Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Designing bioactive scaffold from co-assembled collagen-laminin short peptide hydrogels for controlling cell behaviour

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Table S1: Mass analysis of synthesized peptides using LC-MS.

Peptides	Calculated Mass (m/z) (Chem Draw)	Observed Mass (m/z) (LC-MS)
Nap-FFGSO	753.32	753.61
Nap-IKVAV	712.43	712.66
Nap-YIGSR	778.38	778.65

Table S2: Comparison of morphologies of different nanostructures and the diameter of nano fibrous network of collagen-laminin co-assembled hydrogels.

Peptide composition	Morphology	Diameter (nm)	
		AFM	TEM
Na-FFGSO	Aggregates	--	
Nap-IKVAV	Fibers	40 ± 4.5	35± 3.1
Nap-YIGSR	Fibers	28.7 ± 4.4	20 ± 2.3
Na-FFGSO+ A	Short fibers	62 ± 4.8	38 ± 5.6
Nap-FFGSO+ B	Long entangled fibers	17 ± 2.3	8 ± 2.7
Nap-FFGSO+ A+ B	Mixed fibers (long and short)	26 ±3.1	10.5 ± 2.3

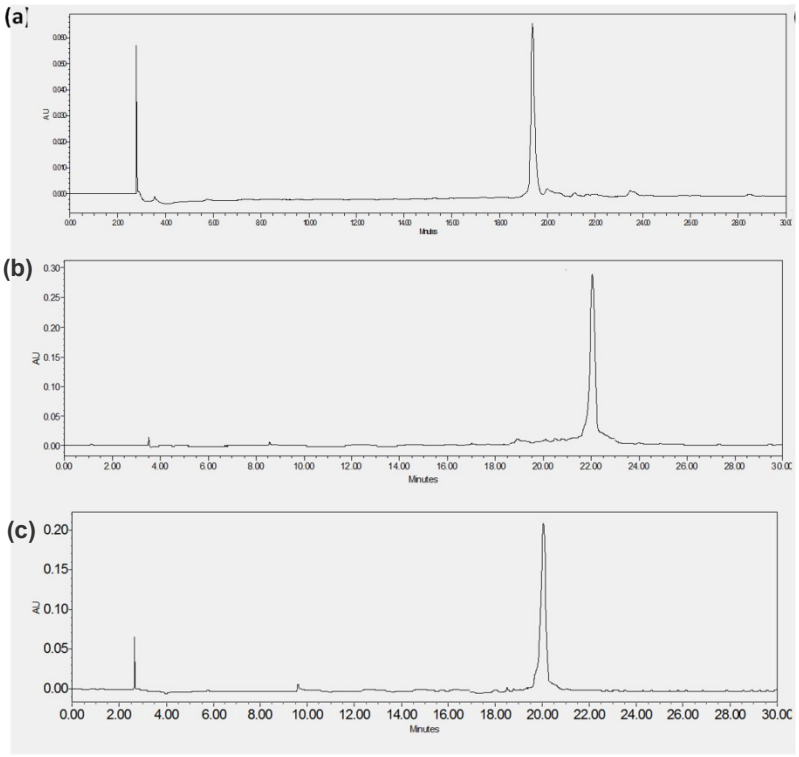

Figure S1: HPLC chromatograms of synthesized peptides (a) Nap-FFGSO, (b) Nap-IKVAV and (c) Nap-YIGSR.

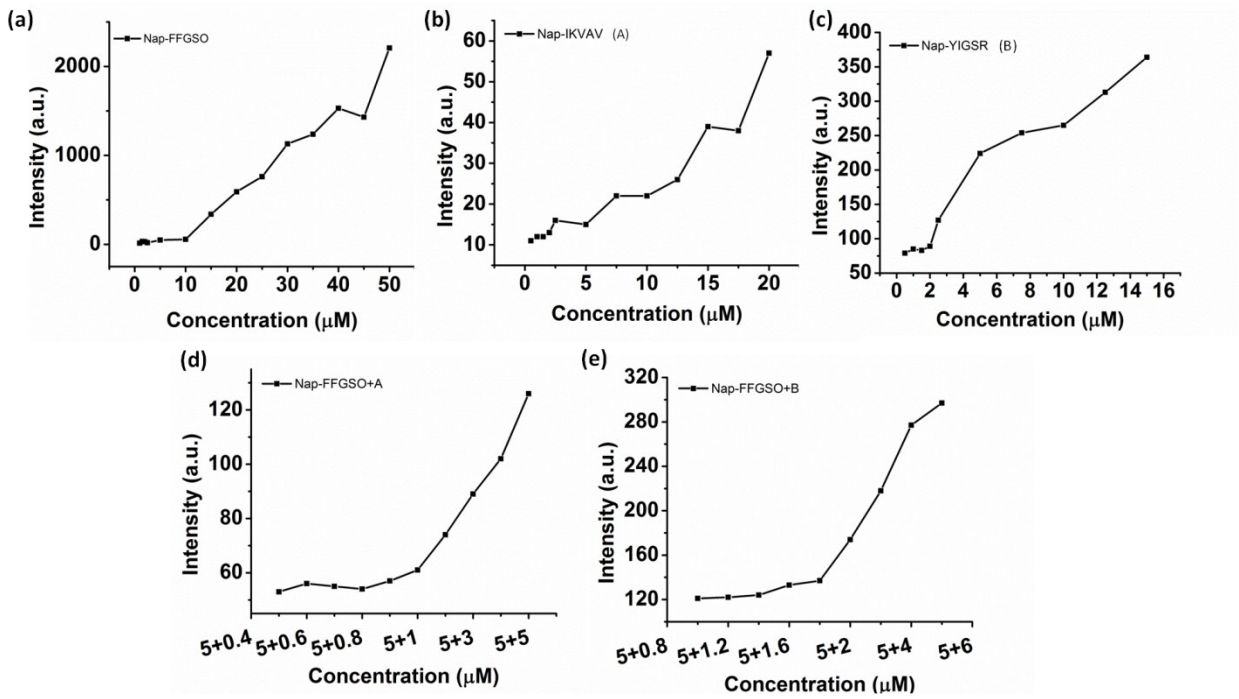

Figure S2: Critical aggregation concentration determination of individual peptides(a) Nap-FFGSO, (b) Nap-IKVAV, (c) Nap-YIGSR and co-assembled peptides (d) Nap-FFGSO+A and (e) Nap-FFGSO+B using Thioflavin T dye.

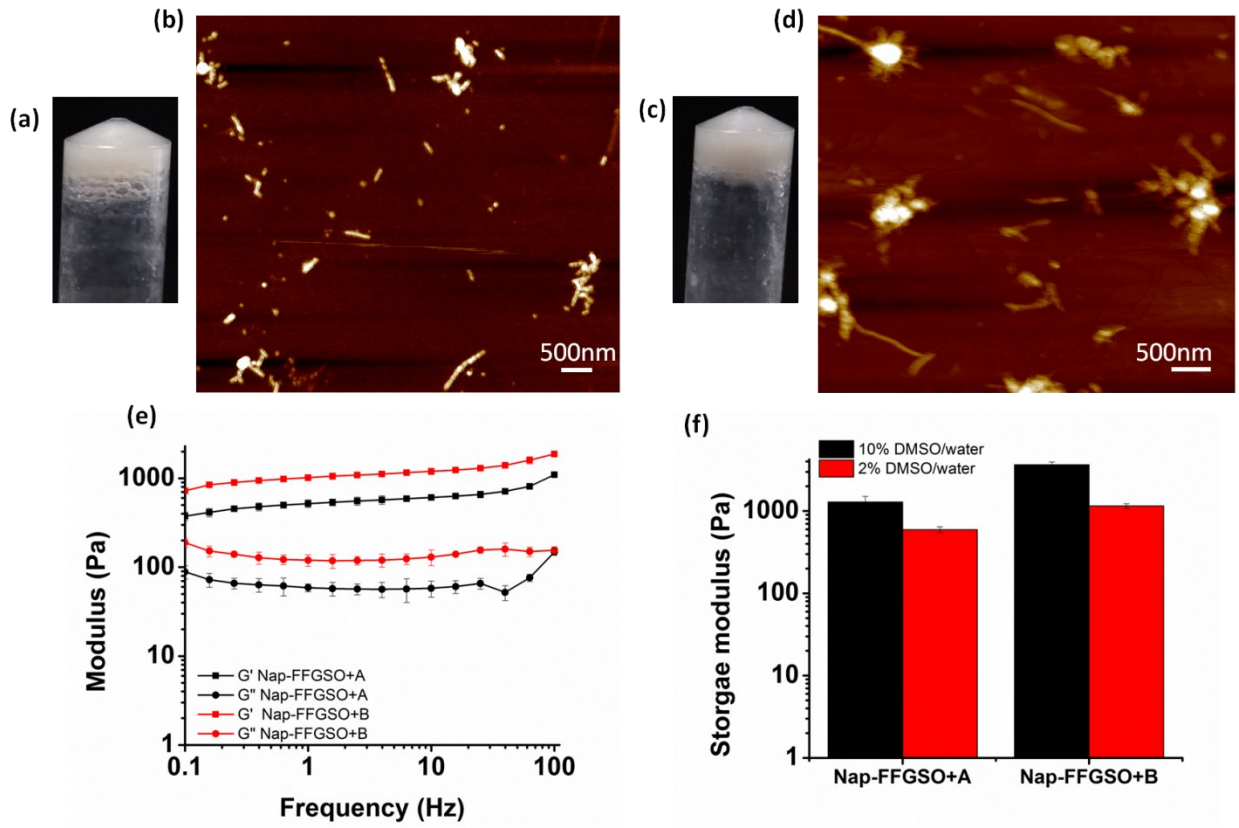

Figure S3: Gelation studies of collagen and laminin inspired co-assembled gels in 2% DMSO/water with optical images and respective AFM microscopic images of gels of (a & b) Nap-FFGSO+A and (c & d) Nap-FFGSO+B; (e) Rheology studies of gels prepared in 2% DMSO/water at the concentration of 30mM of Nap-FFGSO and 3mM of either Nap-IKVAV (A) or Nap-YIGSR (B) and (f) Comparison to gels prepared in 10%DMSO/water and 2% DMSO/water.

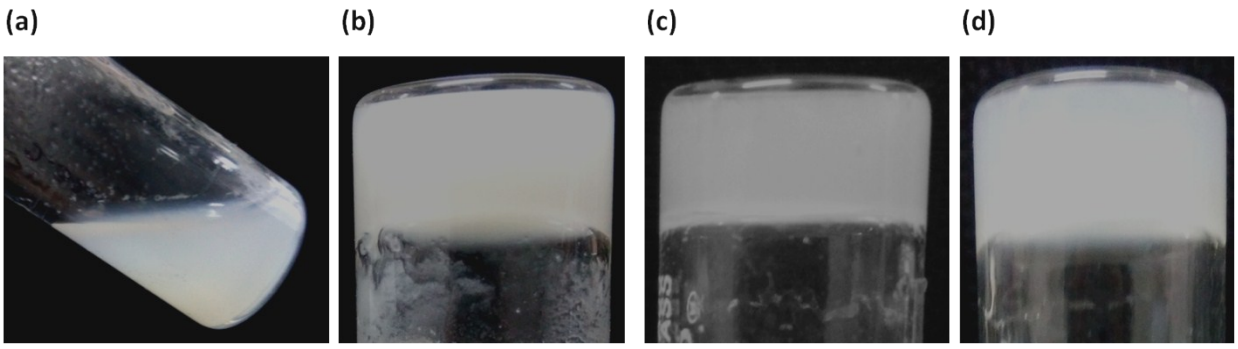

Figure S4: Optical images of (a)Nap-FFGSO as a soution and (b) Nap-FFGSO+A, (c) Nap-FFGSO+B, (d) Nap-FFGSO+A+B as co-assembled gels in 10% DMSO/water.

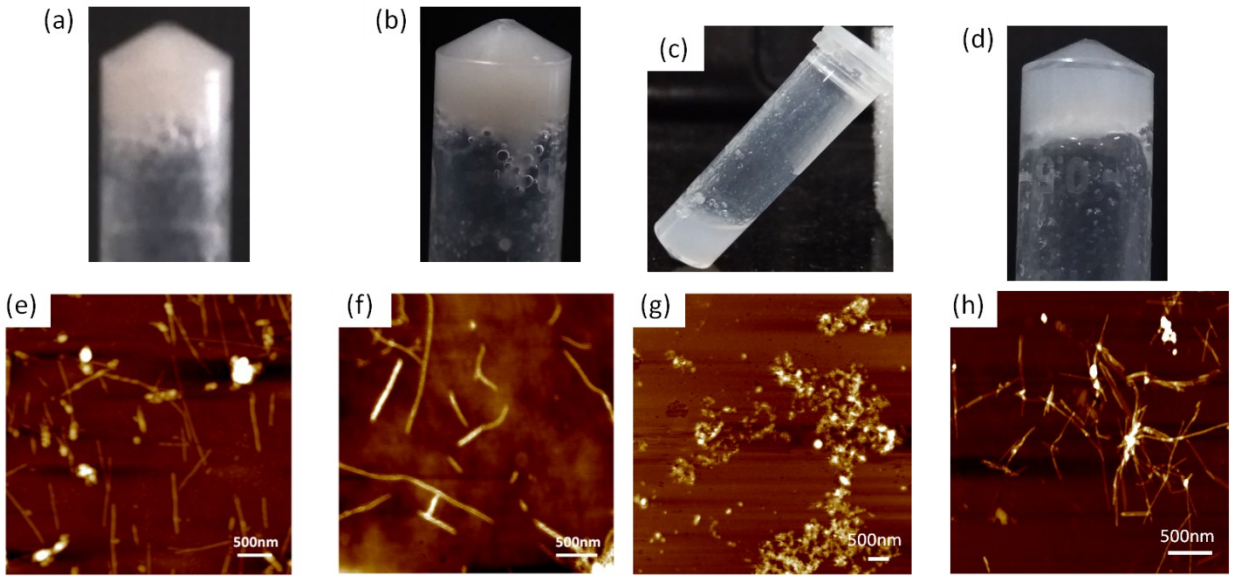

Figure S5: Control hydrogels with varied concentration of CIP and LMP peptide and their respective AFM images (a, e) Nap-FFGSO (20mM)+A (4.5mM), (b, f) Nap-FFGSO (20mM)+B (4.5mM), (c, g) Nap-FFGSO (20mM)+ A(1.5mM)+B (1.5mM) and (d, h) Nap-FFGSO (20mM)+ A(2.25mM)+B (2.25mM)

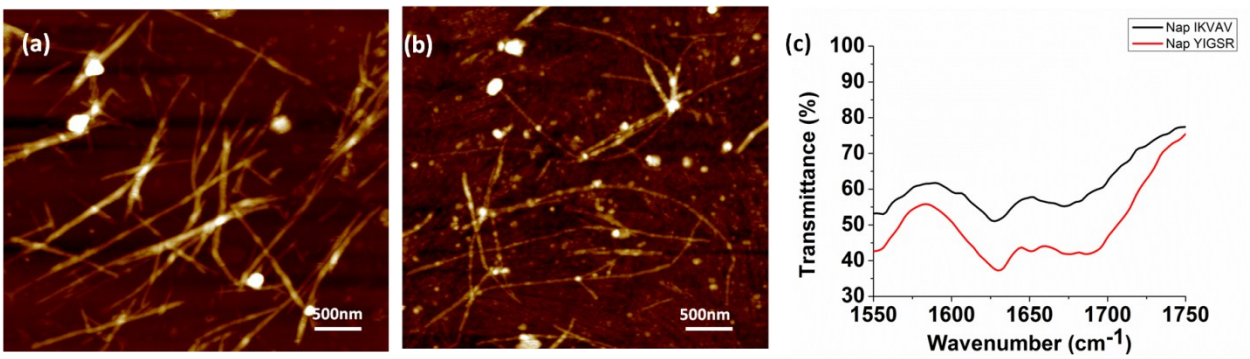

Figure S6: AFM images of (a) Nap-IKVAV and (b) Nap-YIGSR at 5mM concentration. (c) FTIR spectra of LMP's.

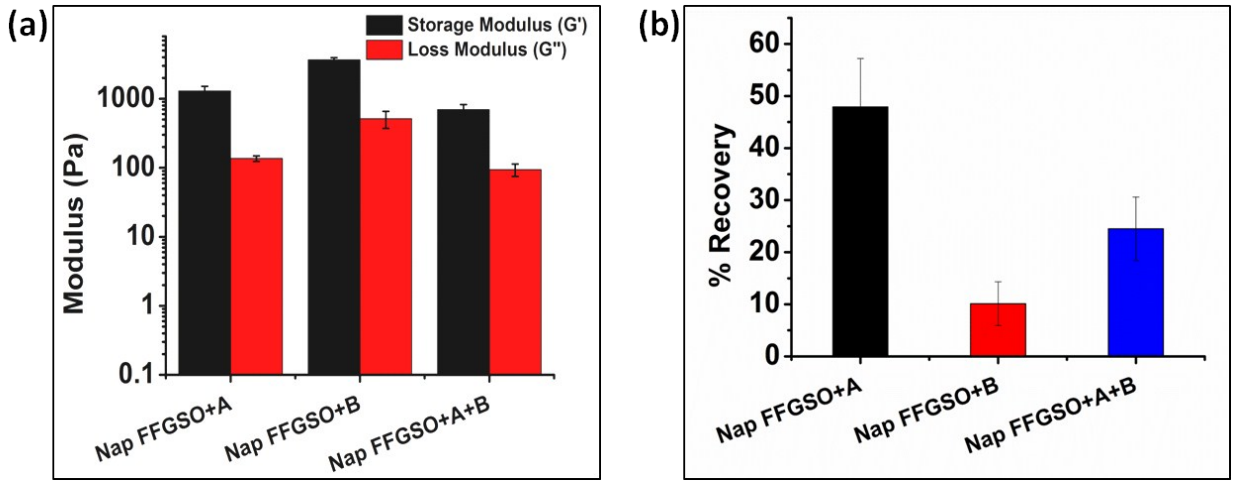

Figure S7: (a) Comparison of mechanical stiffness of the co-assembled peptide gels and (b) Differential percentage recovery of different collagen and laminin inspired peptide co-assembled gels after 100 sec, as evident through thixotropic studies, carried at 50% strain.

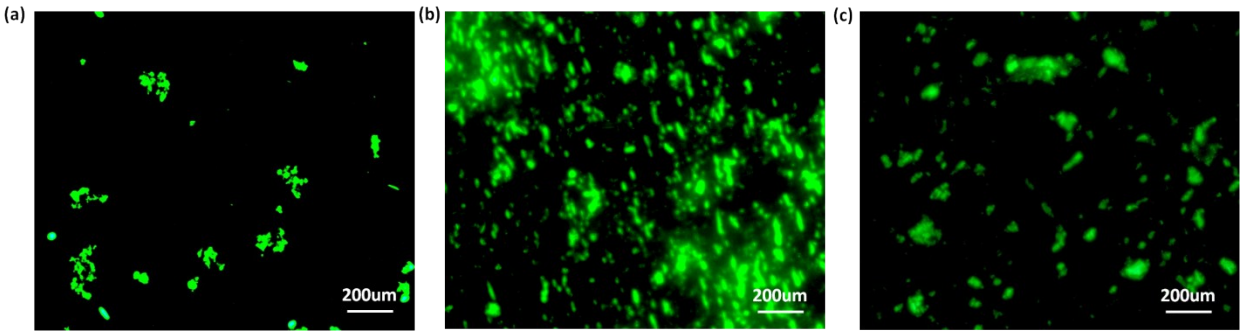

Figure S8: Fluorescence microscopic images of Thioflavin T bound with co-assembled peptide hydrogels (a) Nap-FFGSO + A, (b) Nap-FFGSO+B and (c) Nap-FFGSO+A+B.

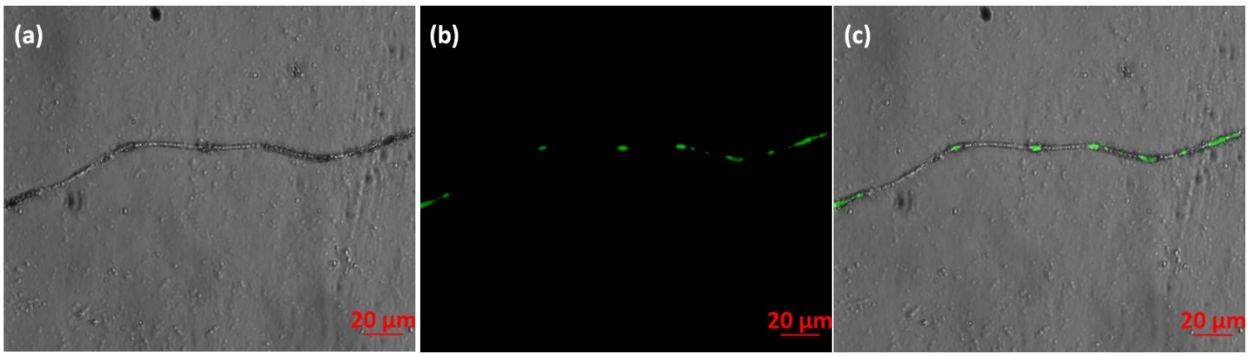

Figure S9: Images of co-assembled peptides with (a) bright field, (b) fluorescence and (c) merged image of bright field and fluorescence showing localization of fluorescent Nap-IKVAV along the non-fluorescent Nap-FFGSO peptide fiber.

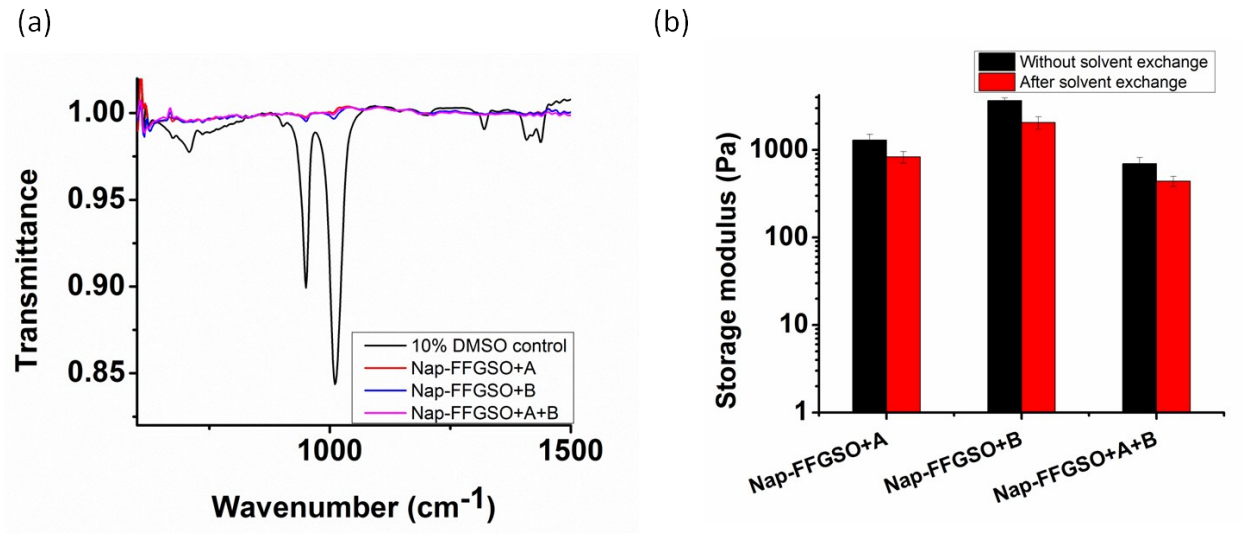

Figure S10: Solvent exchange of gels prepared at concentration of 30mM Nap-FFGSO and 3mM of LMP in 10% DMSO/water (a) FTIR spectra of CIP-LMP gels after solvent exchange showing diminished sulfoxide peak at 1020 cm^{-1} and (b) Rheology measurement of gels without and after solvent exchange. The measurements were done after three consecutive cycles of solvent exchange with water.

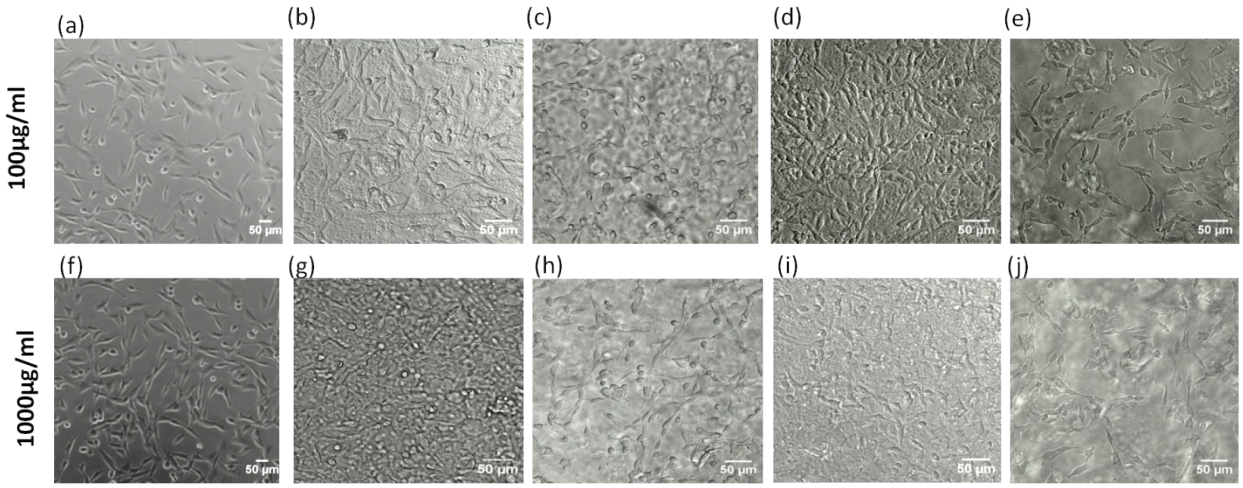

Figure S11: Biocompatibility studies: microscopic images of C6 cells after treatment with (a and f) control (TCP), (b and g) Nap-FFGSO, (c and h) Nap-FFGSO+A, (d and i) Nap-FFGSO+B and (e and j) Nap-FFGSO+A+B at 100µg/ml (0.13mM) and 1000µg/ml (1.3mM) concentrations, after 4 hrs of treatment.

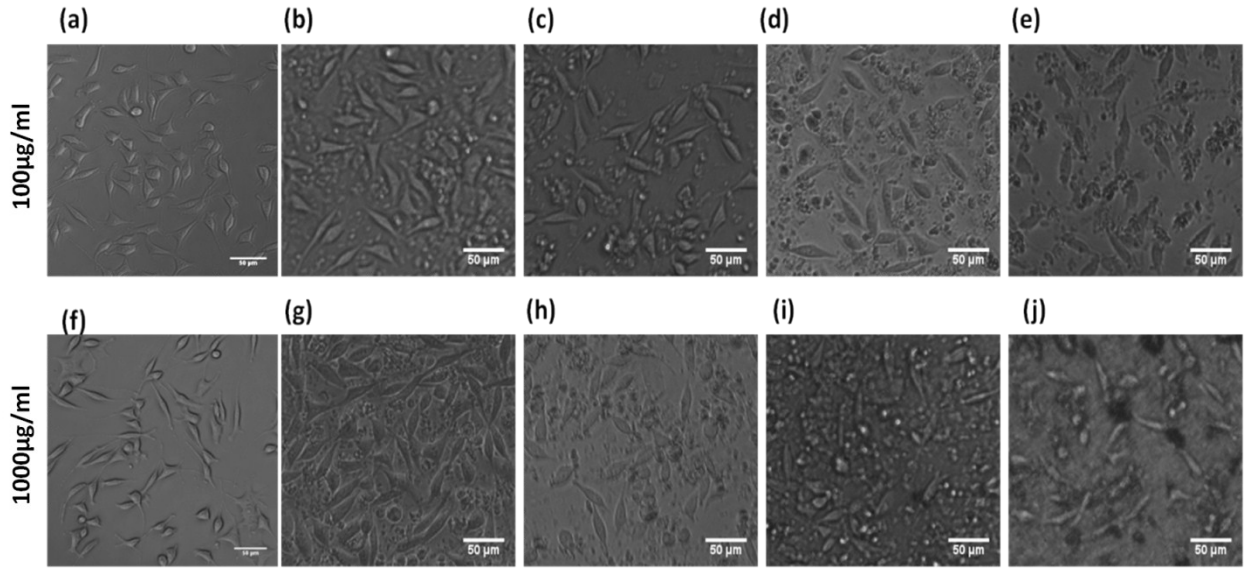

Figure S12: Biocompatibility studies: microscopic images of L929 cells after treatment with (a and f) control (TCP), (b and g) Nap-FFGSO, (c and h) Nap-FFGSO+A, (d and i) Nap-FFGSO+B and (e and j) Nap-FFGSO+A+B at 100µg/ml (0.13mM) and 1000µg/ml (1.3mM) concentrations, after 4 hrs of treatment.

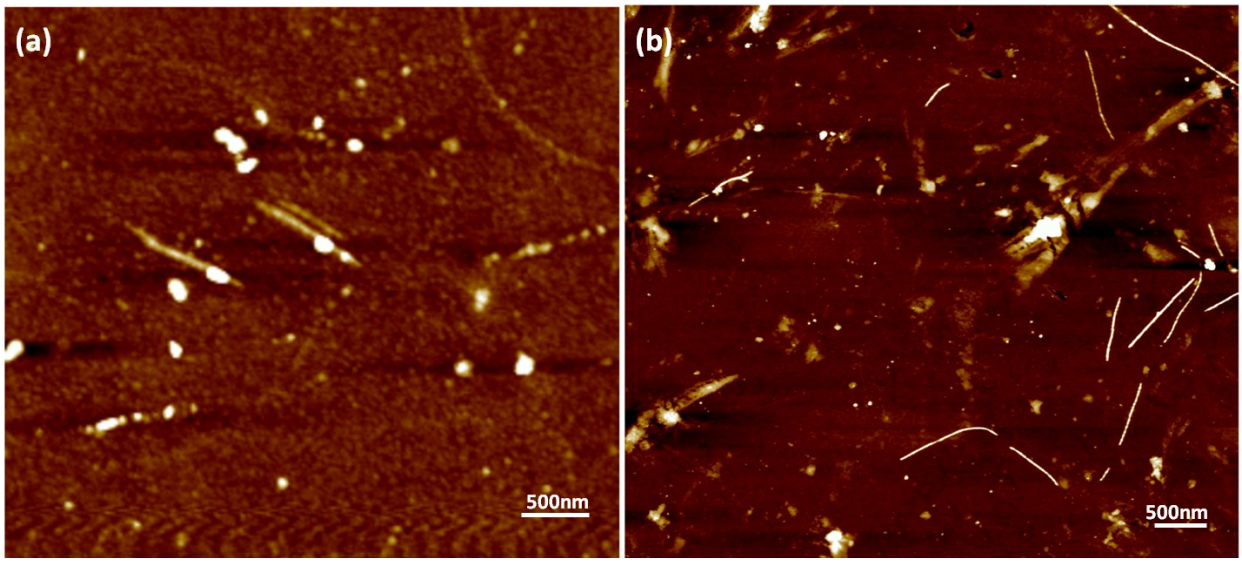

Figure S13: AFM images of 5mg/ml (6.5mM) stock diluted to (a) 100 μg/ml (0.13mM) and (b) 1000μg/ml (1.3mM) peptide concentration.

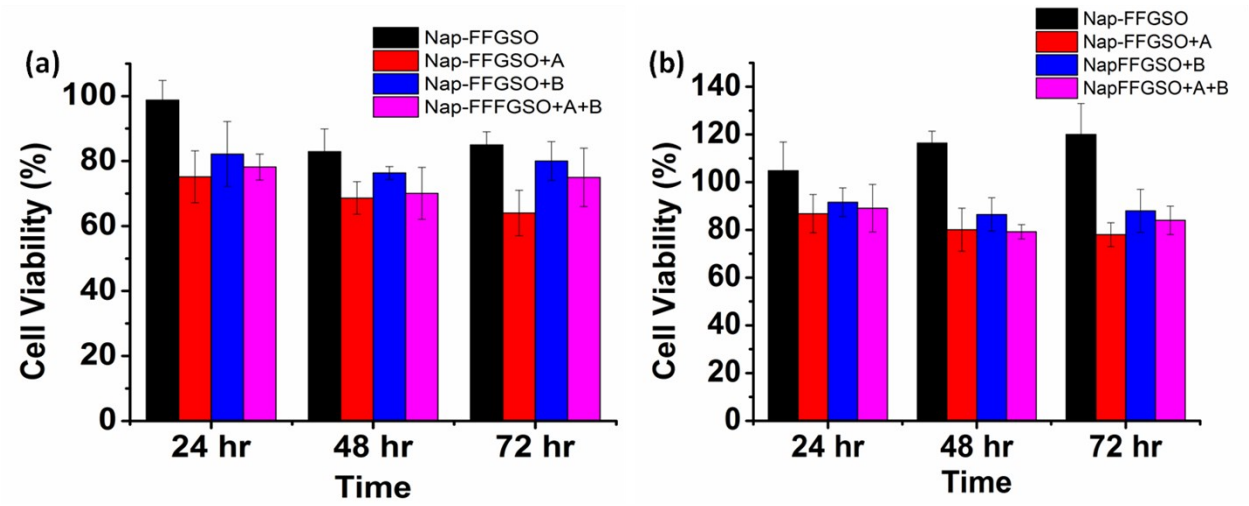

Figure S14: Biocompatability studies of CIP-LMP peptide self-assembled structures with (a) C6 cells and (b) L929 cells when treated with peptide concentration of 1000µg/ml (1.3mM) for 24 hrs, 48 hrs and 72hrs.

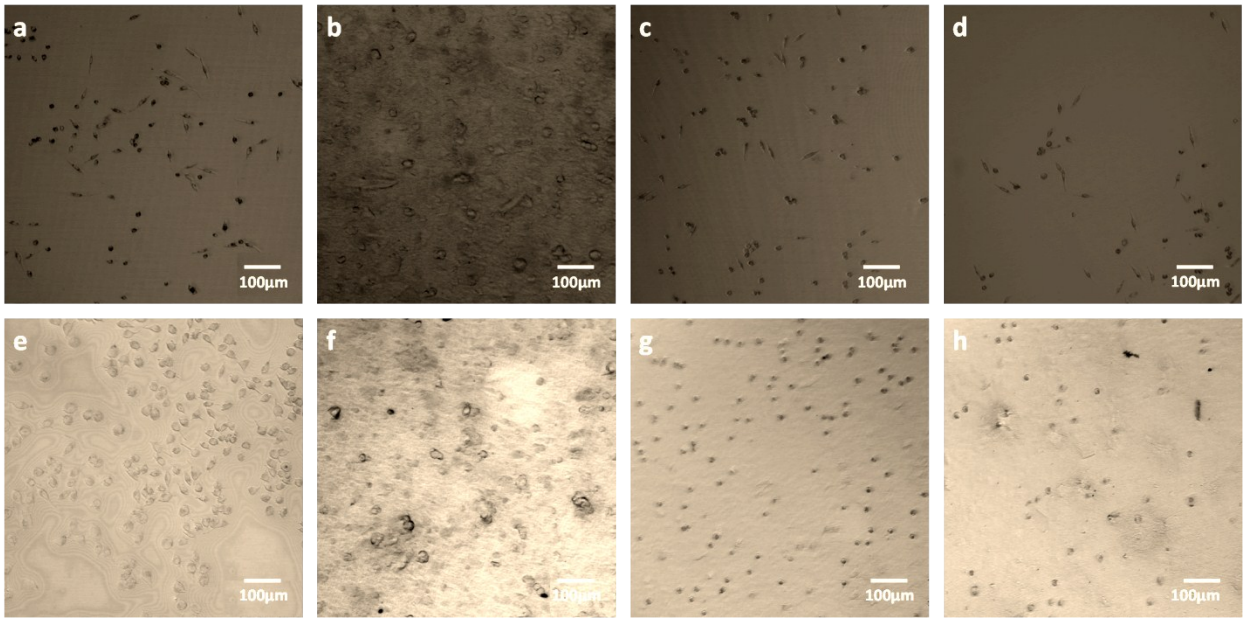

Figure S15: 2D culture phase contrast images of C6 and L929 cells with (a, e) control; (b, f) Nap-FFGSO+A; (c, g) Nap-FFGSO+B and (d, h) Nap-FFGSO+A+B co-assembled gels, respectively, after 24 hrs.

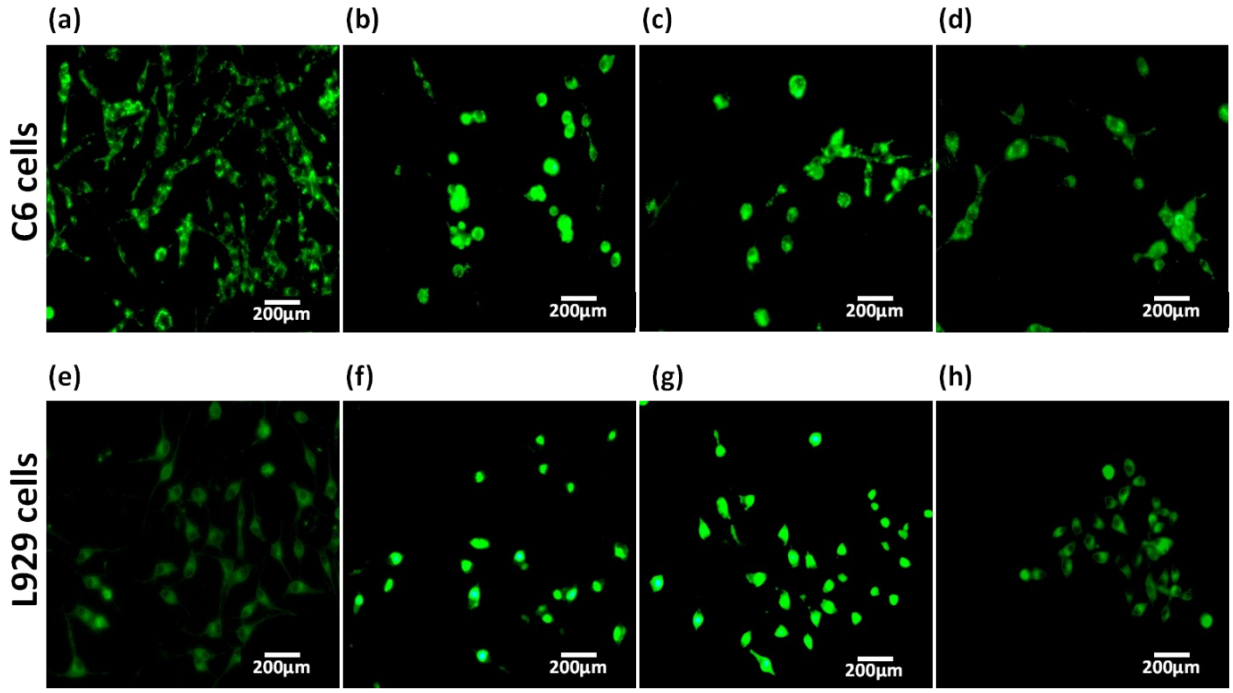

Figure S16: Live dead imaging using fluorescence microscope with C6 cells and L929 cells showing live cells(green) after 24 hrs cultured over (a and e) plastic (control), (b and f) Nap-FFGSO+A gels, (c and g) Nap-FFGSO+B gels and (d and h) Nap-FFGSO+A+B gels. No red signal observed indicating absence of dead cells after 24 hrs.

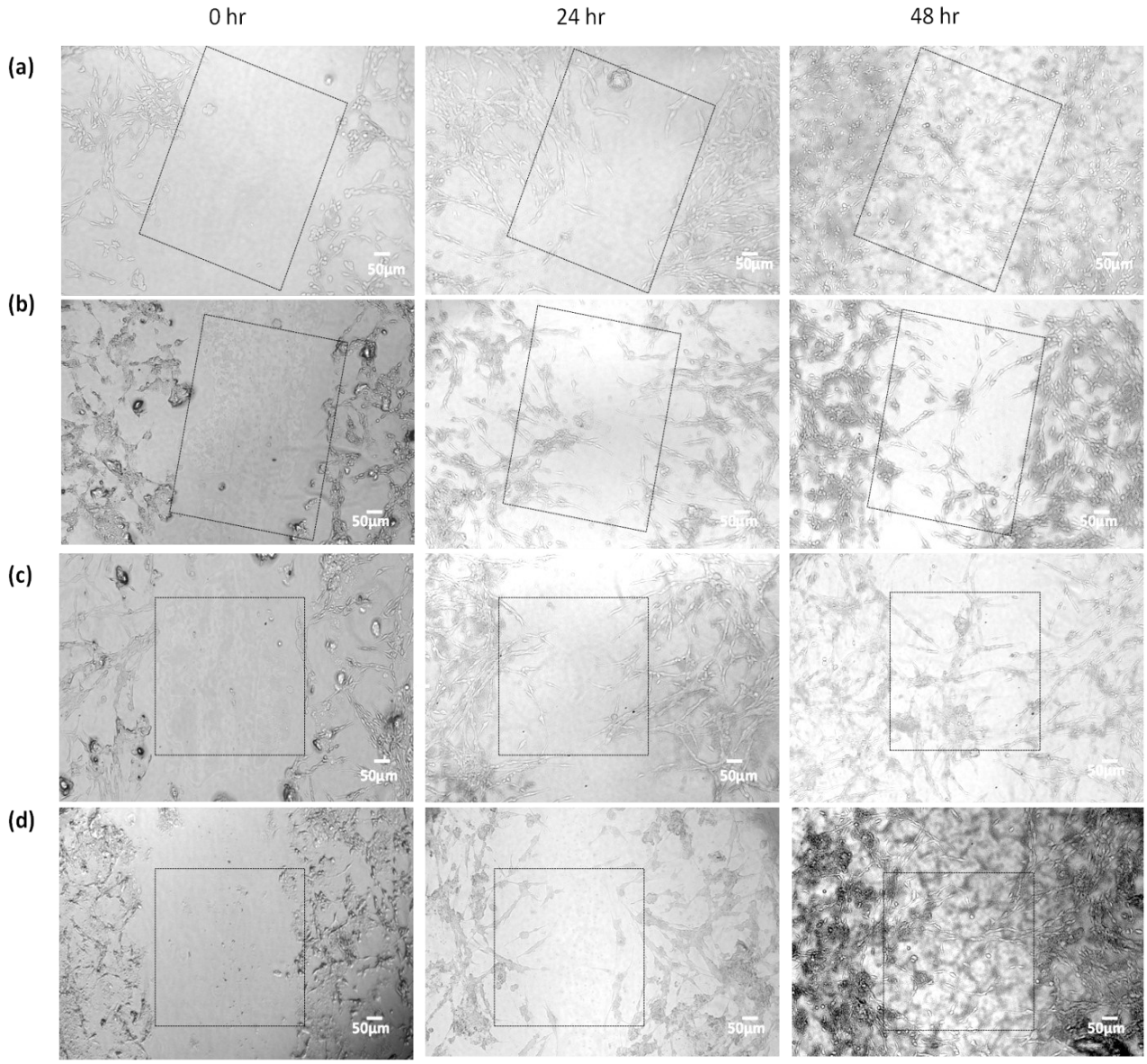

Figure S17: Proliferation and migration response of C6 cells in the presence of diluted peptide gels, assessed by microscopic images of scratched region after 0 hr, 24 hrs and 48 hrs time points after treatment with (a) control (2%DMSO/water, blank), (b) Nap-FFGSO+A, (c) Nap-FFGSO+B and (d) Nap-FFGSO+A+B at concentration of 1000µg/ml (1.3mM).

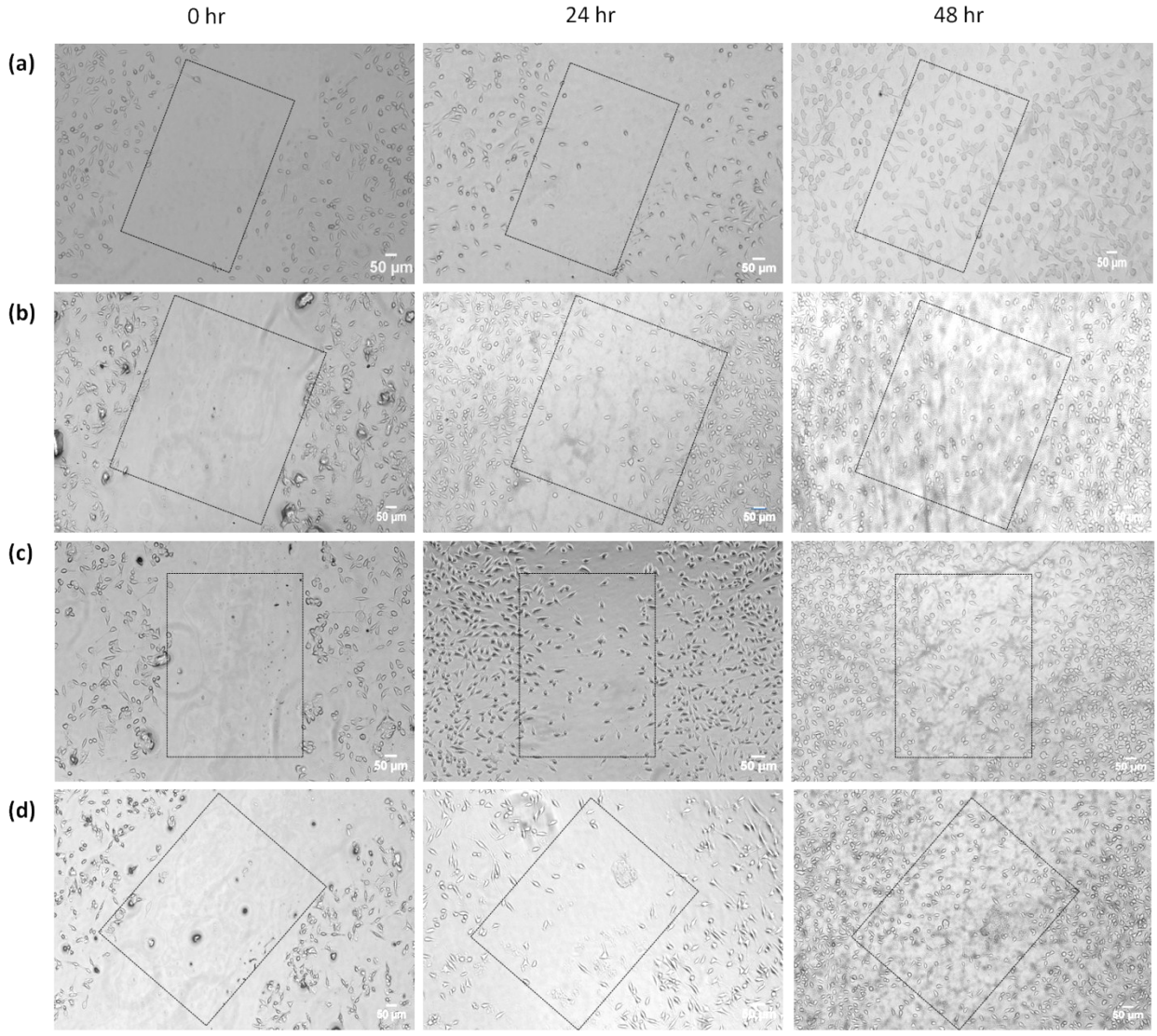

Figure S18: Proliferation and migration response of L929 cells in the presence of diluted peptide gels, assessed by microscopic images of scratched region after 0 hr, 24 hrs and 48 hrs time points after treatment with (a) control (~~10~~ 2%DMSO/water, blank), (b) Nap-FFGSO+A, (c) Nap-FFGSO+B and (d) Nap-FFGSO+A+B at concentration of 1000µg/ml (1.3mM).