Supporting Information

Designing an ECM-inspired supramolecular scaffold by utilizing the interactions between minimalistic neuroactive peptide and heparin

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Table S1. Minimum gelation concentration (MGC) and optical images of hydrogels formed by co-assembly of heparin and Tc peptide at different (w/w) ratio.

Tc (mg/mL)	**Heparin (mg/mL)**	**Ratio Tc: heparin**	**Gelation pH**	**Status after 24 hrs**	**Images**
2.5	2.5	1:1	7.0	Sol	
5 (MGC of composite hydrogel)	5	1:1	7.0	Gel	
5	2.5	2:1	7.0	Gel	
5	1	5:1	7.0	Gel	
5	10	1:2	7.0	Gel	
5	0	Control peptide	7.0	Sol	

Table S2. Optical images of hydrogels formed by mixing heparin and Tc peptide at 10 mg/mL concentration of peptide.

Tc (mg/mL)	**Heparin (mg/mL)**	**Ratio Tc: heparin**	**Gelation pH**	**Status after 24 hrs**	**Images**
10	0	Control peptide	7.0	Gel	
10	10	1:1	7.0	Gel	
10	5	2:1	7.0	Gel	
10	2	5:1	7.0	Gel	
10	20	1:2	7.0	Gel	
0	10	Control heparin	7.0	Sol	

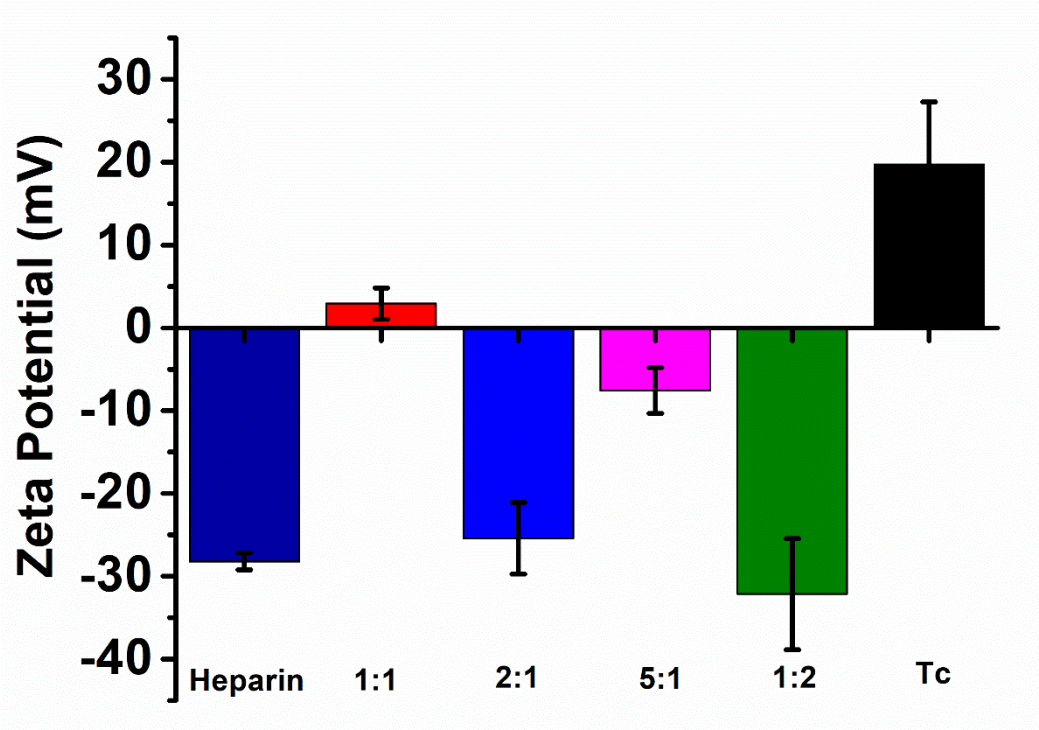

Figure S1. Zeta potential measurement of the combined hydrogels near physiological pH.

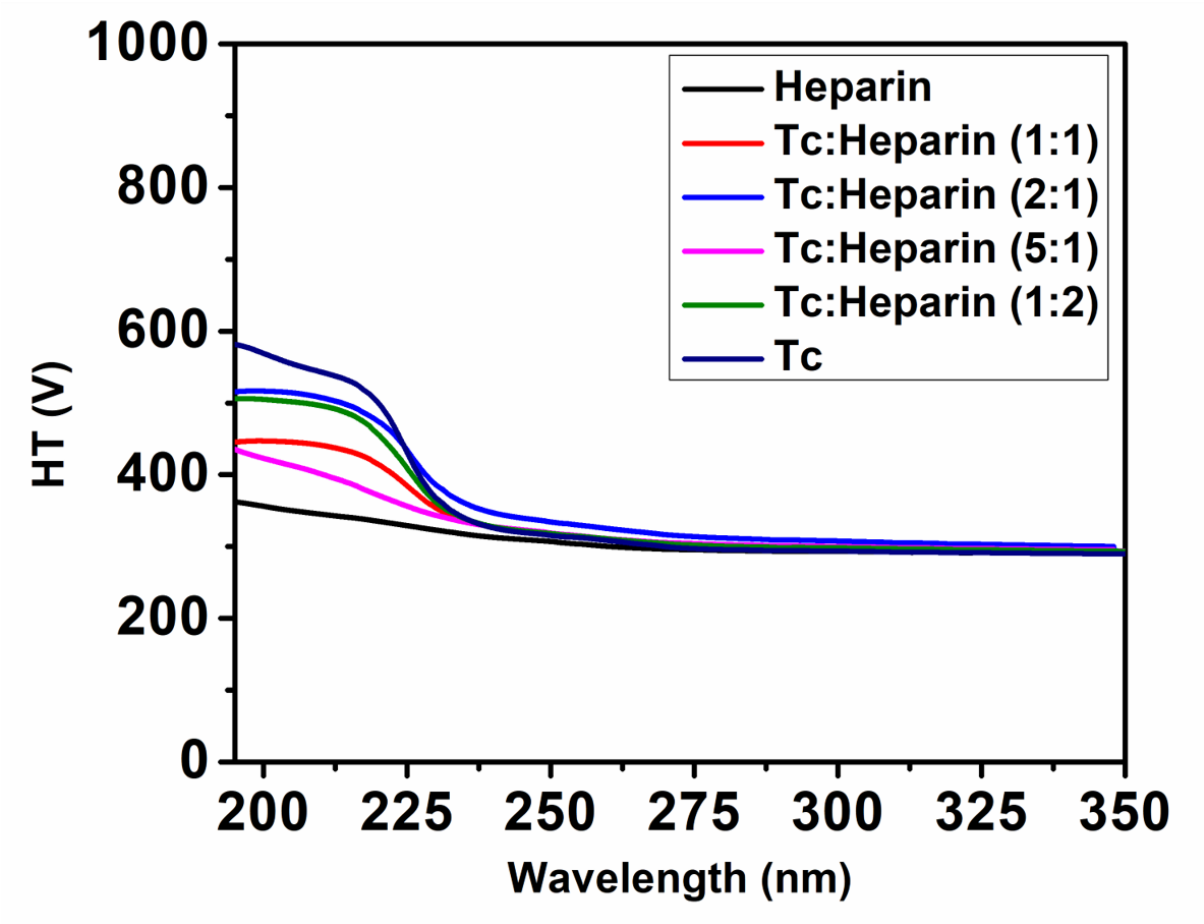

Figure S2. Spectra representing the variation in HT with wavelength for Tc and heparin combined hydrogels at 10 mg/mL concentration.

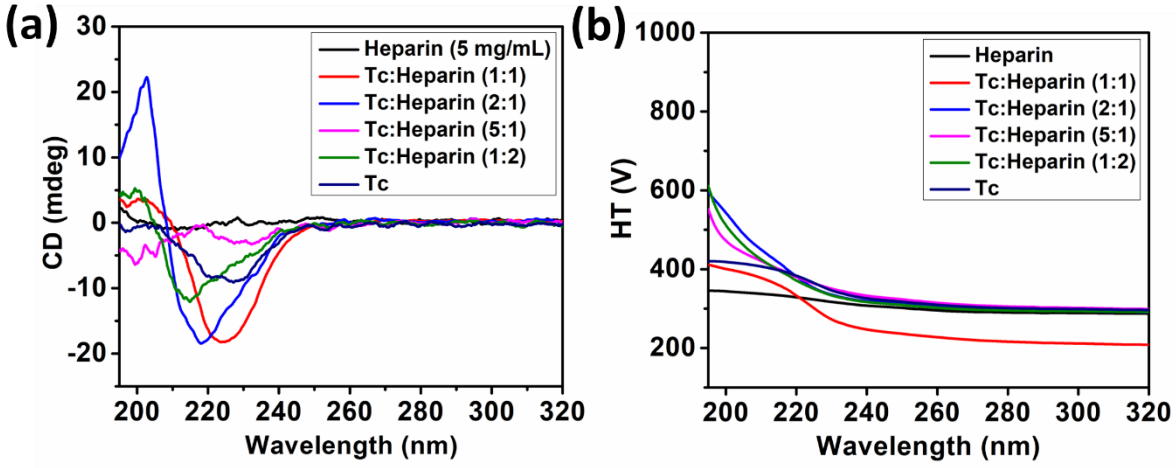

Figure S3. (a) CD spectra of Tc-heparin combined hydrogels at 5mg/mL concentration showing the variation in the supramolecular organization of Tc in the presence of heparin. (b) Spectra representing the variation in HT with wavelength for Tc and heparin combined hydrogels at 5 mg/mL concentration.

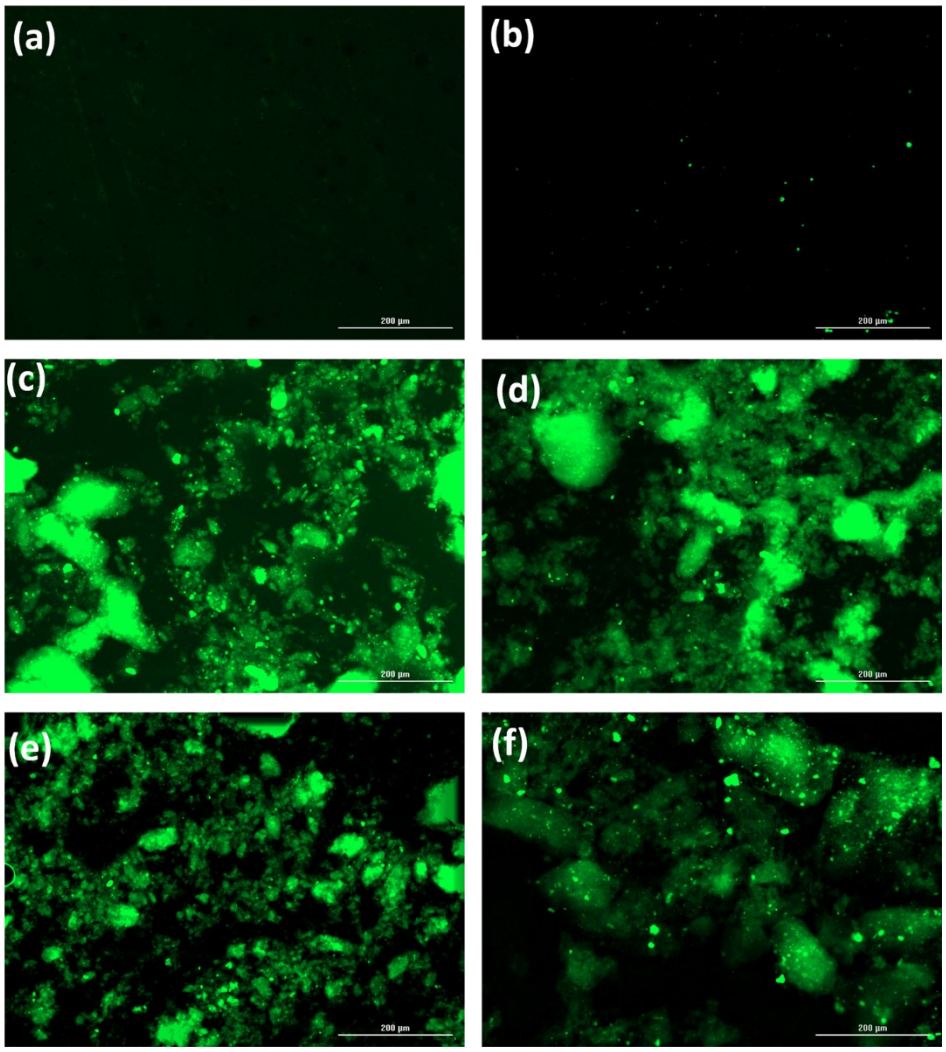

Figure S4. Fluorescence microscopic images of (a) control ThT solution, (b) Heparin, (c) 1:1 combined gel, (d) 2:1 combined gel, (e) 5:1 combined gel, (f) 1:2 combined gel showing binding of hydrogels with ThT. Scale bar is 200 μm.

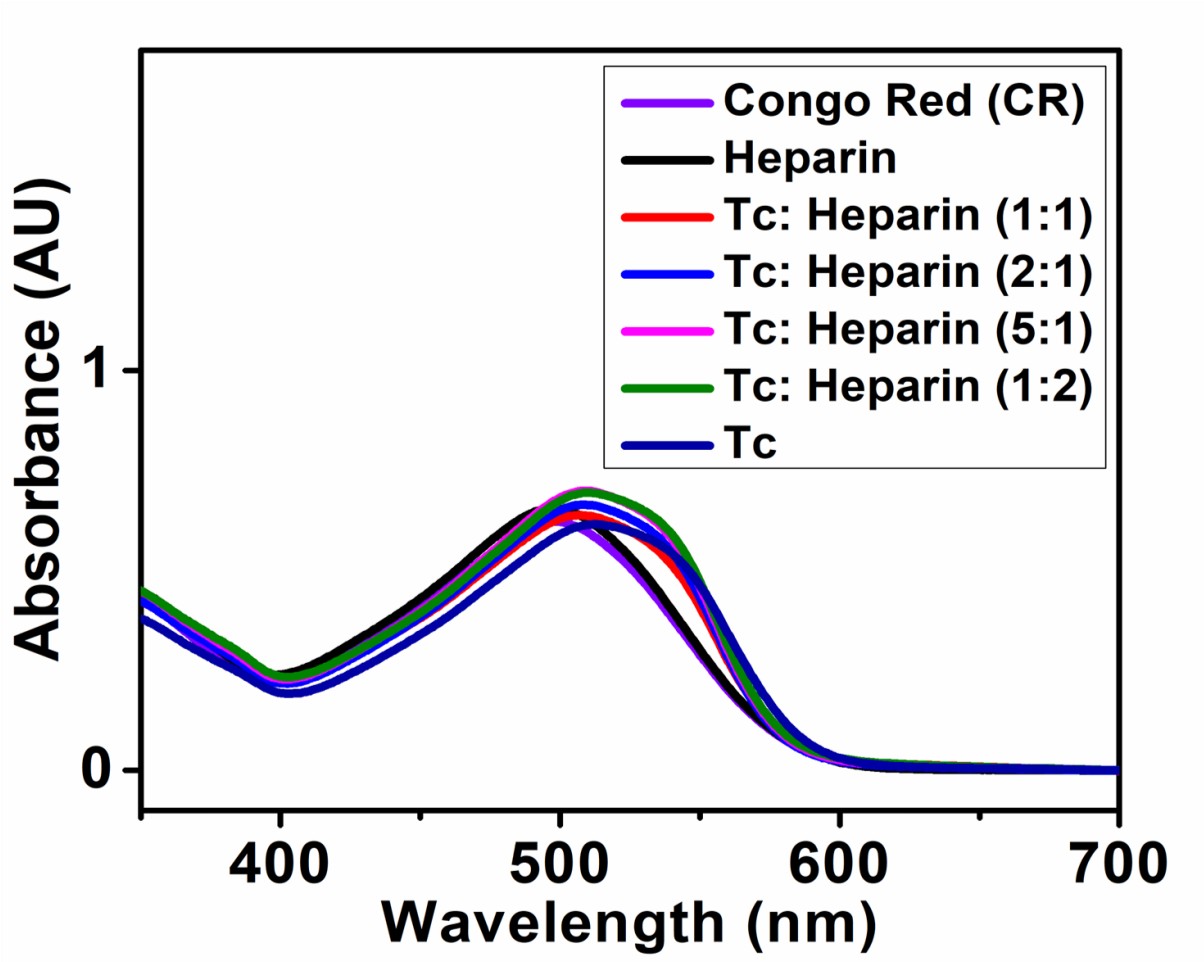

Figure S5. Variation in Congo red spectra upon binding to Tc-heparin combined hydrogel.

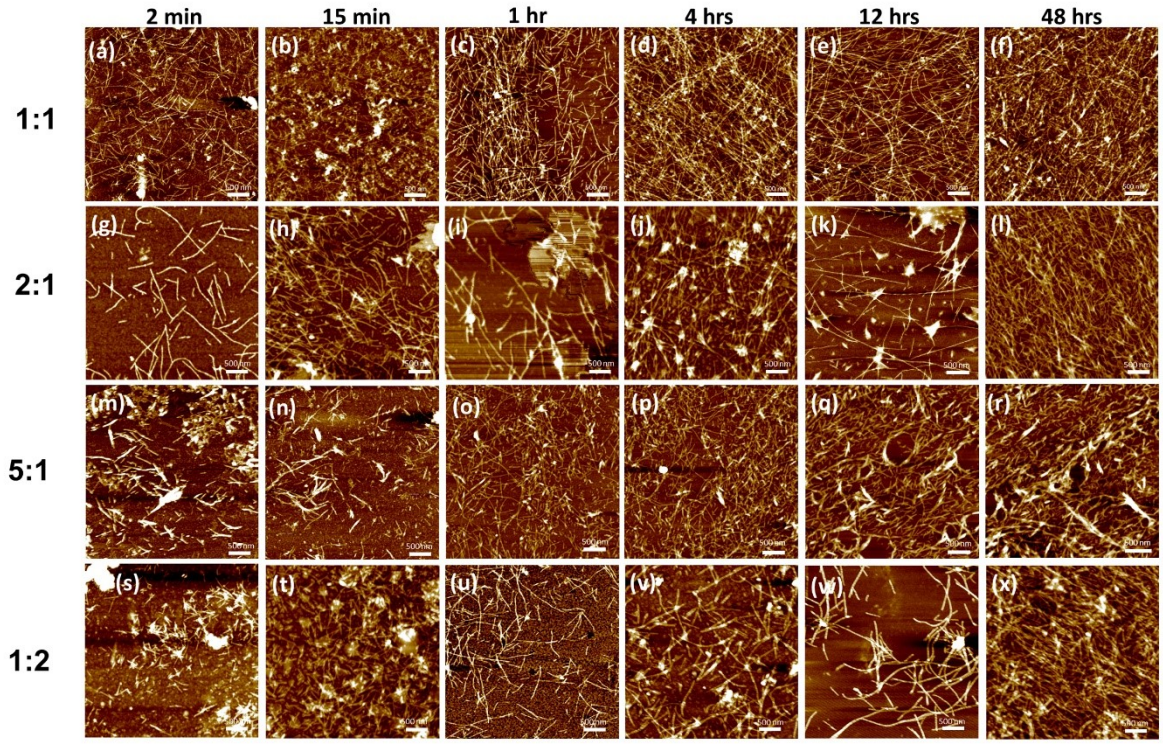

Figure S6. Time dependent AFM images of the combined hydrogels showing the nucleation and growth of the supramolecular nanofibers to form hydrogel scaffold. AFM images of (a-f) 1:1 combined gel, (g-l) 2:1 combined gel, (m-r) 5:1 combined gel and (s-x) 1:2 combined gel. Scale bar is 500 nm.

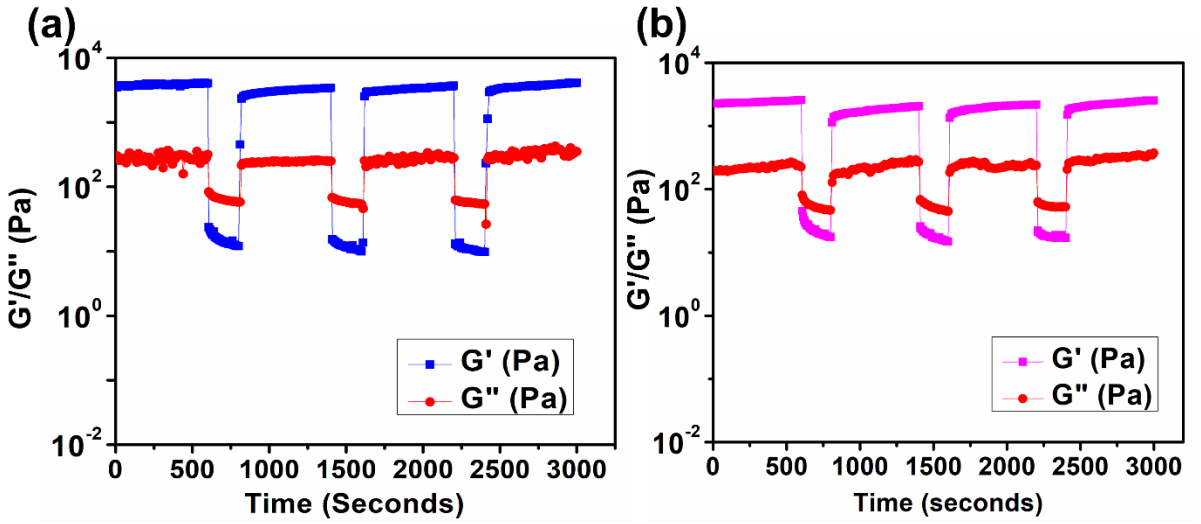

Figure S7. Thixotropic studies of Heparin-Tc co-assembled hydrogels showing mechanoresponsive behaviour. Variation in storage modulus (G') and loss modulus (G") of the (a) 2:1 combined gel and (b) 5:1 combined gel upon application of step strain cycle.

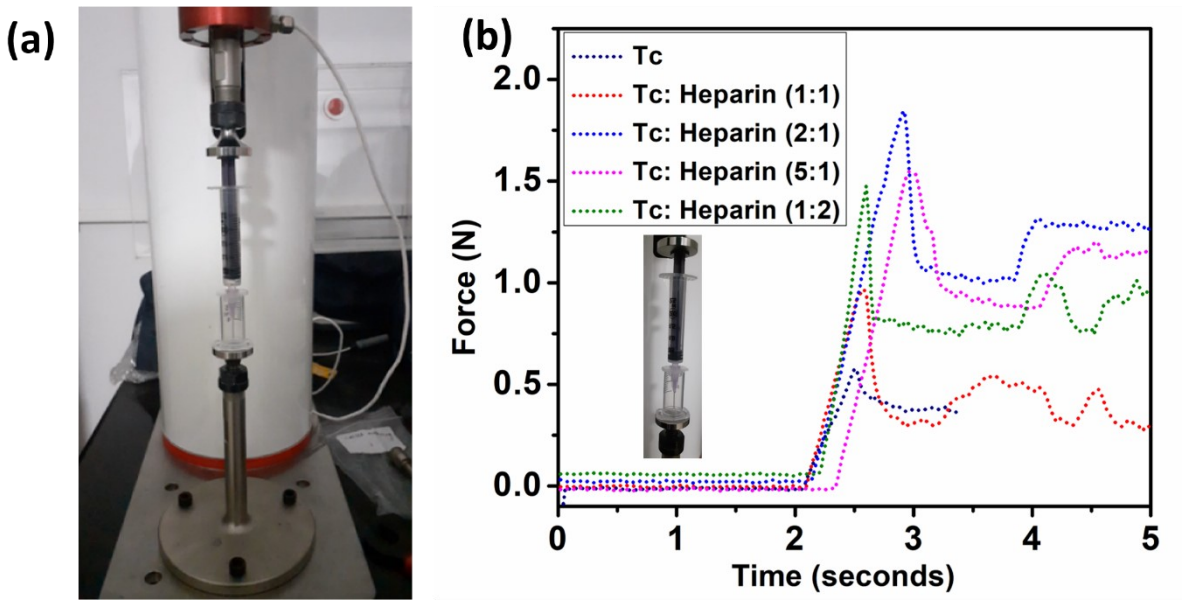

Figure S8. (a) Customized axial compression testing device. (b) The force required to inject the pre-formed hydrogel through 18 G needle from a 2.5 cc syringe at an injection rate of 150 µl/s. Data is represented as force vs time. Inset images show the syringe carrying the hydrogel material attached to custom-made step-up used in the experiment.

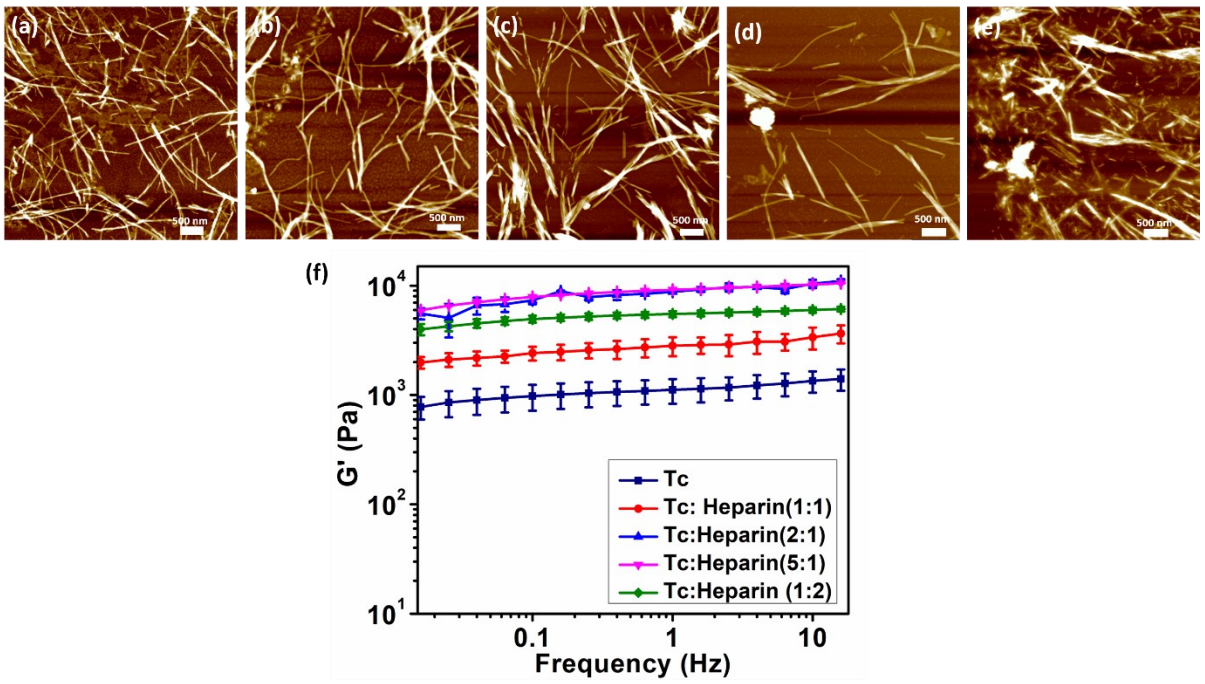

Figure S9. Thermal annealing study of the hydrogel scaffolds at 10 mg/mL concentration. AFM images of (a) 1:1 combined gel, (b) 2:1 combined gel, (c) 5:1 combined gel, (d) 1:2 combined gel, (e) Tc hydrogel after thermal annealing, (f) Frequency sweep analysis of the thermally annealed hydrogels showing the variation in G'(Pa) after heat treatment. Scale bar is 500 nm.

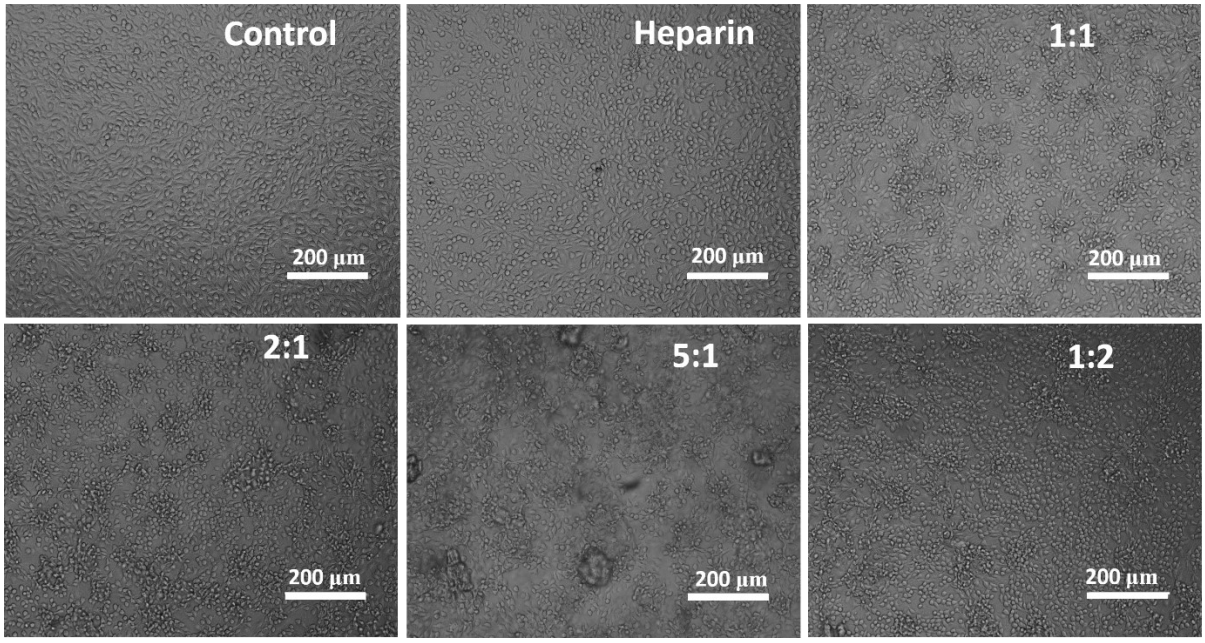

Figure S10. Cell culture images of C6 cells showing the elongated morphology of adhered cells after 48 hours of treatment (1000 µg/mL). Scale bar is 200 µm.

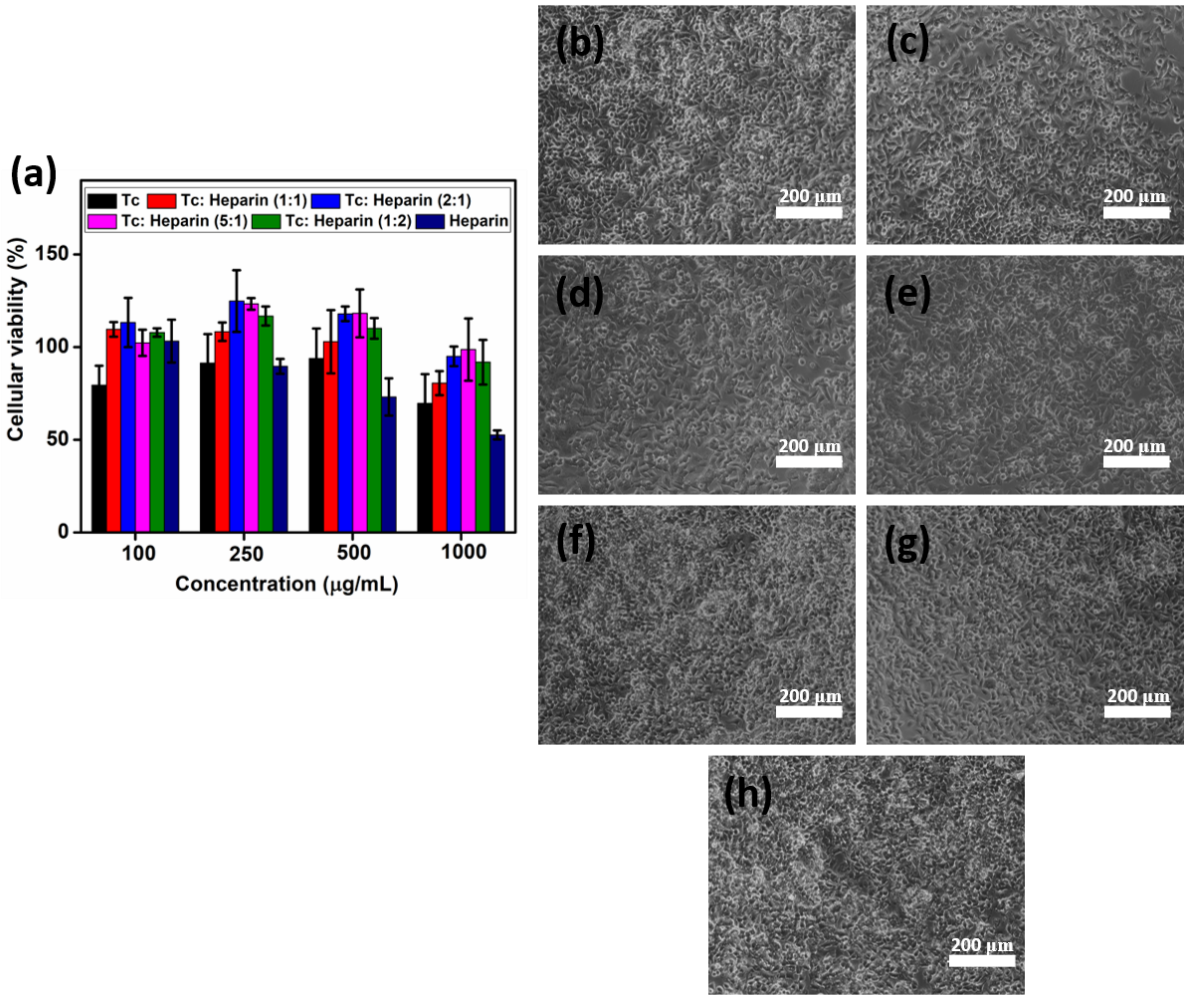

Figure S11. Assessment of cytotoxicity of peptide and combined hydrogels in the oligomeric state at different mixing ratios. (a) Evaluation of cytotoxicity of hydrogel treatment on SH-SY5Y (Human neuroblastoma cell line). Cell culture images showing the morphology of adhered cells after 48 hours of treatment (1000 µg/mL), (b) Heparin, (c) 1:1 combined gel, (d) 2:1 combined gel, (e) 5:1 combined gel, (f) 1:2 combined gel, (g) Tc hydrogel. Scale bar is 200 µm.

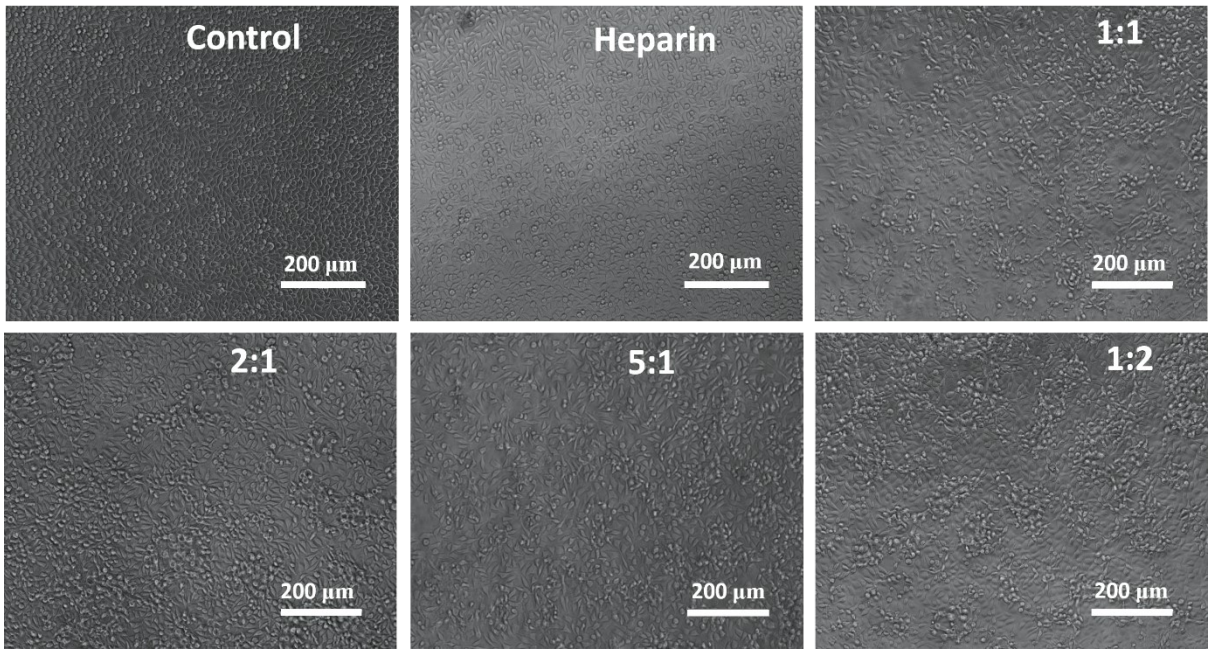

Figure S12. Cell culture images of l929 cells showing the elongated morphology of adhered cells after 48 hours of treatment (1000 µg/mL) . Scale bar is 200 µm.

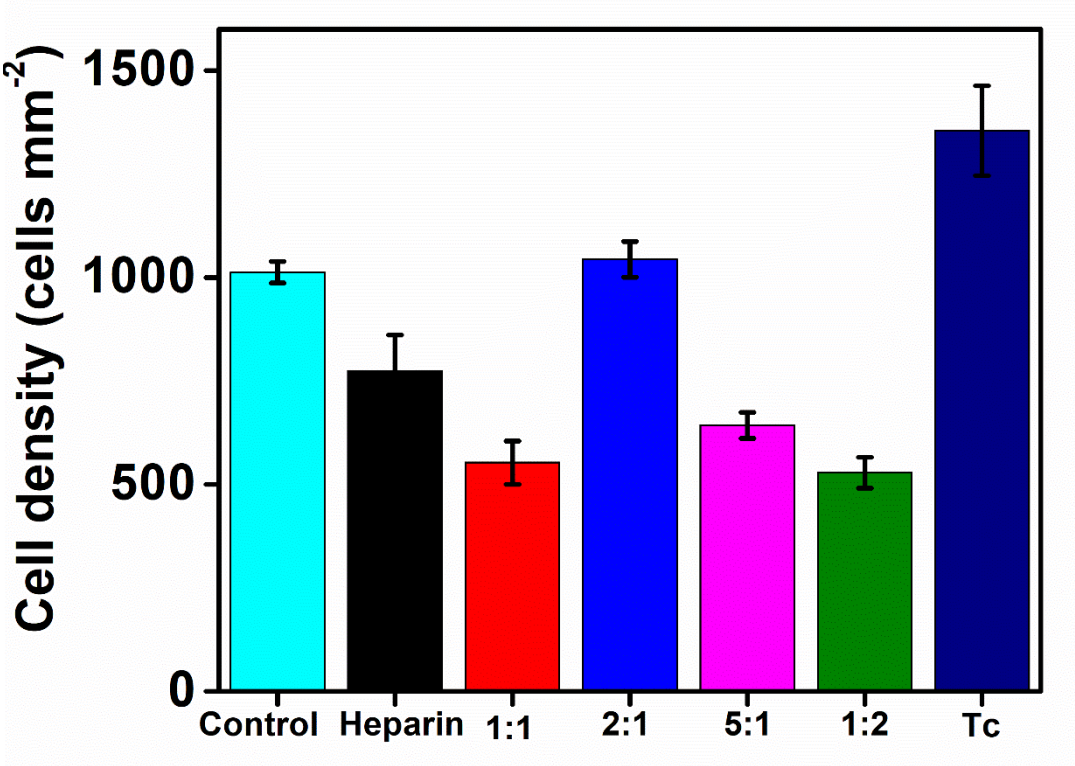

Figure S13. Quantification of cellular density from confocal images of C6 cells after 5 days of cell seeding.

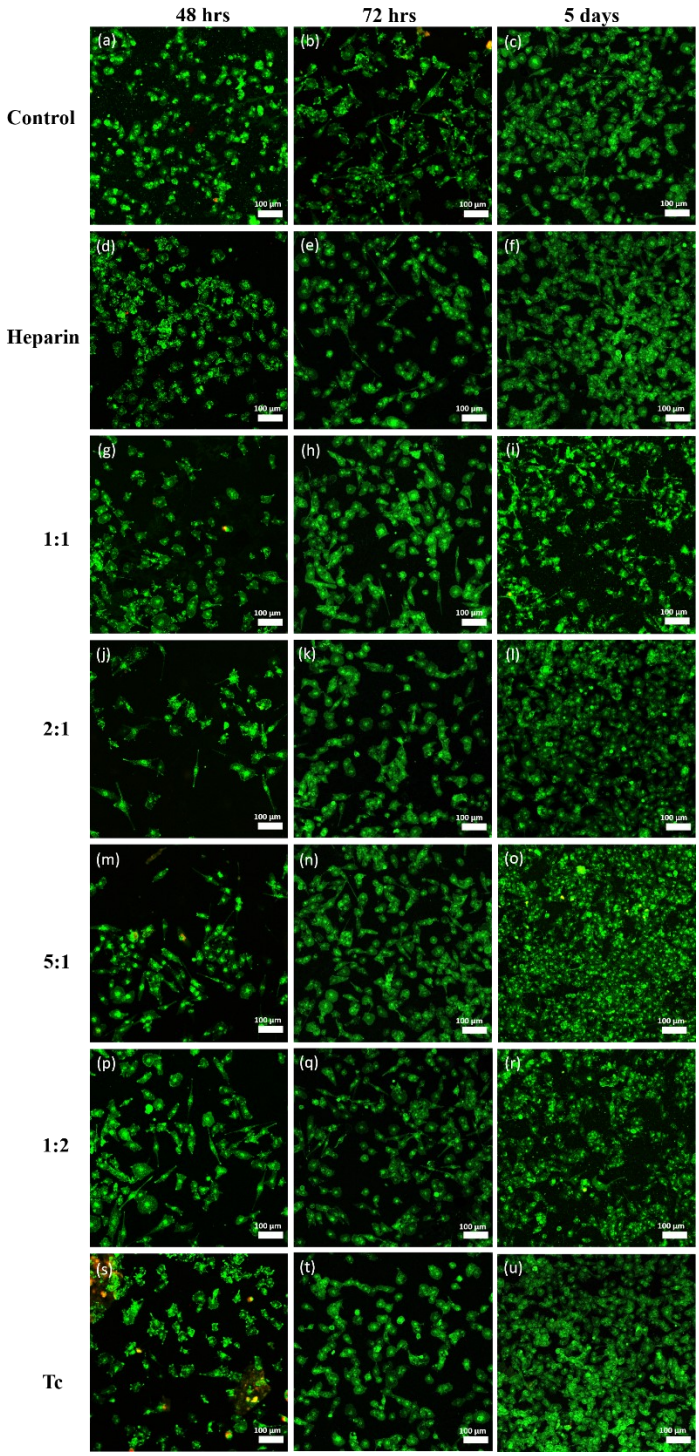

Figure S14. Assessment of cellular adhesion and proliferation on 2-D matrix of hydrogels by Live/Dead staining at 10 mg/mL concentration. Confocal laser scanning microscopic images of human neuroblastoma cell line, SH-SY5Y (a-c) control coverslip, (d-f) Heparin, (g-i) 1:1 combined gel, (j-l) 2:1 combined gel, (m-o) 5:1 combined gel, (p-r) 1:2 combined gel and (s-u) Tc peptide gel. Scale bar is 100 µm.

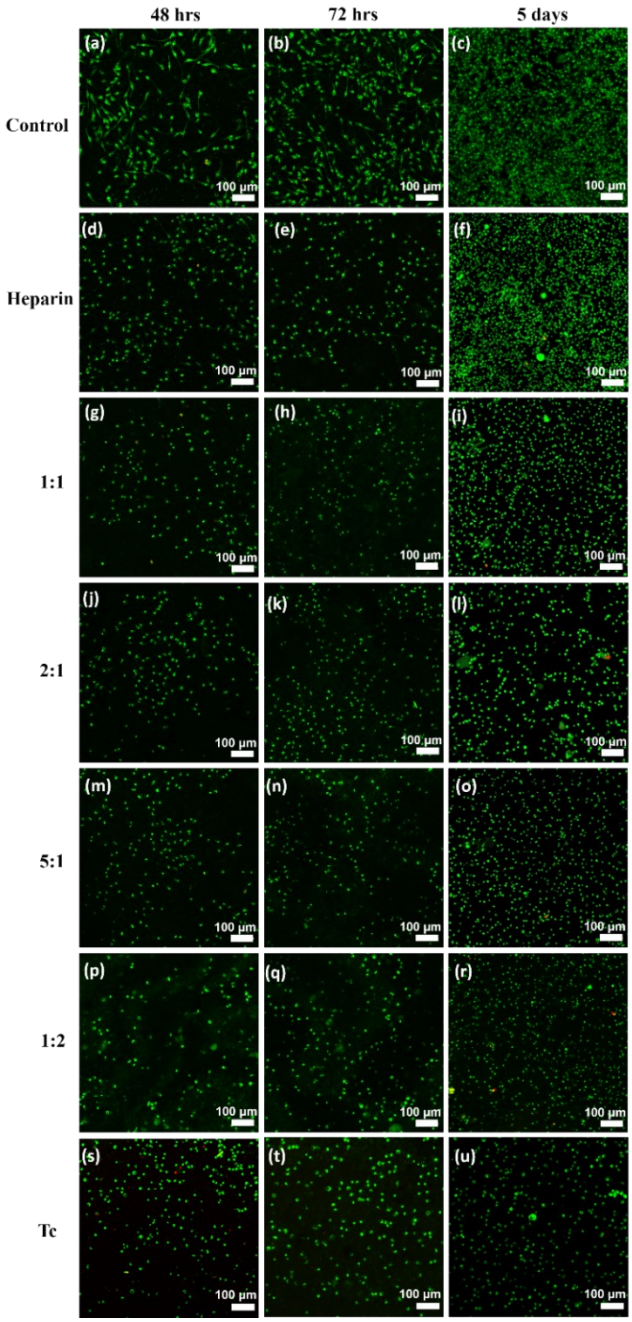

Figure S15. Assessment of cellular adhesion and proliferation on 2-D matrix of hydrogels by Live/Dead staining at 10 mg/mL concentration. Confocal laser scanning microscopic images of mouse fibroblast cell line, L929 (a-c) control coverslip, (d-f) Heparin, (g-i) 1:1 combined gel, (j-l) 2:1 combined gel, (m-o) 5:1 combined gel, (p-r) 1:2 combined gel and (s-u) Tc peptide gel. Scale bar is 100 µm.

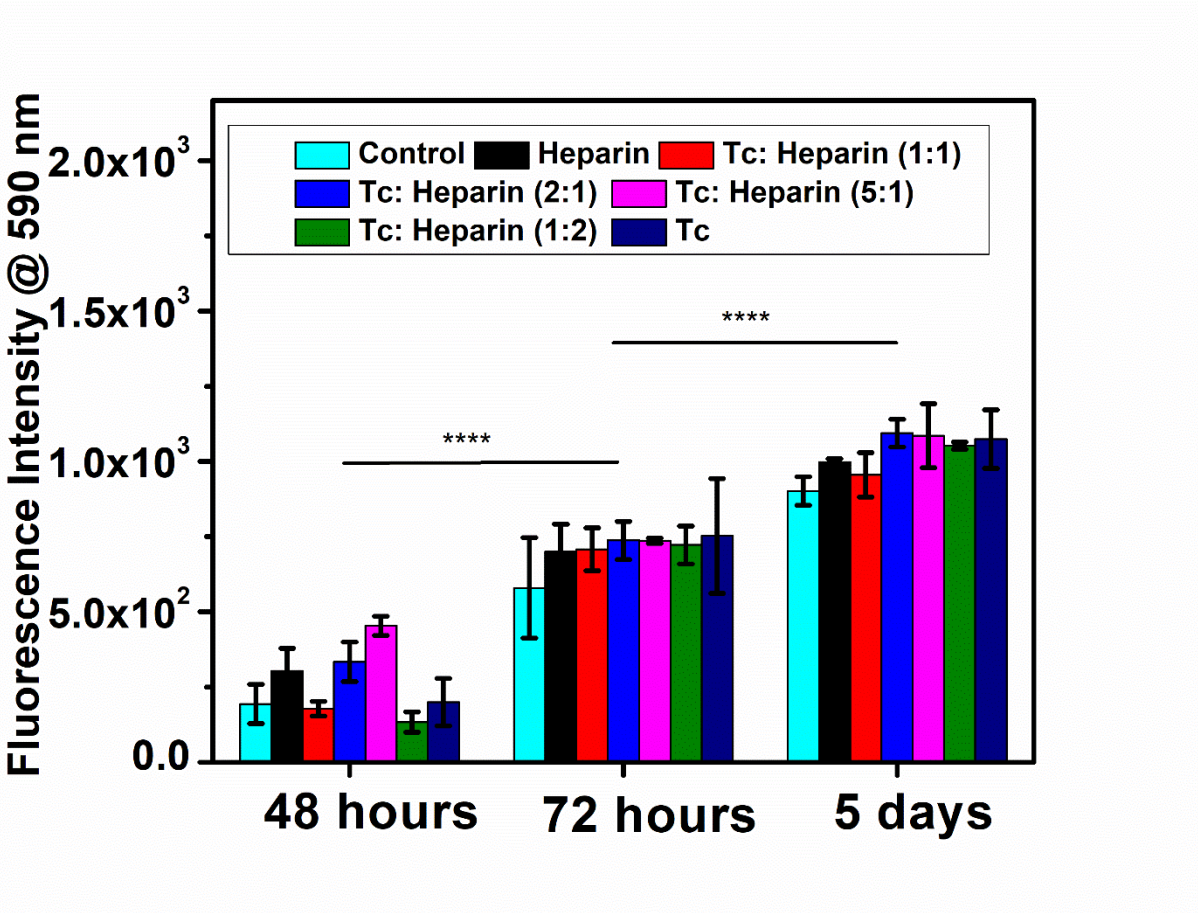

Figure S16. Quantification of cellular adhesion and proliferation on 2-D matrix of hydrogels by alamar blue assay on human neuroblastoma cell line, SH-SY5Y at 10 mg/mL concentration.

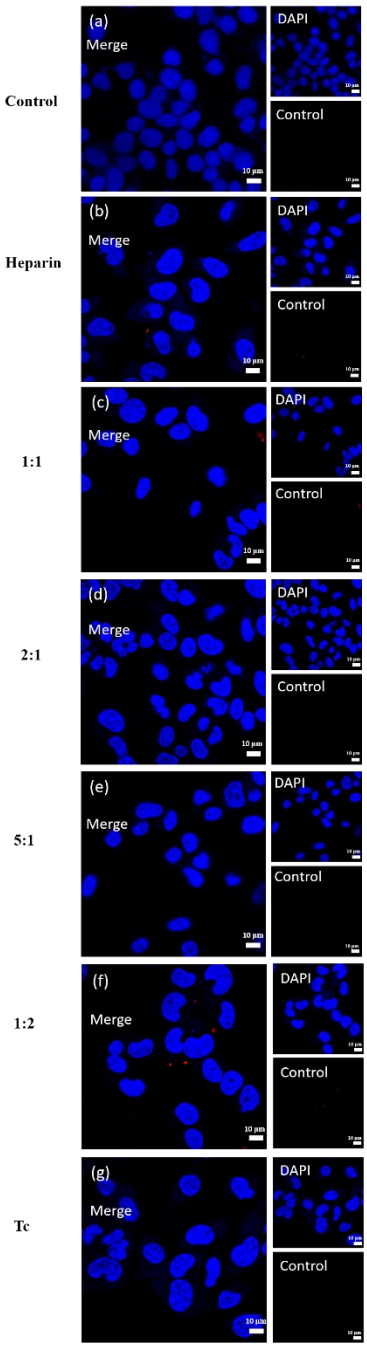

Figure S17. Immunostaining of human neuroblastoma cell line, SH-SY5Y with neural marker protein β-III tubulin after 5 days of 2-D cell culture at 10 mg/mL concentration without β-III tubulin primary antibody. CLSM images of cells after staining (a) control coverslip, (b) Heparin, (c) 1:1 combined gel, (d) 2:1 combined gel, (e) 5:1 combined gel, (f) 1:2 combined gel and (g) Tc peptide gel. Scale bar is 10 μm.

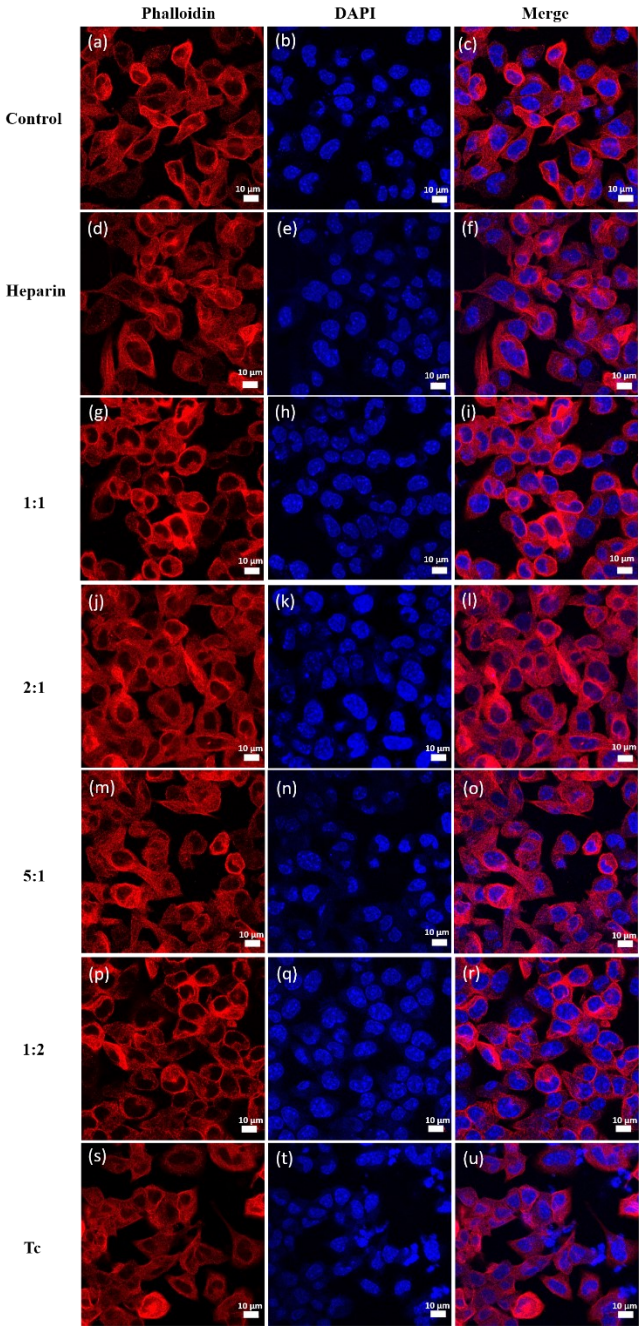

Figure S18. Immunostaining of human neuroblastoma cell line, SH-SY5Y with neural marker protein β-III tubulin after 5 days of 2-D cell culture at 10 mg/mL concentration. CLSM images of cells after staining (a-c) control coverslip, (d-f) Heparin, (g-i) 1:1 combined gel, (j-l) 2:1 combined gel, (m-o) 5:1 combined gel, (p-r) 1:2 combined gel and (s-u) Tc peptide gel. Scale bar is 10 µm.

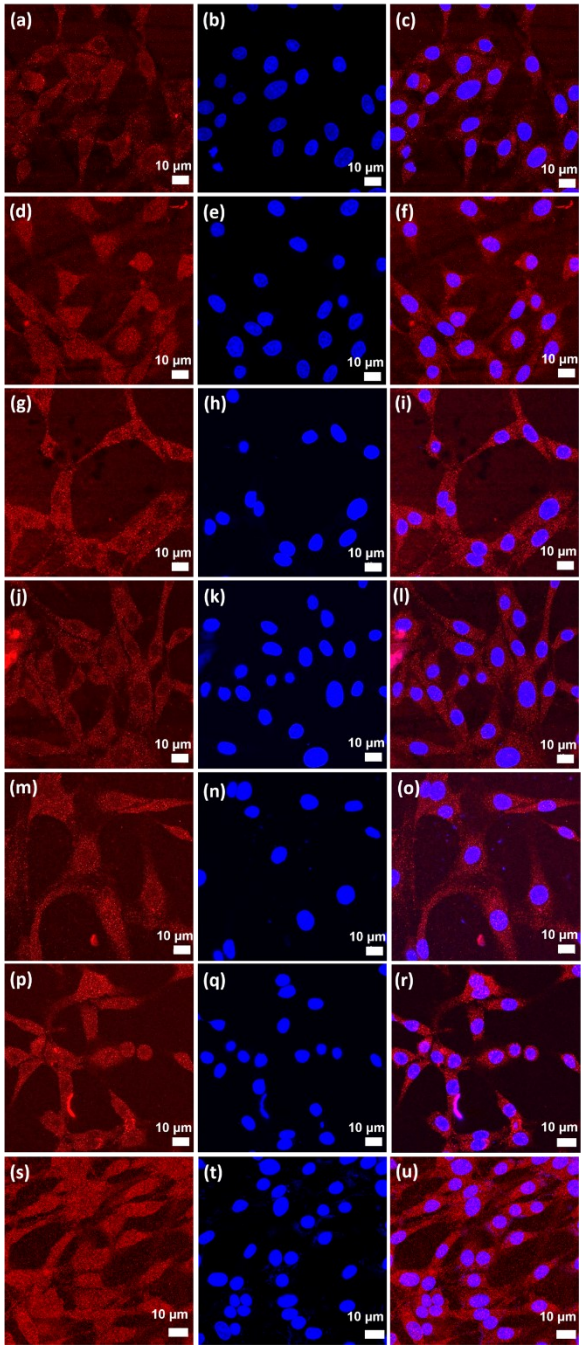

Figure S19. Immunostaining of rat glioma cell line, C6 with neural marker protein β-III tubulin after 5 days of 2-D cell culture at 10 mg/mL concentration. CLSM images of cells after staining (a-c) control coverslip, (d-f) Heparin, (g-i) 1:1 combined gel, (j-l) 2:1 combined gel, (m-o) 5:1 combined gel, (p-r) 1:2 combined gel and (s-u) Tc peptide gel. Scale bar is 10 µm.

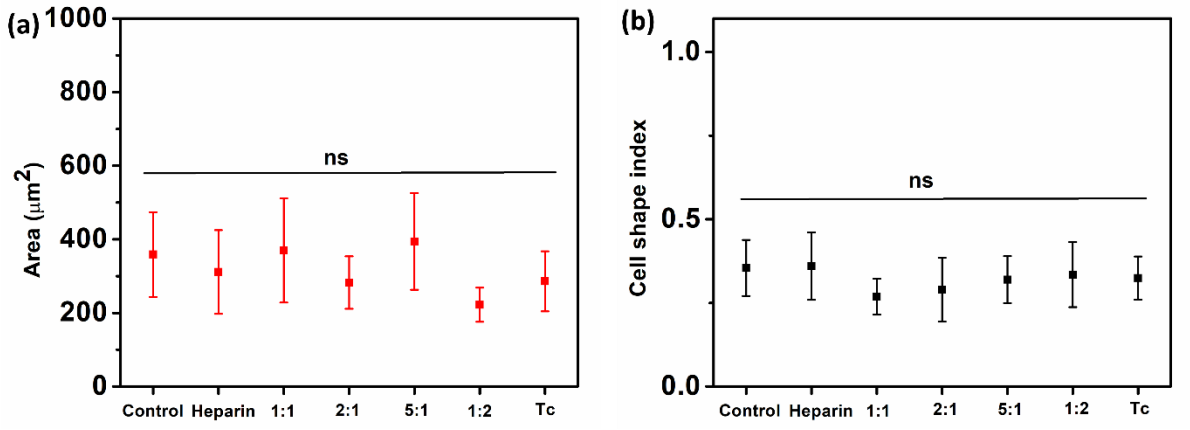

Figure S20. Quantification of (a) cells spreading area and (b) cell shape index of rat glioma cell line, C6 on uncoated coverslip and combined hydrogels. Data are represented as mean ± SD. "ns" stands for nonsignificant difference (one-way ANOVA, Bonferroni's multiple comparisons tests)

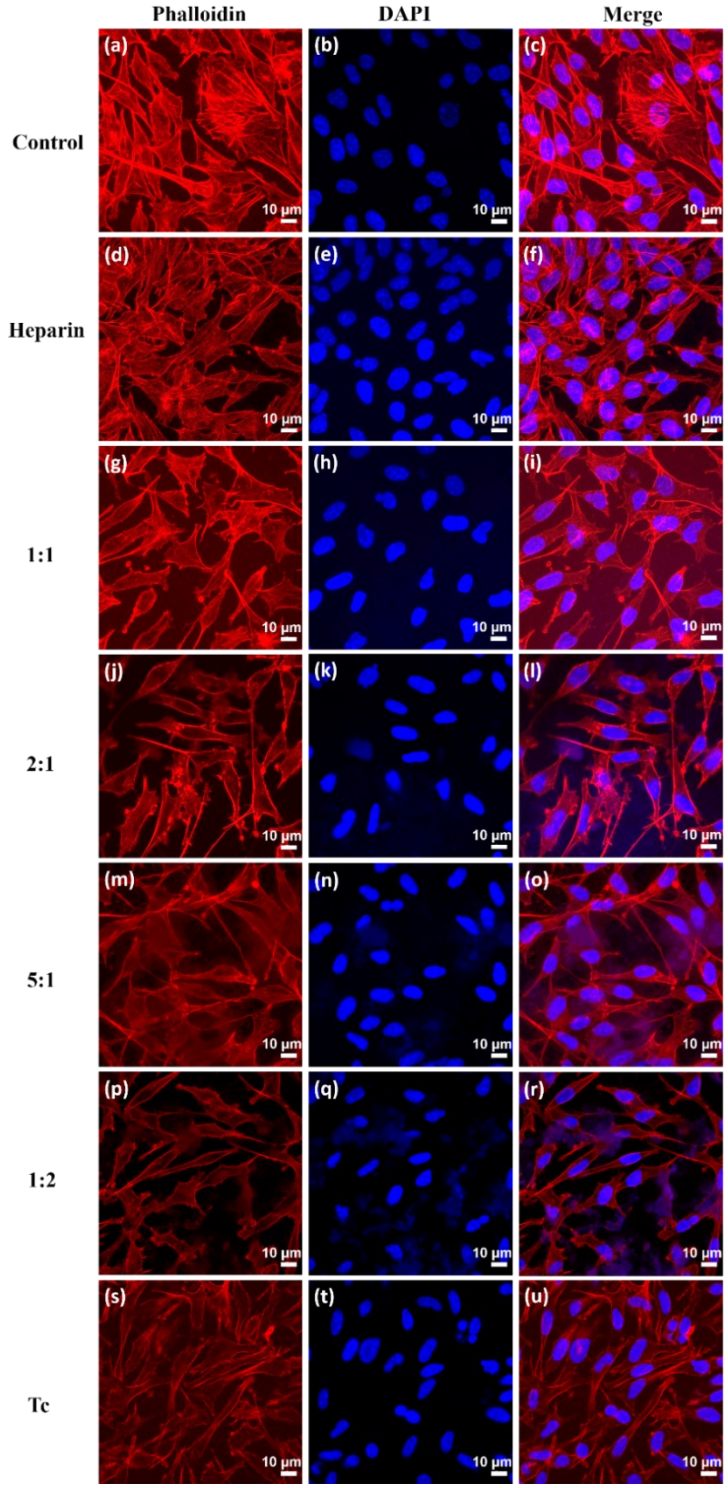

Figure S21. Cytoskeleton staining mouse fibroblast cell line, L929 after 5 days of 2-D cell culture at 10 mg/mL concentration. CLSM images of cells after staining (a-c) control coverslip, (d-f) Heparin, (g-i) 1:1 combined gel, (j-l) 2:1 combined gel, (m-o) 5:1 combined gel, (p-r) 1:2 combined gel and (s-u) Tc peptide hydrogel. Scale bar is 10 µm.

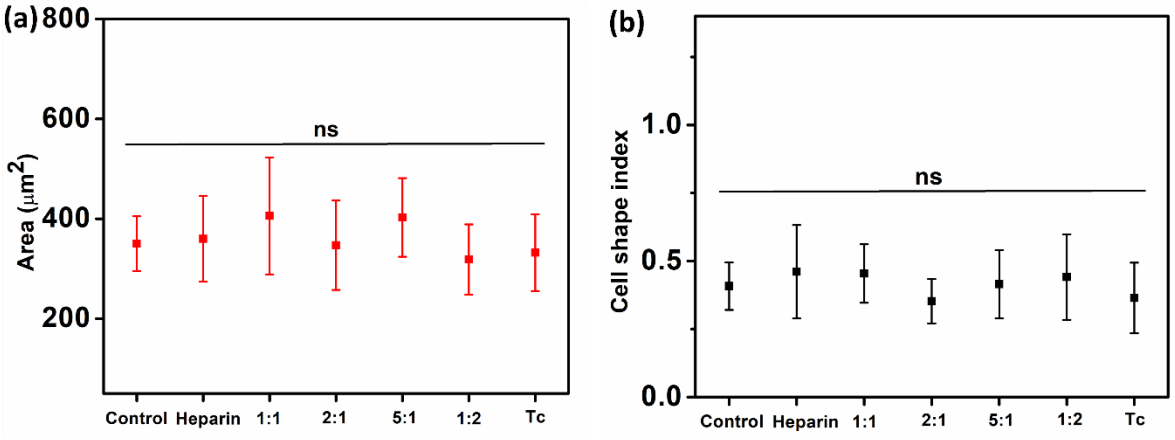

Figure S22. Quantification of (a) cells spreading area and (b) cell shape index of mouse fibroblast cell line, L929 on uncoated coverslip and combined hydrogels. Data are represented as mean ± SD. "ns" stands for nonsignificant difference (one-way ANOVA, Bonferroni's multiple comparisons tests)

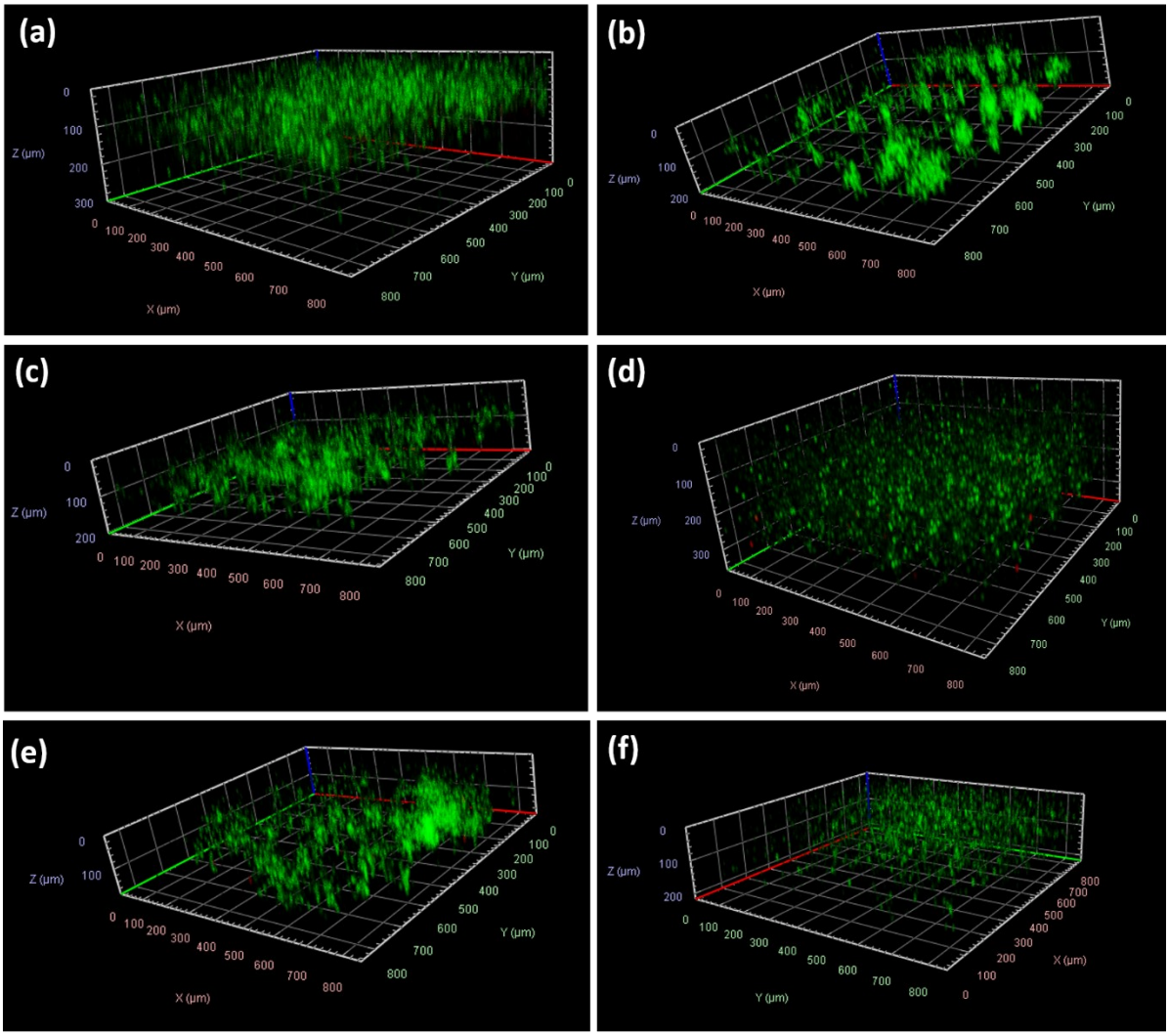

Figure S23. Z-stack rendering images of the SH-SY5Y cells after live/dead staining in the 3-D cell culture conditions in case of (a) Matrigel, (b) 1:1 combined gel, (c) 2:1 combined gel, (d) 5:1 combined gel, (e) 1:2 combined gel and (f) Tc peptide gel.

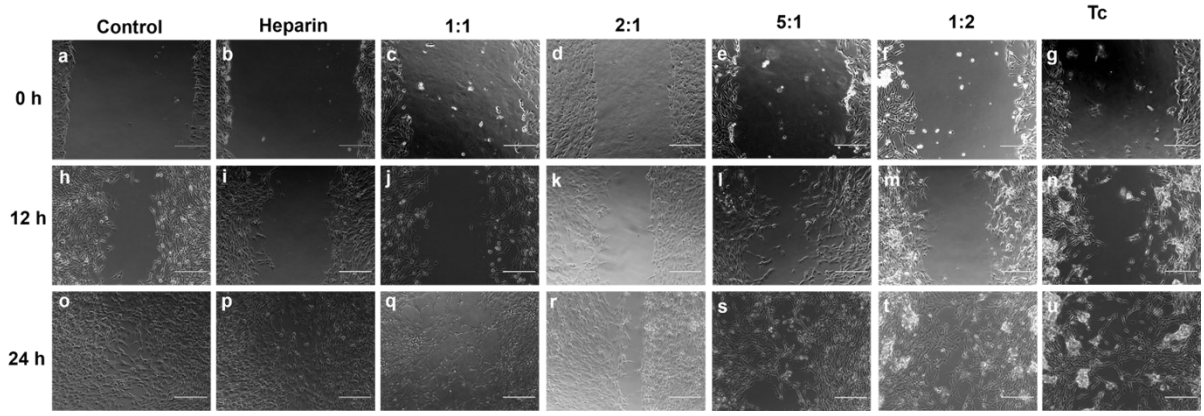

Figure S24. Assessment of cellular migration of C6 cells in the presence hydrogel treatment (1000 μg/mL). Scratch closure in (a,h,o) control, (b,i,p) Heparin (c,j,q) 1:1 combined gel, (d,k,r) 2:1 combined gel, (e,l,s) 5:1 combined gel, (f, m, t) 1:2 combined gel and (g,n,u) Tc peptide gel at 0, 12 and 24 hours respectively. Scale bar is 200 μm.

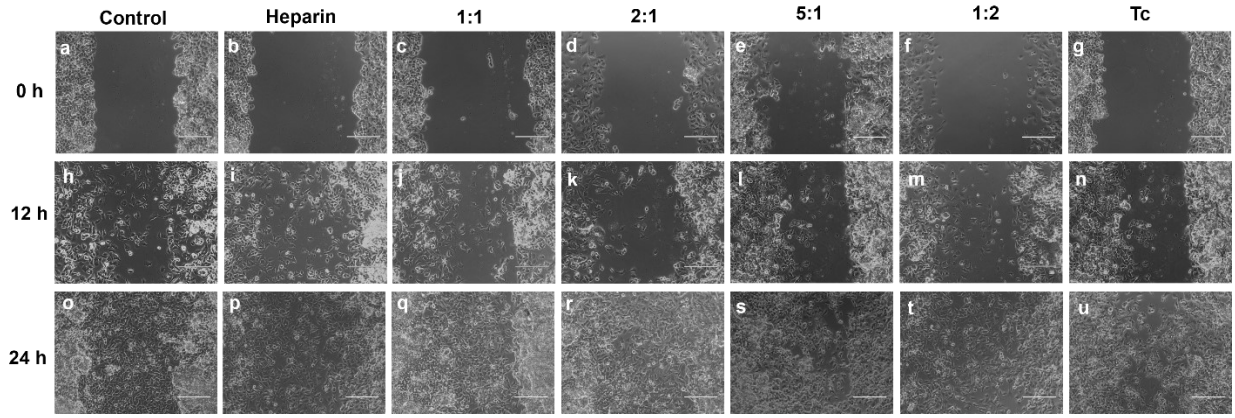

Figure S25. Assessment of cellular migration of SH-SY5Y cells in the presence hydrogel treatment (1000 µg/mL). Scratch closure in (a,h,o) control, (b,i,p) Heparin (c,j,q) 1:1 combined gel, (d,k,r) 2:1 combined gel, (e,l,s) 5:1 combined gel, (f, m, t) 1:2 combined gel and (g,n,u) Tc peptide gel at 0, 12 and 24 hours respectively. Scale bar is 200 µm.

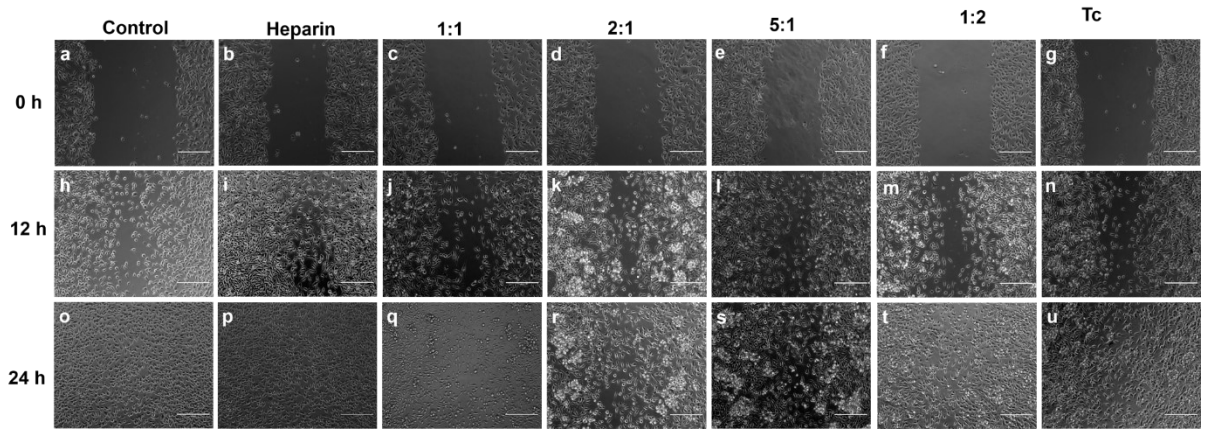

Figure S26. Assessment of cellular migration of L929 cells in the presence hydrogel treatment (1000 µg/mL).. Scratch closure in (a,h,o) control, (b,i,p) Heparin (c,j,q) 1:1 combined gel, (d,k,r) 2:1 combined gel, (e,l,s) 5:1 combined gel, (f, m, t) 1:2 combined gel and (g,n,u) Tc peptide gel at 0, 12 and 24 hours respectively. Scale bar is 200 µm.

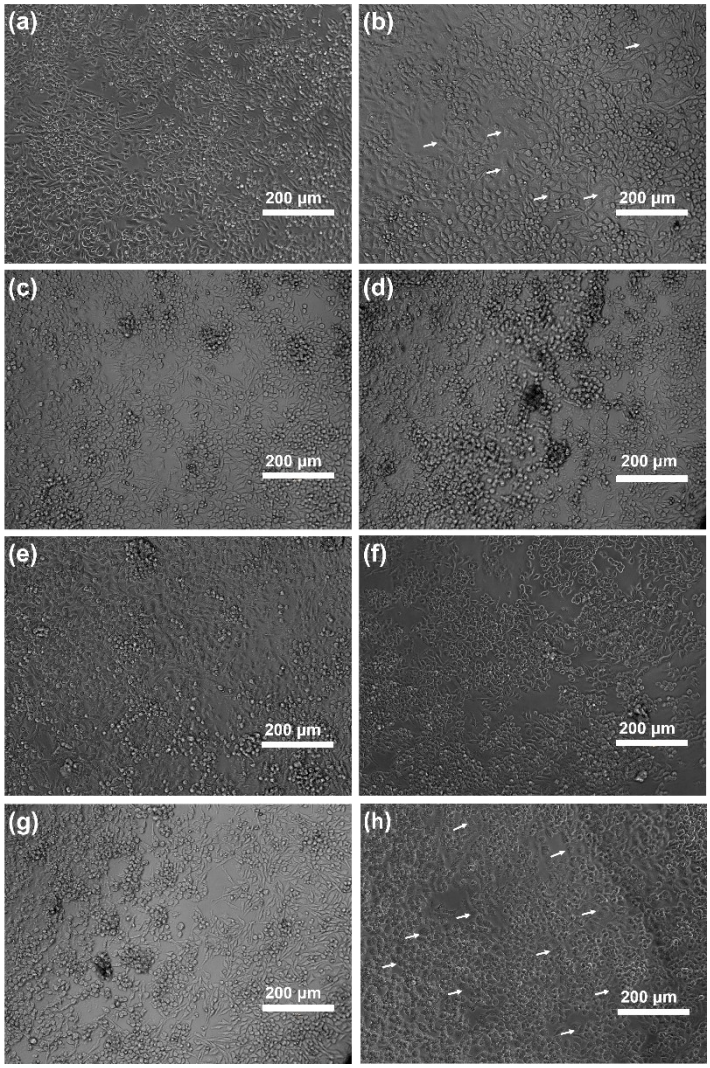

Figure S27. Microscopic Raw cell images showing the changes in cellular morphology on hydrogel at 10 mg/ml concentration. Optical images of the Raw cells after culturing on (a) control (no treatment), in the presence of (b) Heparin, (c) 1:1 combined gel, (d) 2:1 combined gel, (e) 5:1 combined gel, (f) 1:2 combined gel, (g) Tc peptide gel and (h) LPS (10 µg/mL).