Supplementary Information (SI) for Journal of Materials Chemistry B.

Supplementary Information for

Improving the bioactivity and mechanical properties of poly(ethylene glycol)-based hydrogels through a supramolecular support network

Yuzhu Liu[1], Md Shariful Islam[1], Anna Bakker[2], Zihao Li[3], Alaa Ajam[2], Jamie J. Kruzic[2], Kristopher A. Kilian[1,3]

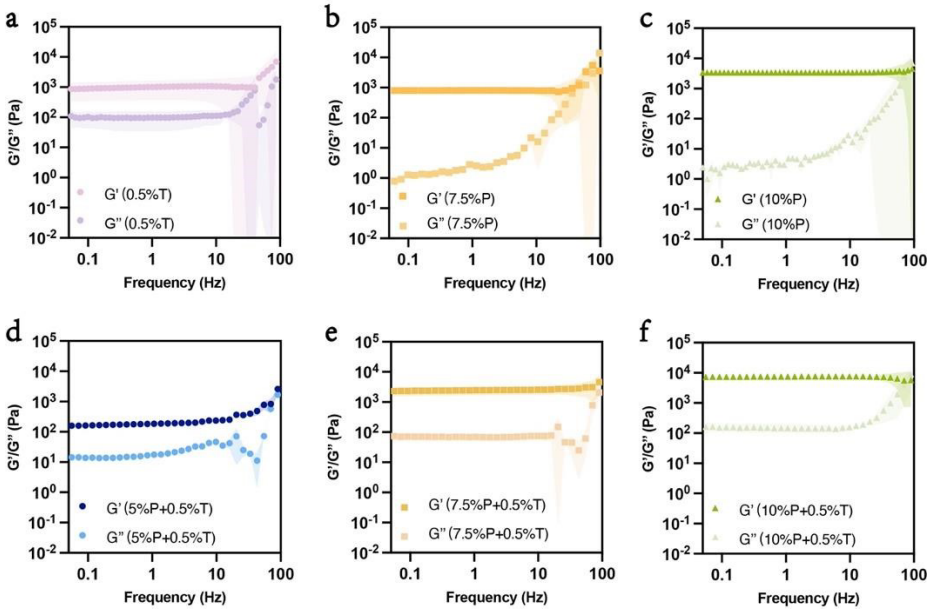

Figure S1. Rheological Analysis of PEGDM/Trpzip Hydrogels. (a-c) Frequency sweep measurements of the storage (G') and loss modulus (G") for hydrogels composed of 0.5% (w/v) Trpzip, 7.5% (w/v) PEGDM, and 10% (w/v) PEGDM, showing the response to increasing strain. (d-f) Frequency sweep measurements of the storage (G') and loss modulus (G") for hybrid hydrogels with 5%, 7.5%, and 10% (w/v) PEGDM combined with 0.5% (w/v) Trpzip, indicating the influence of Trpzip on the mechanical behavior and frequency tolerance of the PEGDM network. All tests were performed in triplicate (n=3).

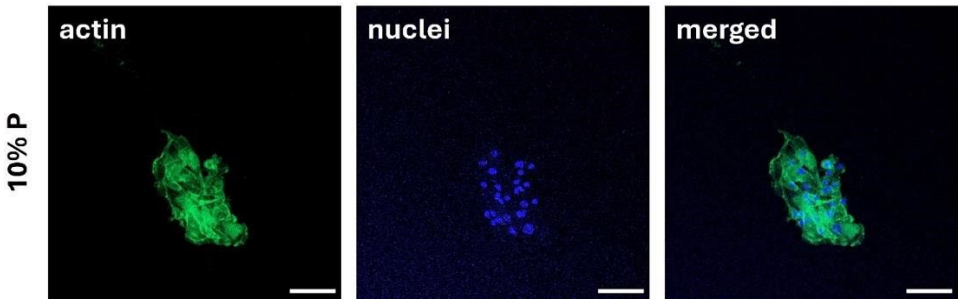

Figure S2. Morphology and Quantitative Analysis of ADSCs Cultured on PEGDM Hydrogels After 2 Days. Confocal microscopy images of adiposederived stem cells (ADSCs) cultured on 10% (w/v) PEGDM hydrogel formulations stained for nuclei (blue, DAPI) and actin (green, phalloidin) after 2 days. Scale bar: 100 μm.

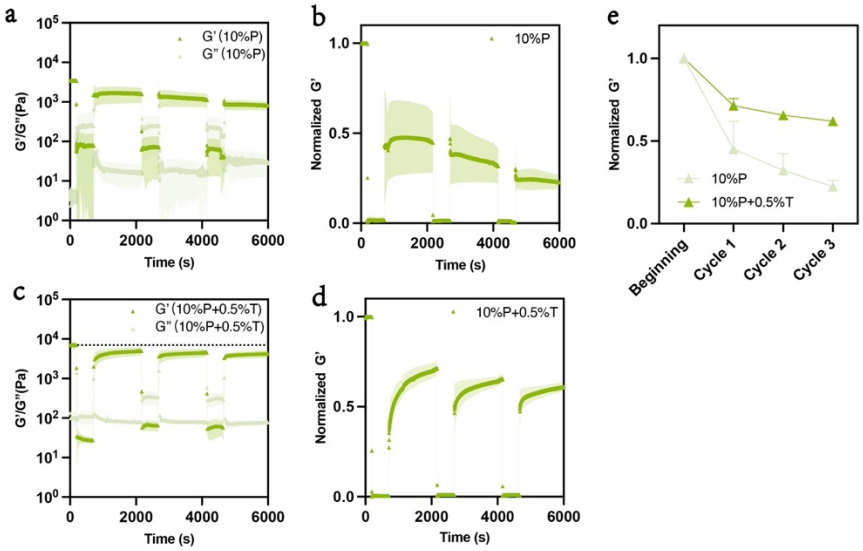

Figure S3. Self-healing properties of PEGDM/Trpzip hydrogel. (a) and (c) are thixotropic measurements of storage modulus (G') and loss modulus (G'') of 10% (w/v) PEGDM and 10%PEGDM/ 0.5% (w/v) Trpzip hydrogels. Their normalized storage modulus (G') changes are shown in (b) and (d). (e) The changes of the normalized mean value of storage modulus (G') in each cycle of 10% (w/v) PEGDM and 10%PEGDM/ 0.5% (w/v) Trpzip hydrogels. All tests were performed in triplicate (n=3)

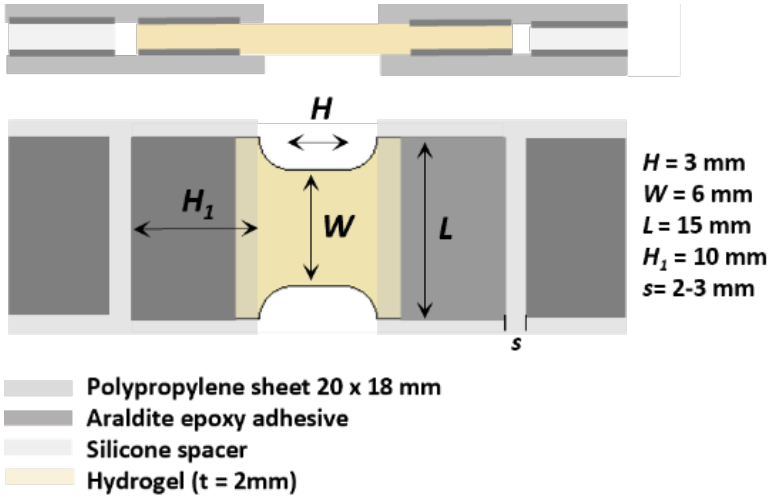

Figure S4. Tensile testing sample design.

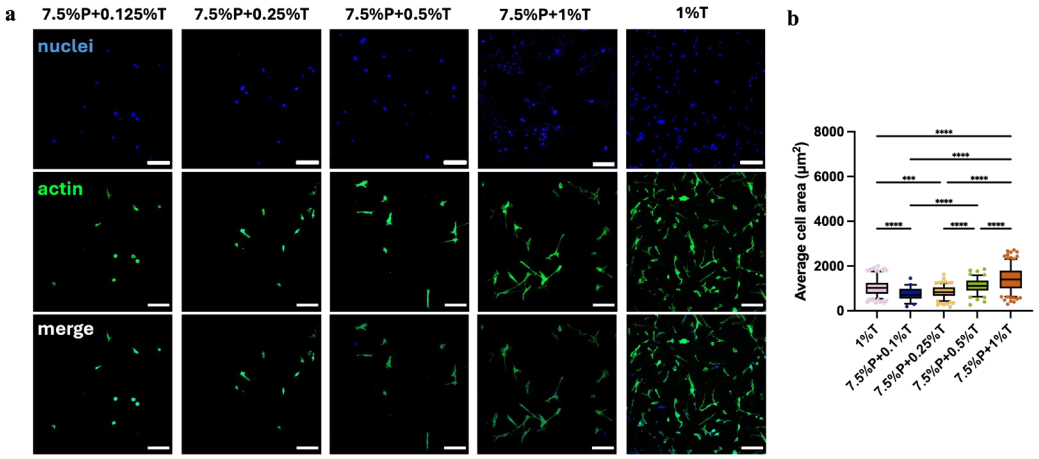

Figure S5. Morphology and Quantitative Analysis of ADSCs Cultured on PEGDM/Trpzip Hydrogels After 2 Days. (a) Confocal microscopy images of adipose-derived stem cells (ADSCs) cultured on various hydrogel formulations stained for nuclei (blue, DAPI) and actin (green, phalloidin) after 2 days. The different hydrogel formulations include 0.5% (w/v) Trpzip, 7.5% PEGDM + 0.125% Trpzip, 7.5% PEGDM + 0.25% Trpzip, 7.5% PEGDM + 0.5% Trpzip, and 7.5% PEGDM + 1% Trpzip. Scale bar: 100 μm. (b) Column plot showing the average cell area (μm²) of ADSCs across different hydrogel formulations. (c) Box plot depicting the aspect ratio of ADSCs on various hydrogels, providing insights into cell elongation. (d) Box plot representing cell roundness on different hydrogel formulations, indicating the degree of circularity of ADSCs. Data were collected from 100 cells per condition, with whiskers showing the 5th to 95th percentile. Statistical significance was determined using one-way ANOVA with p-values indicated by '*'p<0.1, '**'p<0.01, '***'p<0.001, '****'p<0.0001.

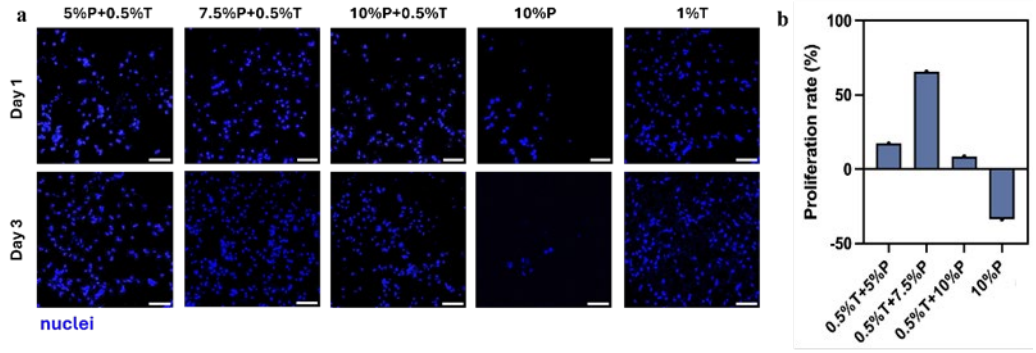

Figure S6. Cell proliferation of ADSCs Cultured on PEGDM/Trpzip Hydrogels After 1 and 3 Days. (a) Confocal microscopy images of adipose-derived stem cells (ADSCs) cultured on various hydrogel formulations stained for nuclei (blue, DAPI) after 1 and 3 days. The different hydrogel formulations include 0.5% (w/v) Trpzip, 5% PEGDM + 0.5% Trpzip, 7.5% PEGDM + 0.5% Trpzip, and 10% PEGDM + 0.5% Trpzip. Scale bar: 100 μm. (b)The proliferation rate of each hydrogel comparing from Day 1 and Day 3 culturing. Statistical significance was determined using one-way ANOVA with p-values indicated by '*'$p<0.1$, '**'$p<0.01$, '***'$p<0.001$, '****'$p<0.0001$.