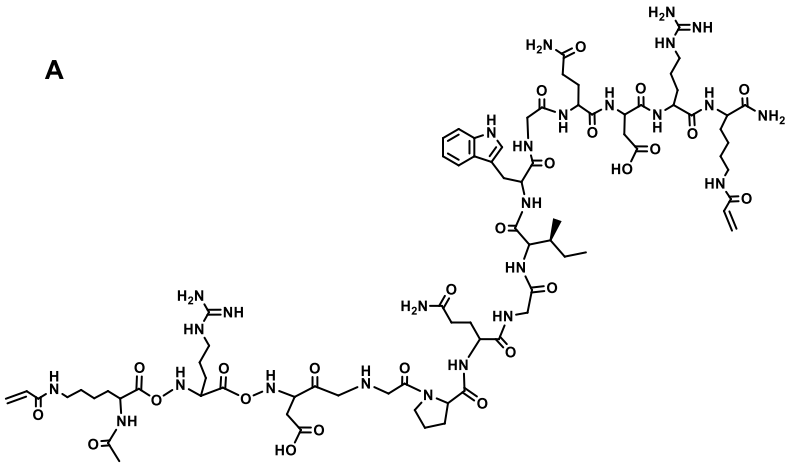
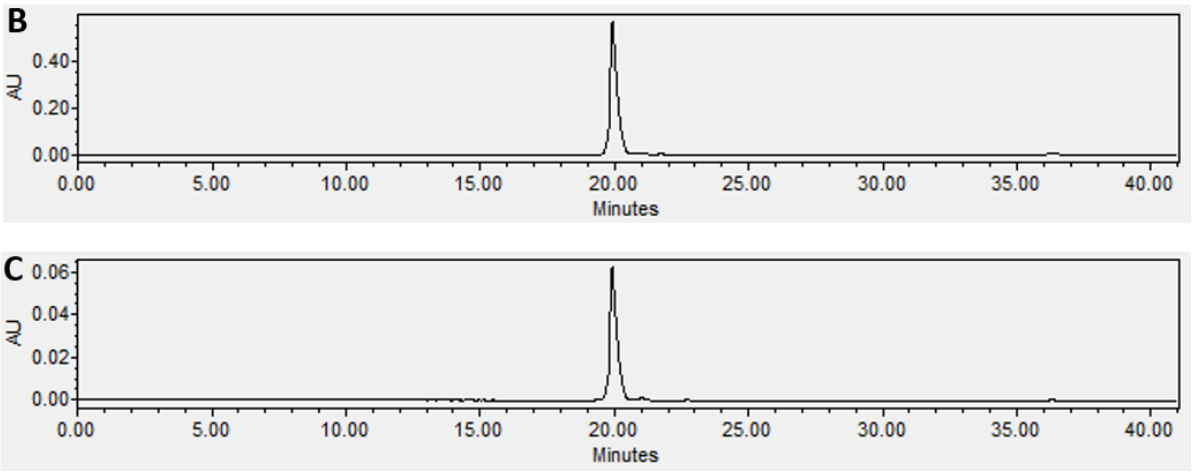


Figure S3. (A): Synthesis of HA-TCO. (B): ^{1}H NMR spectrum of TCO-hydrazide in $CDCl_3$ (i) and HA-TCO in D_2O (ii).





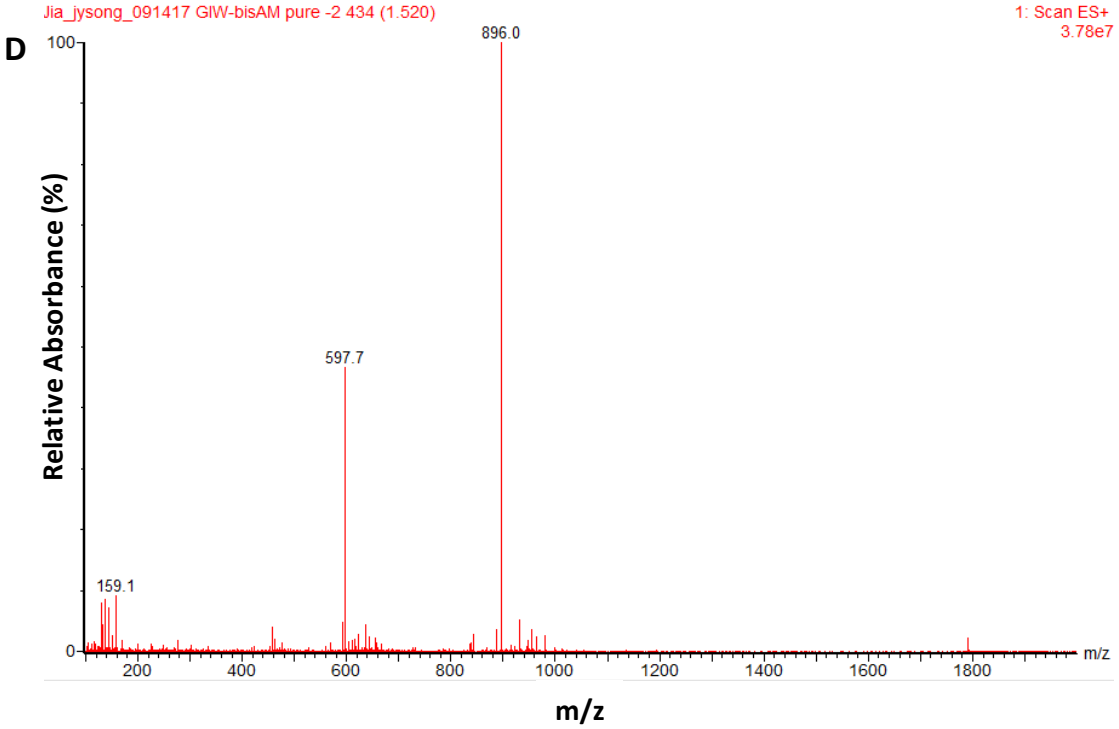
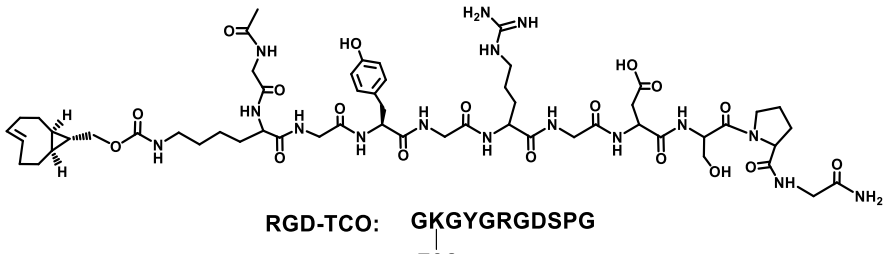
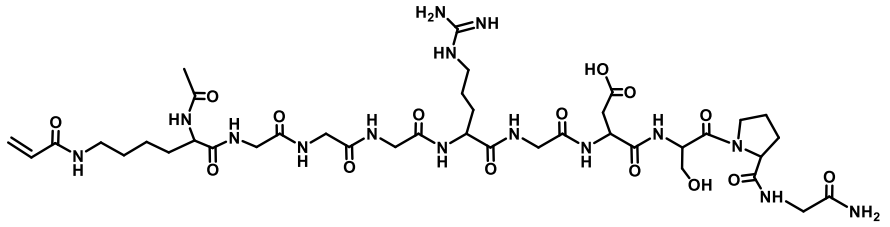


Figure S4. (A): Chemical structure of MMP-cleavable peptide crosslinker, GIW-bisAM; (B, C): Analytical HPLC chromatograms of GIW-bisAM monitored at λ= 214 (B) 280 (C) nm; (D): ESI-MS (positive ion mode) of GIW-bisAM (1790 g/mol).



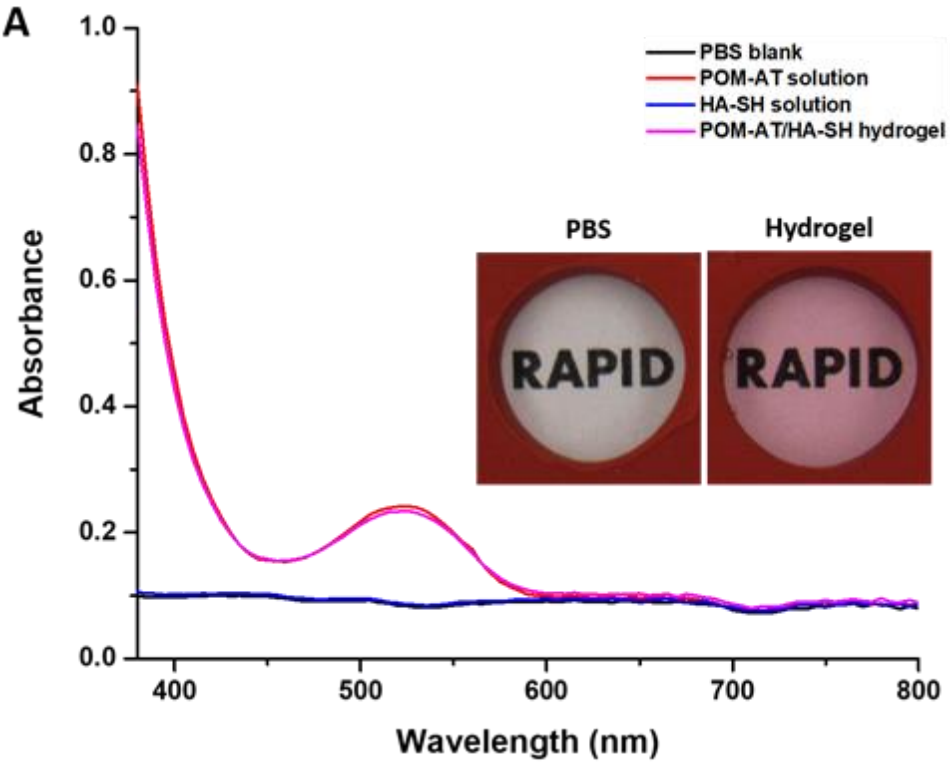
RGD-TCO: GKGYGRGDSPG
TCO



RGD-AM: AM-KGGGRGDSPG

Figure S5. Chemical structures of RGD-TCO and RGD-AM.

Hydrogel Homogeneity. Hydrogel homogeneity was characterized spectroscopically and microscopically.[1-2] First, stock solutions of HA-SH (20 mg/mL) and POM-AT (40 mg/mL) were mixed at a volume ratio of 23/77 and pH was adjusted to 7.9 using 1 mM NaOH. The mixture was introduced between two glass slides with a 2.5-mm silicone spacer. Homopolymer controls were similarly prepared using HA-SH or POM-AT alone at the respective final gel concentrations. The absorbance was monitored at 380-800 nm at room temperature with a path length of 2.87 mm using a UV-vis spectrometer (Agilent Technologies, Cary 60). Separately, hydrogels were prepared as described above. Two hours later, hydrogels were introduced to an aqueous bath containing Cy3-TCO (0.2 μM, Ex: 555 nm; Em: 565 nm, AAT Bioquest) to label POM via interfacial tetrazine ligation for 24 h. Samples were inspected using a Zeiss LSM 880 confocal microscope. Images were recorded using a Z-stack interval of 5 μm and 16.99 μm with 20X and 5X objectives, respectively.



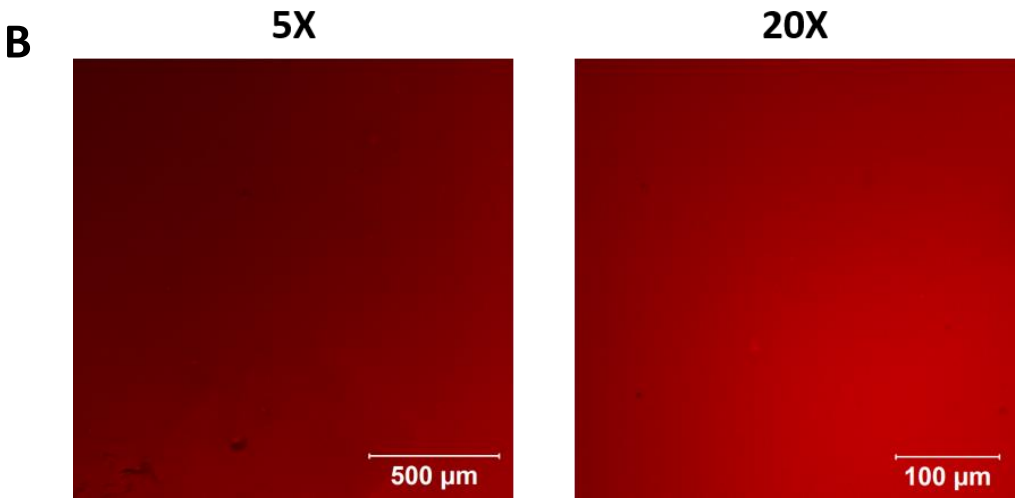


Figure S6. Characterization of hydrogel homogeneity by UV-vis spectroscopy (A) and confocal microscopy (B). (A): UV-vis absorbance from blank PBS, POM-AT solution, HA-SH solution and the hydrogel derived from Michael addition using HA-SH and POM-AT. Inserts show the appearance of the word "RAPID" below the blank PBS solution the hydrogel. (B): Representative confocal z-stack images of Cy 3-labled POM/HA gels.

Hydrogel Degradation. Degradation of Michael gels containing GIW crosslinks was analyzed gravimetrically. Hydrogel disks were immersed in PBS with or without Collagenase type IV (100 U/mL). The degradation medium was refreshed every day. At a predetermined time, hydrogel mass was recorded and mass loss was determined based on the initial gel mass at time zero. Three independent measurements were averaged for each sample. Results were expressed as the mean ± standard deviation (SD).

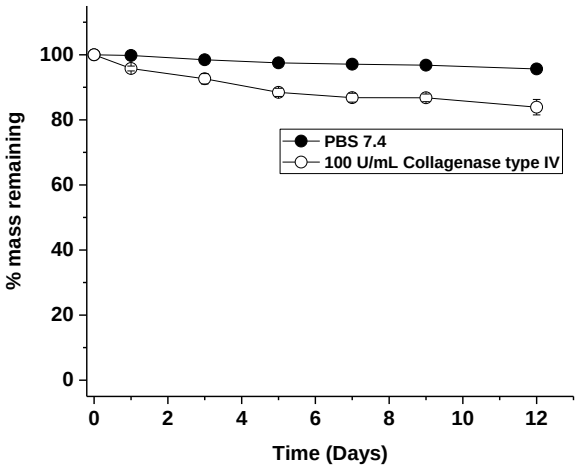


Figure S7. Gravimetrical characterization of hydrogel degradation in the presence or absence of Collagenase type IV.

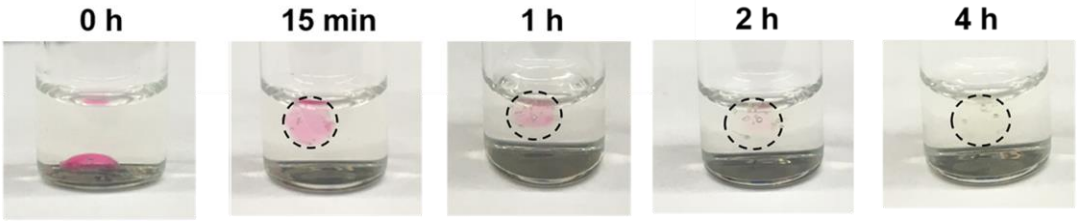


Figure S8. Photographs capturing the establishment of the secondary tetrazine network. A Michael gel disk was dropped in a bath of HA-TCO. Tetrazine ligation occurred at the gel-liquid interface to introduce additional crosslinking. Black circle outlines the gel border.

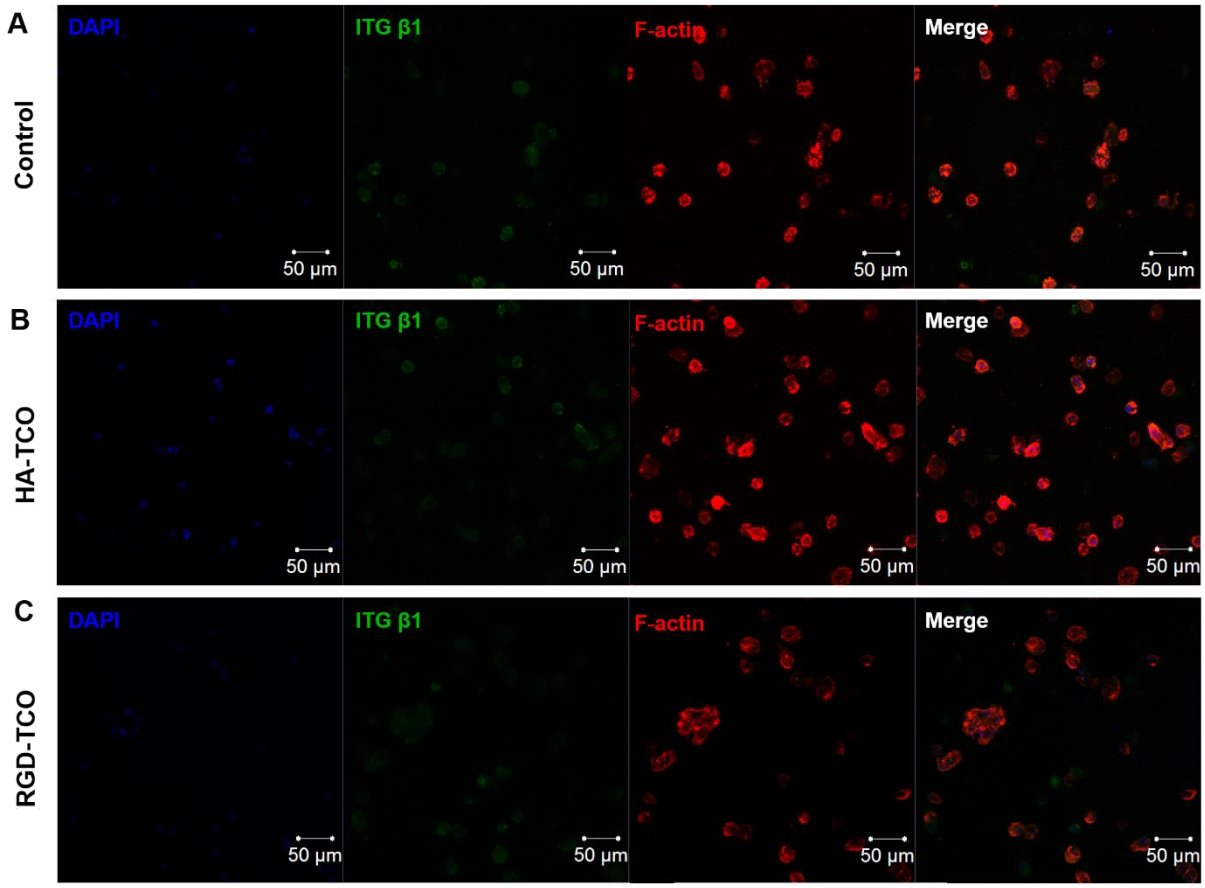


Figure S9: Representative confocal images of immunofluorescently stained hMSC cultured in GIW-free Michael gel controls (A) or HA-TCO (B) or RGD-TCO (C) treated cultures. HA-TCO or RGD-TCO were introduced on day 2 and images were captured after 9 days of 3D culture. Cell nuclei, ITG β1 and F-actin, were stained blue, green and red, respectively.

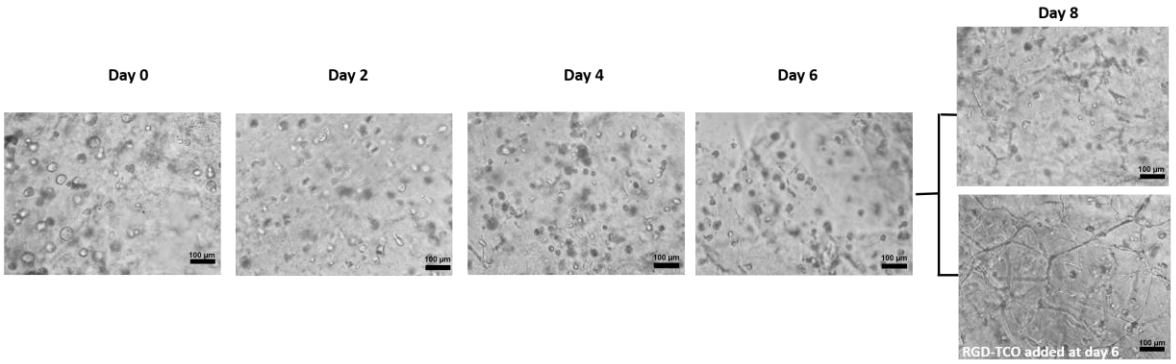


Figure S10: Effects of delayed tagging at day 6 on hMSC morphology. hMSCs were cultured in MMP-degradable Michael gels until day 6, at which time RGD-TCO was introduced to the media and constructs were maintained until day 8.

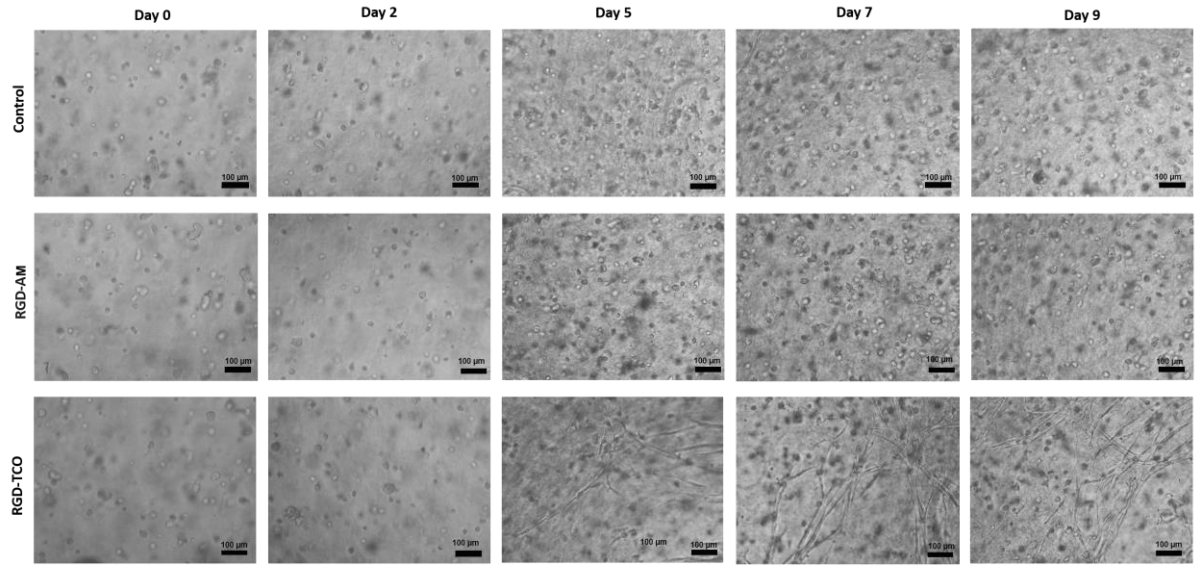


Figure S11. Representative brightfield microscopy images of hMSC cultured in MMP-degradable Michael gels and treated with RGD-AM or RGD-TCO at day 2. Scale bar: 100 µm.

References

(1) Lau, H. K.; Li, L.; Jurusik, A. K.; Sabanayagam, C. R.; Kiick, K. L. Aqueous Liquid–Liquid Phase Separation of Resilin-Like Polypeptide/Polyethylene Glycol Solutions for the Formation of Microstructured Hydrogels. *ACS Biomater. Sci. Eng.* **2016,** *3*, 757-766.

(2) Abbadessa, A.; Landín, M.; Blenke, E. O.; Hennink, W. E.; Vermonden, T. Two-Component Thermosensitive Hydrogels: Phase Separation Affecting Rheological Behavior. *Eur. Polym. J.* **2017,** *92*, 13-26.