

# Designing highly tunable nanostructured peptide hydrogels using differential thermal history to access variable cellular response

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**Table S1** Gelation behaviour of the Cbz-FF-OH dipeptide at room temperature with variable thermal history. The peptide solutions were heated at different gelation induction temperature to induce gelation along with the optical images of the solution/hydrogels formed using different thermal history.

**Table S2** Gelation behaviour of collagen.

**Table S3** Fiber diameter of different hydrogels formed by heating at different gelation temperatures 40°C-90°C as revealed from AFM study.

**Table S4** Stress relaxation time ( $\tau_{1/2}$ ) obtained from stress-relaxation curve for different hydrogels at different thermal history and collagen hydrogel.

**Table S5** Fiber diameter of the gel (Gel<sub>50°C</sub>  $\xrightarrow{\Delta}$  80°C), i.e., after the heating of the Gel<sub>50°C</sub> at 80°C for 1 hour, revealed from AFM study.

**Figure S1** Fluorescence microscopic images of (a) ThT control, ThT bound to (b) Gel<sub>40°C</sub>, (c) Gel<sub>50°C</sub>, (d) Gel<sub>60°C</sub>, (e) Gel<sub>70°C</sub>, (f) Gel<sub>80°C</sub>, (g) Gel<sub>90°C</sub> and (h) collagen.

**Figure S2** Amplitude sweep study of (a) Gel<sub>50°C</sub>, (b) Gel<sub>60°C</sub>, (c) Gel<sub>70°C</sub>, (d) Gel<sub>80°C</sub> and (e) Gel<sub>90°C</sub>.

**Figure S3** Morphological analysis of different hydrogels in DMEM at different thermal history, FESEM images of (a) Gel<sub>40°C</sub>, (b) Gel<sub>50°C</sub>, (c) Gel<sub>60°C</sub>, (d) Gel<sub>70°C</sub>, (e) Gel<sub>80°C</sub> and (f) Gel<sub>90°C</sub>.

**Figure S4** (a) MTT assay of the peptide solution, (b) bright field images of cells adhered on peptide solution (Concentration 500 µg/ml)

**Figure S5** Bright field images of cells adhered on (a) control, (b) collagen and different hydrogels with diverse nanostructures (c) Gel\_40°C, (d) Gel\_50°C, (e) Gel\_60°C, (f) Gel\_70°C, (g) Gel\_80°C and (h) Gel\_90°C. (Gel concentration 500 µg/ml)

**Figure S6** (a) Biocompatibility study of collagen and different hydrogels at different gelation induction temperatures on neuroblastoma SH-SY5Y cells. Bright field images of cells adhered on (b) control, (c) collagen and different hydrogels with diverse nanostructures (d) Gel\_40°C, (e) Gel\_50°C, (f) Gel\_60°C, (g) Gel\_70°C, (h) Gel\_80°C and (i) Gel\_90°C. (Gel concentration 500 µg/ml)

**Figure S7** FESEM study of the different hydrogels (a) Gel\_40°C, (b) Gel\_50°C, (c) Gel\_60°C, (d) Gel\_70°C, (e) Gel\_80°C and (f) Gel\_90°C on the surface of the glass after rewetting with media in the 2D cell culture experiment.

**Figure S8** FACS analysis of fibroblast L929 cells (a) in control and after treated with (b) collagen and different gels of Cbz-FF-OH fabricated with variable thermal history (c) Gel\_40°C, (d) Gel\_50°C, (e) Gel\_60°C, (f) Gel\_70°C, (g) Gel\_80°C, (h) Gel\_90°C. Percentage of live and dead cells indicated by the quadrant Q1 and Q3, respectively.

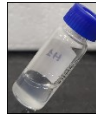





**Figure S9** Biocompatibility study of Cbz-FF-OH peptide hydrogels formed by heating at 50°C and the thermoreversible hydrogel that is formed by melting Gel\_50°C at 80°C for 1 hour followed by cooling to RT to fabricate new hydrogel (Gel\_50°C  $\xrightarrow{\Delta}$  80°C).

**Figure S10** Bright field images of cells adhered on different hydrogels with diverse nanostructures (a) Cbz-FF-OH peptide hydrogels formed by heating at 50°C and (b) the thermoreversible hydrogel that is formed by melting Gel\_50°C at 80°C for 1 hour followed by cooling to RT. (Gel concentration after dilution 500 µg/ml)


**Figure S11** Alamar study of Cbz-FF-OH peptide hydrogels formed by heating at 50°C and the thermoreversible hydrogel that is formed by melting Gel\_50°C at 80°C for 1 hour followed by cooling to RT resulting in new hydrogel (Gel\_50°C  $\xrightarrow{\Delta}$  80°C).

**Table S1** Gelation behaviour of the Cbz-FF-OH dipeptide at room temperature with variable thermal history. The peptide solutions were heated at different gelation induction temperature

to induce gelation along with the optical images of the solution/hydrogels formed using different thermal history.

[ CbzFFOH ] (mM)	Gelation induction temperature (°C)	Observations after 24 hours	Digital images of the gels
30	40	Weak Gel	
30	50	Gel	
30	60	Gel	
30	70	Gel	
30	80	Gel	
30	90	Gel	

**Table S2** Gelation behaviour of collagen.

Concentration of Collagen	Observation after 24 hours	Digital image of the gel
1 mg/ml	Self-supporting hydrogel	

**Table S3** Fiber diameter of different hydrogels formed by heating at different gelation temperatures 40°C-90°C as revealed from AFM study.

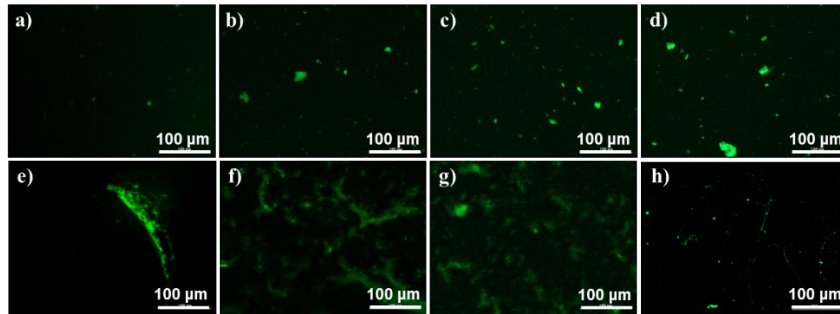
Name of the gels	Fiber diameter (nm)
Gel_40°C	66 ± 4.5
Gel_50°C	63 ± 3.5
Gel_60°C	55 ± 4.1
Gel_70°C	52 ± 2.8
Gel_80°C	41 ± 2.1
Gel_90°C	39 ± 2.4
Collagen	40 ± 3.1

**Table S4** Stress relaxation time ( $\tau_{1/2}$ ) obtained from stress-relaxation curve for different hydrogels at different thermal history and collagen hydrogel.

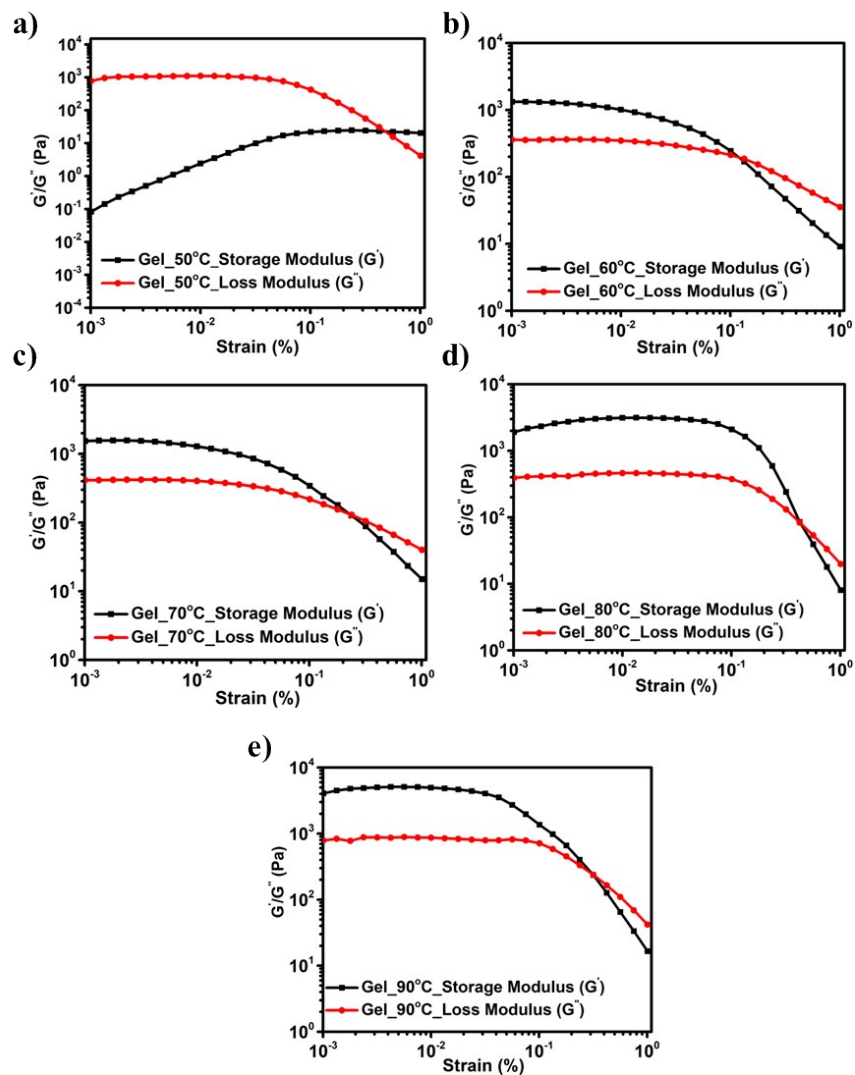
Name of the gels	Stress relaxation time ( $\tau_{1/2}$ ) (Sec)
Gel_50°C	34.008
Gel_60°C	3.427
Gel_70°C	4.781
Gel_80°C	2.447
Gel_90°C	5.194
Collagen	0.92

**Table S5** Fiber diameter of the gel (Gel\_50°C  $\xrightarrow{\Delta}$  80°C), i.e., after the heating of the Gel\_50°C at 80°C for 1 hour, revealed from AFM study.

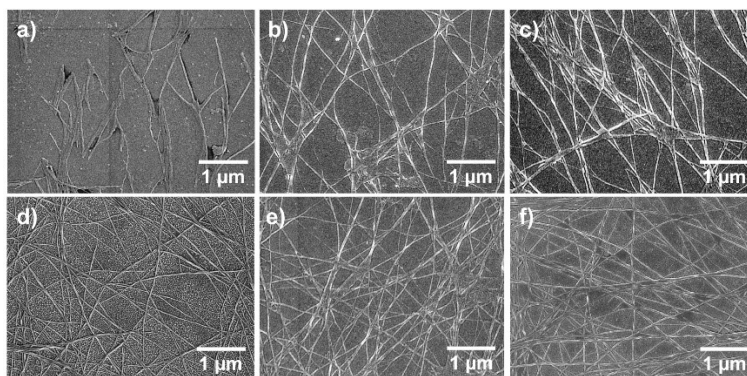
Name of the gels	Fiber diameter (nm)
$\Delta$ (Gel_50°C $\rightarrow$ 80°C)	43 $\pm$ 3.2



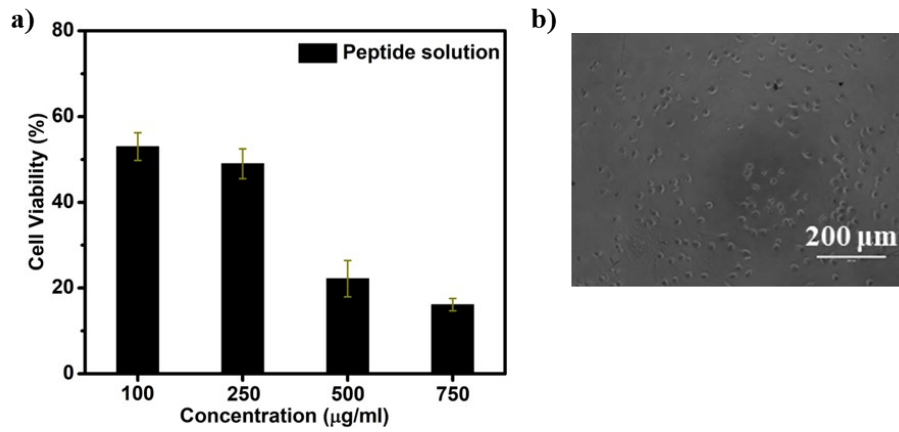
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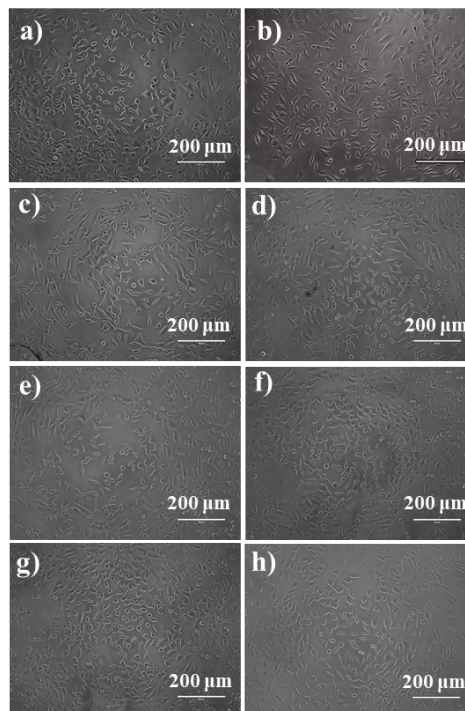
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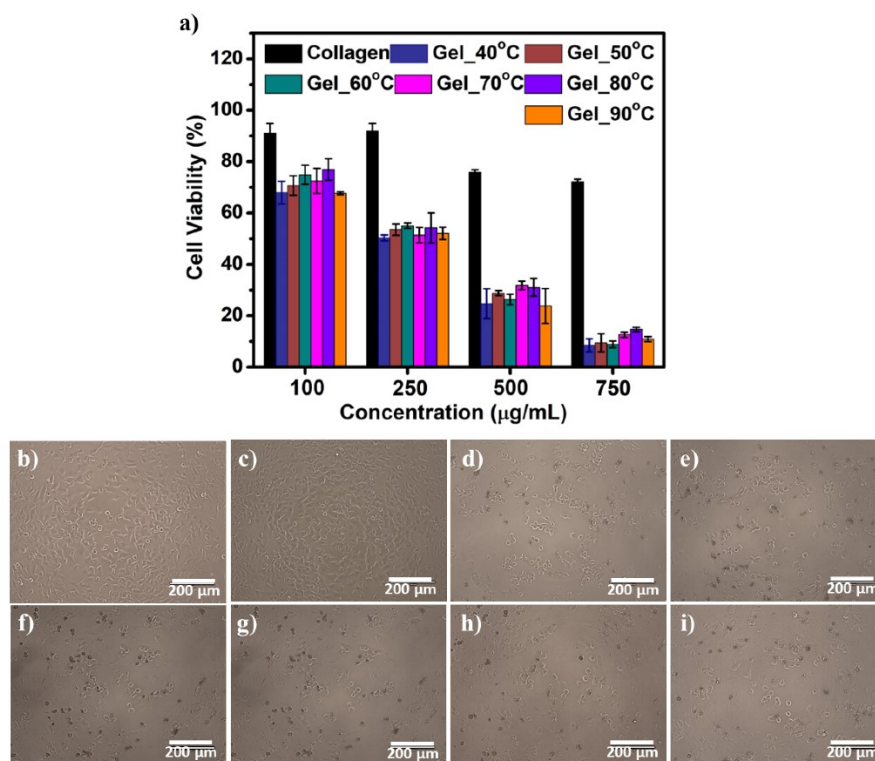
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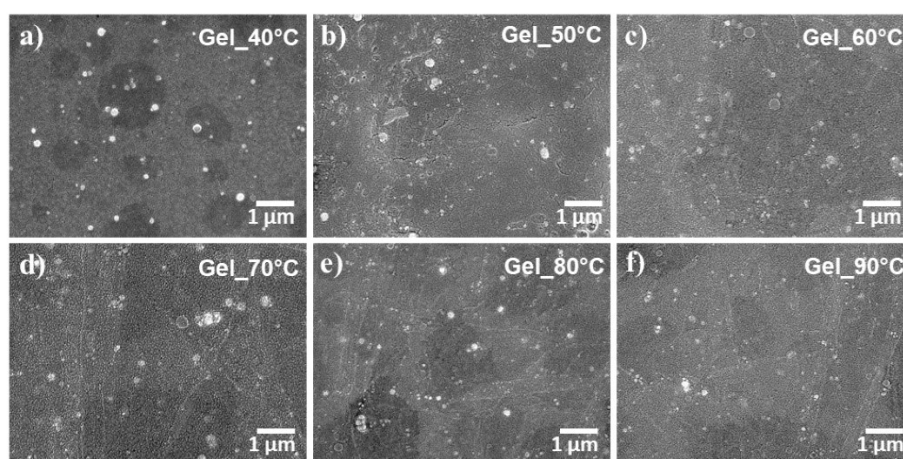
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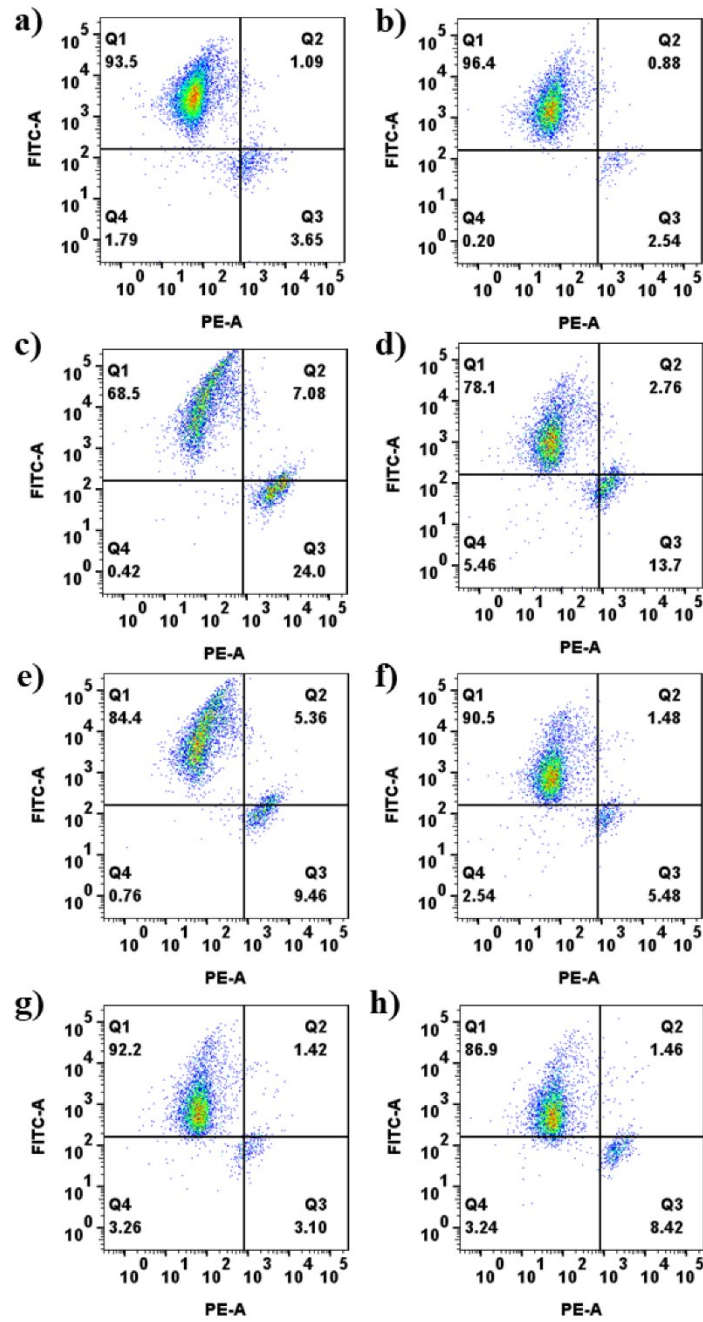


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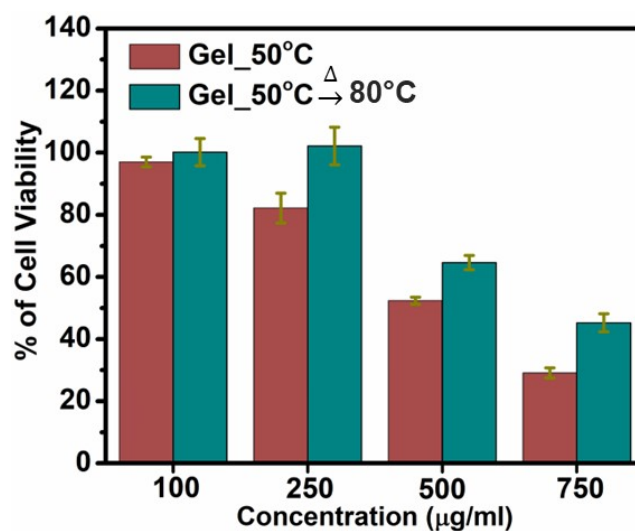


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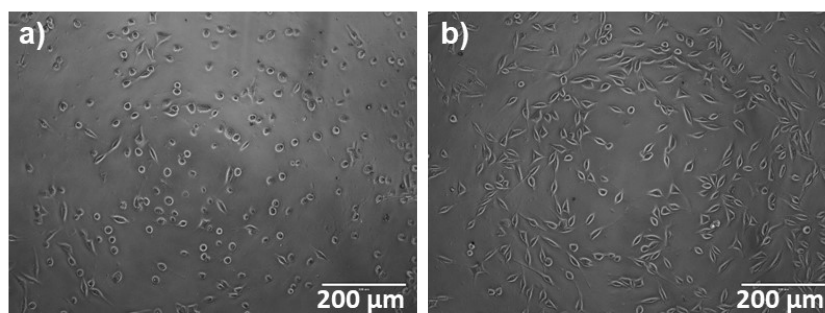




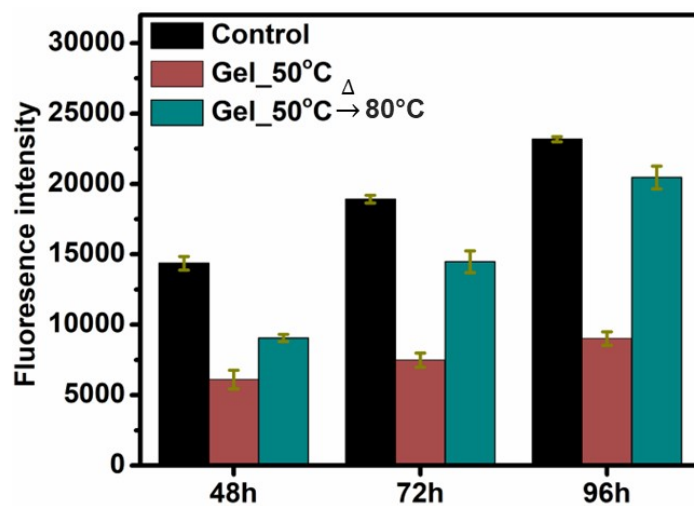
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