Resilin-like polypeptide hydrogels engineered for versatile biological function (Supplementary Information)

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Cloning RLP Constructs

We incorporated 5 enzymatic restriction sites along the RLP DNA template to allow insertion of short DNA oligos that are encoded for various biological sequences to fulfill this purpose. Shown in the RLP sequence in **Table 2**, these 5 enzymatic restriction sites to incorporate biological domains at amino acid level are shown as short amino-acid dyads between various modules. Sites that are employed to generate RLP-RGD, RLP-RDG and RLP-MMP in this manuscript are highlighted with underlines which are position ***VD*** and ***GT*** encoded via Sal I (RLP-MMP) and Kpn I (RLP-RGD and RLP-RDG) restriction sites, respectively. Other positions are included to permit further manipulation of cross-linking density, additional biological sequences or/and optimization of the concentration of certain biological domains. DNA plasmids encoding RLP were mini-prepped and digested either with Sal I or Kpn I to generate vectors for creating other RLP constructs. These agarose-gel extracted linearized vectors were ligated with DNA oligos that encode RGD, RDG and MMP sequences.

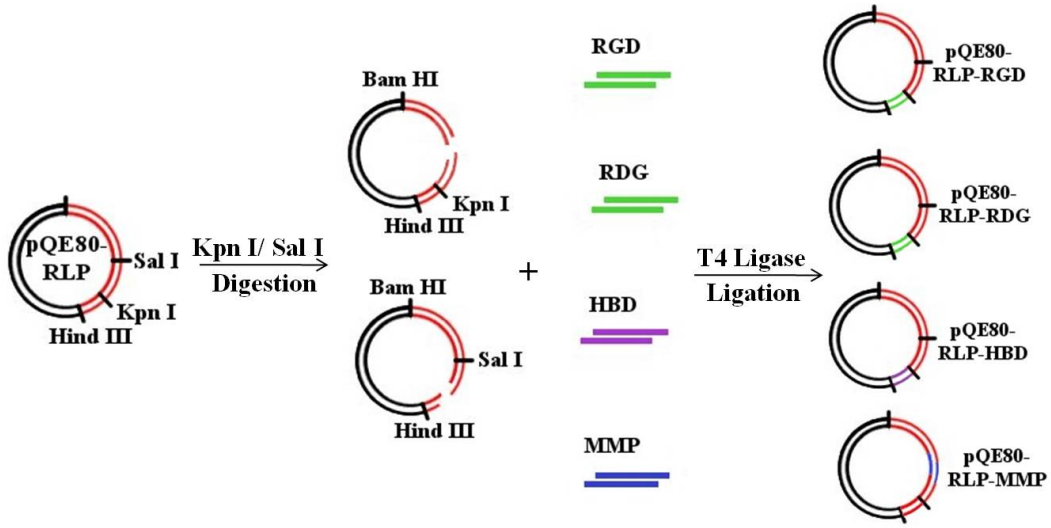

Figure S1. The schematic genetic construction of RLP constructs

RLP 798bps

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CGATTCTATGGGTGCCCCGGGCGGTGGTAATGGTGGTCGTCCGAGTGATAGCTTCGGTGCAC
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TT

RLP-RGD 825bps

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GTCTGATAGCATGGGTGCACCGGGCGGTGGTAATGGTGGTCGCCCGAGCGATAGCTTTGGTG
CACCGGGCGGTGGCAATGAGCTCGGTGGCAAAGGCGGCAAAGGTGGCAAAGGCGGTGAGC
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CGATTCTATGGGTGCCCCGGGCGGTGGTAATGGTGGTCGTCCGAGTGATAGCTTCGGTGCAC
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GAGATAGCCCAGGCGGTACCTAAAAGCTT

RLP-RDG 825 bps

ATGAGAGGATCGCATCACCATCACCATCACGGATCCAGATCTGGTGGTAAAGGTGGTAAAG
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GTCGACGGCGGCCGCCCGAGTGATTCTTTTGGTGCACCGGGTGGCGGCAATGGTGGTCGTCC
GTCTGATAGCATGGGTGCACCGGGCGGTGGTAATGGTGGTCGCCCGAGCGATAGCTTTGGTG
CACCGGGCGGTGGCAATGAGCTCGGTGGCAAAGGCGGCAAAGGTGGCAAAGGCGGTGAGC
TCGGTGGTCGCCCGAGCGATAGCTTCGGCGCGCCGGGCGGCGGTAATGGTGGTCGCCCGAG
CGATTCTATGGGTGCCCCGGGCGGTGGTAATGGTGGTCGTCCGAGTGATAGCTTCGGTGCAC
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RLP-MMP 825bps

ATGAGAGGATCGCATCACCATCACCATCACGGATCCAGATCTGGTGGTAAAGGTGGTAAAG
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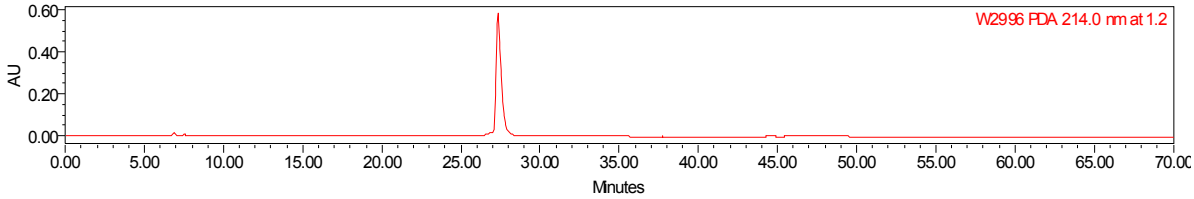
GGTCGTCCGAGTGATAGCTTCGGTGCACCGGGCGGCGGCAATGGCGGTAAAGGCGGTAAAG
GTGGCAAAGGCGGTGGTACCTAAAAGCTT

Figure S2. DNA Sequences of all RLPs

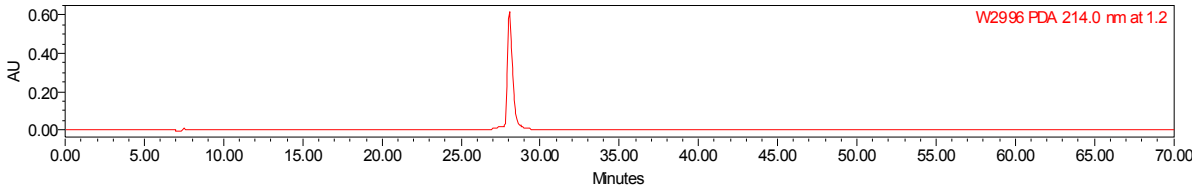
Expression and Purification of RLP Constructs

A single colony of *E coli* M15[pREP4] containing each RLP construct was inoculated in 100ml sterile LB media containing appropriate amount of antibiotics (30 μg/mL kanamycin and 100 μg/mL ampicillin) and grown overnight. 10mL of overnight culture media was subsequently used to inoculate 500mL of 2xTY media (yeast 10g/L, NaCl 5g/L, tryptone 16g/L) for protein expression. The 500mL cultures were grown in a shaker flask at 37 °C until the OD_{600} reached 0.6-0.8 (approximately 2 hours) and then IPTG (final concentration of 1mM) was added to induce the protein expression. Cells were harvested by centrifugation (10000 g for 30 mins at 4 °C), and the cell pellets were stored at -80 °C. The frozen cell pellets were lysed by freeze-thaw cycles and the lysed cell pellets were suspended in pH 8.0 native lysis buffer (50mM NaH_2PO_4, 300mM NaCl, 10mM imidazole). Lysed cells were further disrupted via sonication on ice, using a Fisher Scientific model 500 Sonic Dismembrator (10 mm tapered horn) for 30 mins with a 10-second recovery time. The cell lysate was then centrifuged at 40000 g for 60 mins, the supernatant collected, and the pH adjusted to 8.0, followed with incubation with Ni-NTA resin overnight at 4 °C. The resin was then loaded into a gravitational flow column, washed with native lysis buffer, native wash buffer (50mM NaH_2PO_4, 300mM NaCl, 20mM imidazole, pH 8.0), and finally eluted with native elution buffer (50mM NaH_2PO_4, 300mM NaCl, 250mM imidazole, pH 8.0). 100mL elution fractions were carefully transferred and dialyzed (MWCO 10000) against deionized water (5L) at 4°C with at least 6 changes of water before lyophilization.

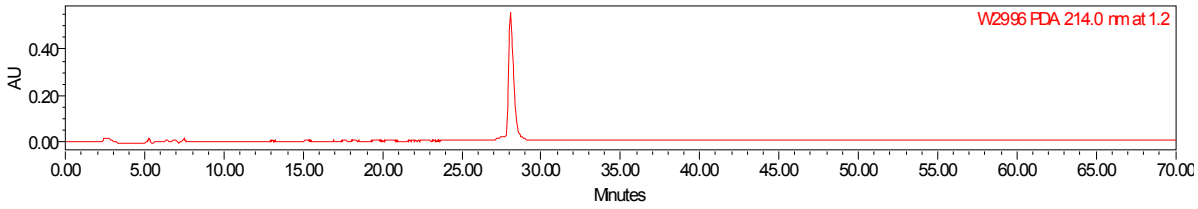
RLP



RLP-RGD



RLP-RDG



RLP-MMP

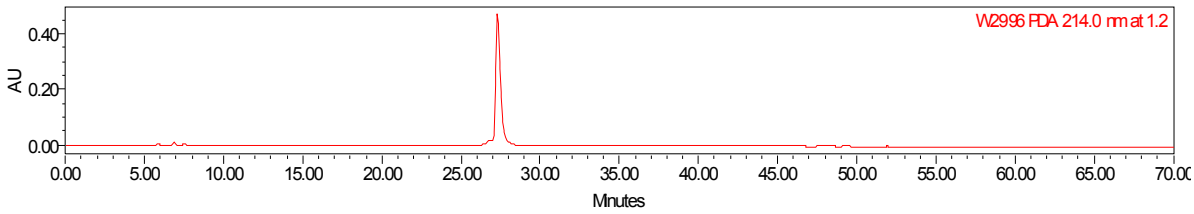


Figure S3. HPLC analysis of RLPs

Table S1. MALDI-TOF mass spectroscopy results for each RLP construct.

Polypeptides	Expected Mass (Da)	Observed Mass (Da)
RLP	23126.5	23125
RLP-RGD	23911	23906
RLP-RDG	23911	23907
RLP-MMP	24007	23993

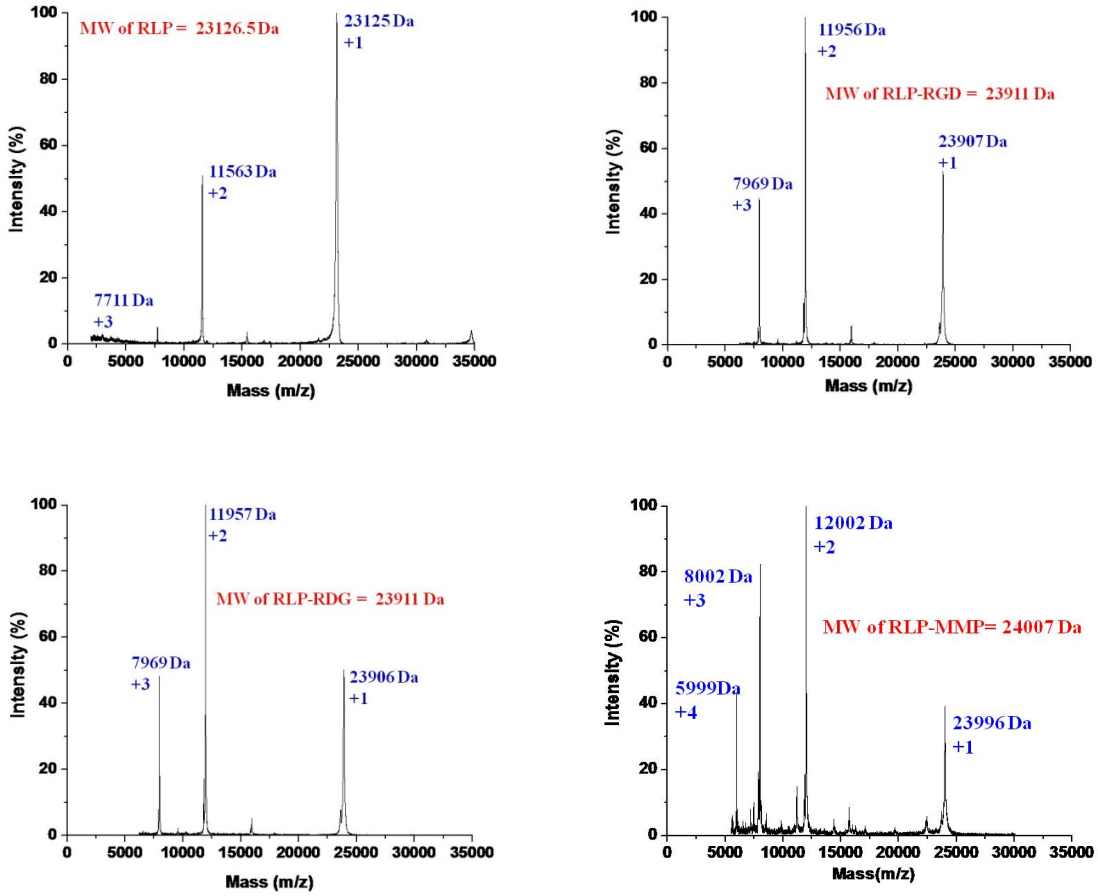


Figure S4. MALDI-TOF Mass Spectrometry of RLPs

Table S2. Amino acid analysis of RLPs

Polypeptides	RLP		RLP-RGD		RLP-RDG		RLP-MMP	
Amino acid	Calc mol%	Obs mol%	Calc mol%	Obs mol%	Calc mol%	Obs mol%	Cal mol%	Obs mol%
Asx	9.88	9.97	9.93	10.23	9.93	10.29	9.56	10.25
Ser	10.27	9.7	10.29	8.95	10.29	9.01	9.93	9.11
Glx	1.52	1.52	1.47	1.50	1.47	1.51	2.21	2.96
Pro	9.13	10.03	9.19	9.31	9.19	9.30	9.19	9.26
Gly	43.73	43.79	43.75	44.10	43.75	44.27	43.75	44.23
Ala	4.56	4.99	4.41	4.71	4.41	4.73	4.41	4.68
Phe	3.04	3.04	2.94	3.10	2.94	3.09	2.94	2.89
Lys	5.71	6.31	5.52	6.61	5.52	6.51	5.51	5.89
Arg	5.32	5.56	5.52	5.76	5.52	5.76	5.15	5.22
His	2.28	2.31	2.2	2.41	2.2	2.31	2.21	1.71
Val	0.76	0.74	0.74	0.76	0.74	0.76	0.74	0.82
Lue	1.52	1.49	1.47	1.48	1.47	1.48	1.47	1.54
Thr	0.38	0.35	0.74	0.65	0.74	0.66	0.37	0.43
Met	1.9	0.5	1.84	0.40	1.84	0.37	1.84	0.49

Circular Dichroic Spectroscopy (CD).

Circular dichroic spectra were recorded on a Jasco J-810 spectropolarimeter (Jasco Inc, Easton, MD) equipped with a Jasco PTC-424S temperature controller. Background scans of DI H_2O were recorded and subtracted automatically from the sample scans. Samples were made at a concentration of approximately 10 μM via dilution. The samples (400 μL) were loaded into a 1 mm path length quartz cuvette. Data points for the wavelength-dependent CD spectra were recorded at every nanometer with a 1 nm bandwidth and an averaging time of 10s for each data point from 190 to 260 nm, at a temperature of 37 °C. The mean residue ellipticity $[\theta]_{MRE}$ (degrees cm^2 $dmol^{-1}$), was calculated via use of the concentration, molecular weight of the samples, number of residues and cell path length.

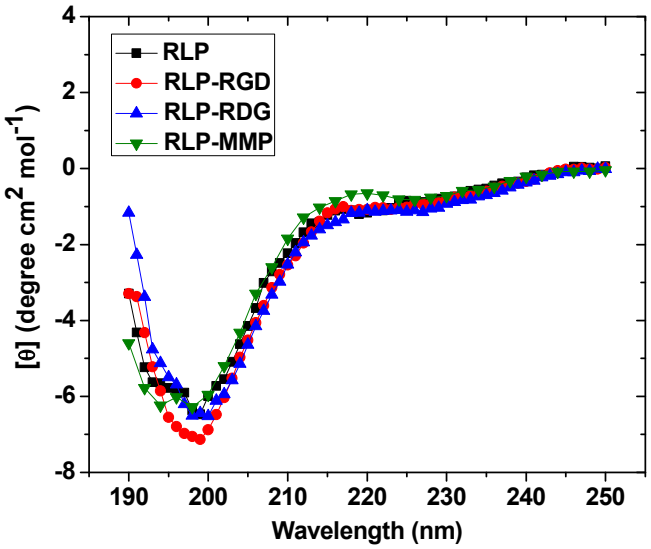


Figure S5. Circular dichroic (CD) characterization of all RLP constructs. Spectra were collected at a protein concentration of 10μM in PBS pH 7.4 at 37 °C.

FTIR-ATR Spectroscopy

FTIR-ATR experiments were performed using a Nexus 670 FTIR spectrometer (Thermo Nicolet, Madison, Wisconsin) with unpolarized light and a MCT detector. The PIKE MIRacle™ single reflection ATR with high IR throughput was used as a universal ATR sampling accessory for analysis of RLP solutions and hydrogels. Spectra recorded at a resolution of 4 cm^{-1} from 650 to 4000 cm^{-1} were obtained by signal averaging 256 scans. 10μl of uncross-linked RLP solution, cross-linked RLP hydrogel, cross-linked 50% RLP + 50% RLP-RGD hydrogel, and cross-linked 50% RLP-RGD + 50% RLP-MMP in D_2O was either dropped or squeezed tightly in order to be in good contact with the diamond crystal plate before collecting the spectra. Uncross-linked RLP solution samples were prepared by the dissolution of RLP in D_2O at concentrations of 20wt% and all RLP hydrogels were formed at a 1:1 (lysine:HMP) ratio with 20wt% polypeptide concentrations in D_2O. The Amide-I region (1600 cm^{-1} to 1700 cm^{-1}) was deconvoluted (Gaussian peaks) using the multiple-peak fitting function in Origin Data Analysis software

(OriginLab, Northampton, MA). Five peaks were employed for fitting the data, which was determined to be the optimal number of peaks for the fit based on assessment of R^2 values.

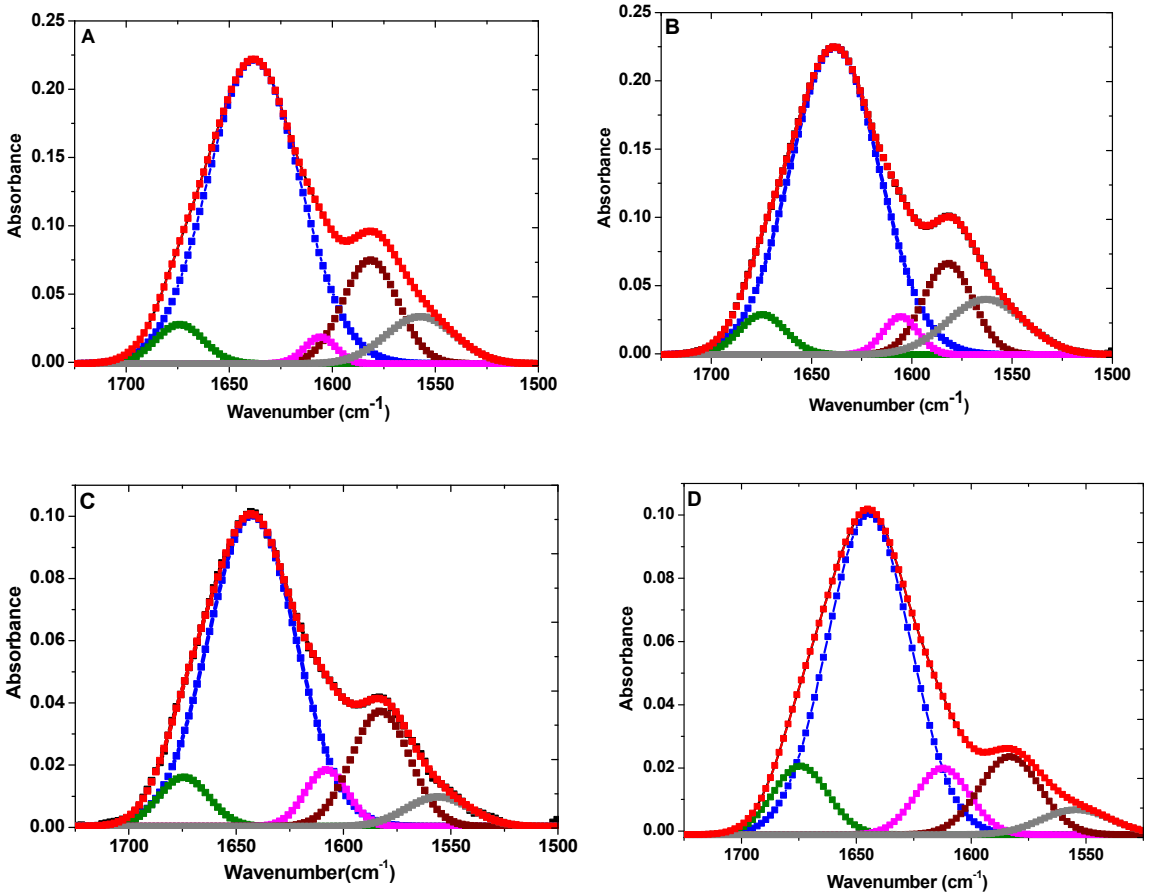


Figure S6. Deconvoluted FTIR-ATR spectra of (A) an uncross-linked, 20wt% RLP solution; (B) 20wt% cross-linked 100% RLP hydrogel; (C) 20wt% cross-linked 50% RLP - 50% RLP-RGD hydrogel; and (D) 20wt% cross-linked 50% RLP-RGD and 50% RLP-MMP hydrogel. All samples were prepared in D_2O at a1:1 (lysine : HMP) cross-linking ratio. In the Amide I region (1700 cm^{-1} to 1600 cm^{-1}), the original experimental data (black) was deconvoluted into peaks corresponding to β-turn (green), random coil (blue), and β-sheet (pink) structures. The final resulting fit is shown in red with a R^2 value of 0.999 for all compositions.

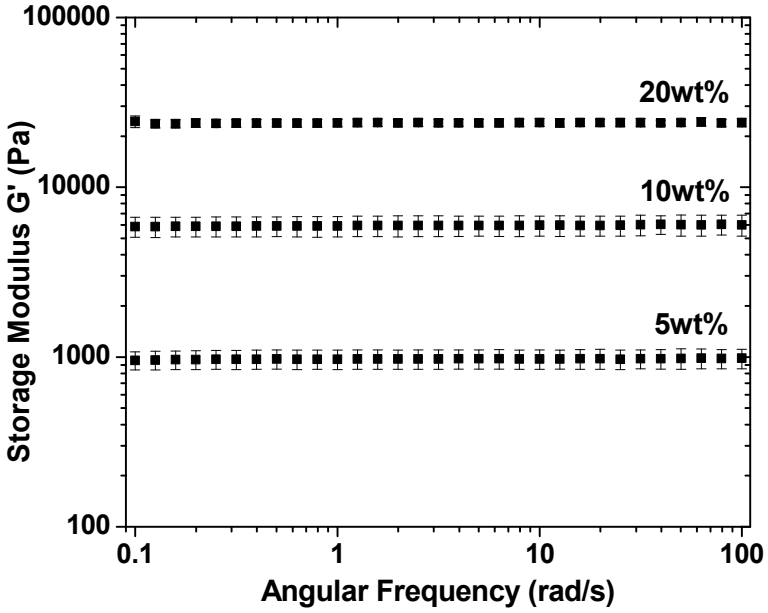


Figure S7. Frequency sweep of 5wt%, 10wt% and 20wt% RLP protein concentrations. G'' is less than 20 Pa under all three protein concentrations.

Swelling Ratio and Water Content Measurements

RLP films were lyophilized before measuring the dry weight. After the dry weights were measured, samples were immersed in phosphate buffered saline (PBS) for 24 hours at 37 ^{o}C, and the wet weights were measured after blotting the excess water with filter paper. The swollen weight was measured again after an additional 24 hours of immersion; no significant change was observed, suggesting that samples reached equilibrium after 24 hours of soaking. The water content of these hydrogels was evaluated in terms of the percentage water content (WC) and swelling ratio (q) at equilibrium. $WC = (W_s-W_d)/W_s \times 100$, $q= W_s/W_d$, where W_s and W_d are the weights of swollen and dry hydrogels. Data reported are an average 5 measurements of 3 different hydrogel compositions, with error reported as the standard deviation.

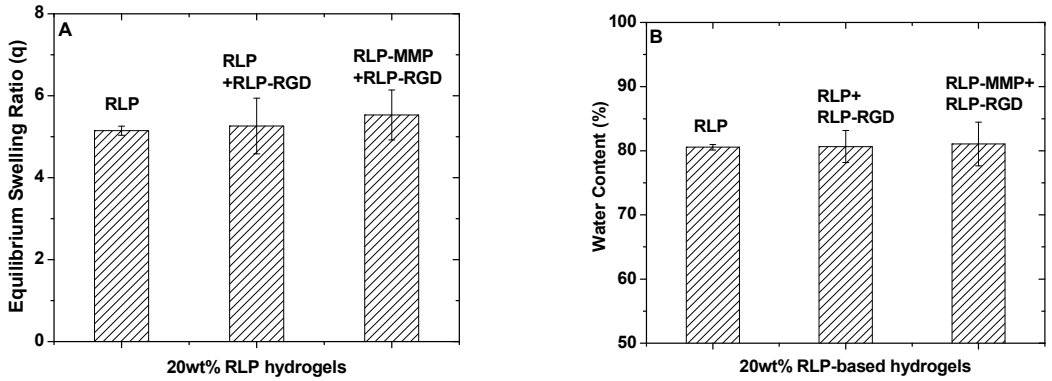


Figure S8. Swelling ratio (A) and water content (B) of RLP-based hydrogels with different compositions. Equilibrium swelling ratio (q) and percentage water content (%) for 20wt% RLP-based hydrogels were tested on an average of 5 repeat measurements at a 1:1 (lysine : HMP) cross-linking ratio, with error reported as the standard deviation.

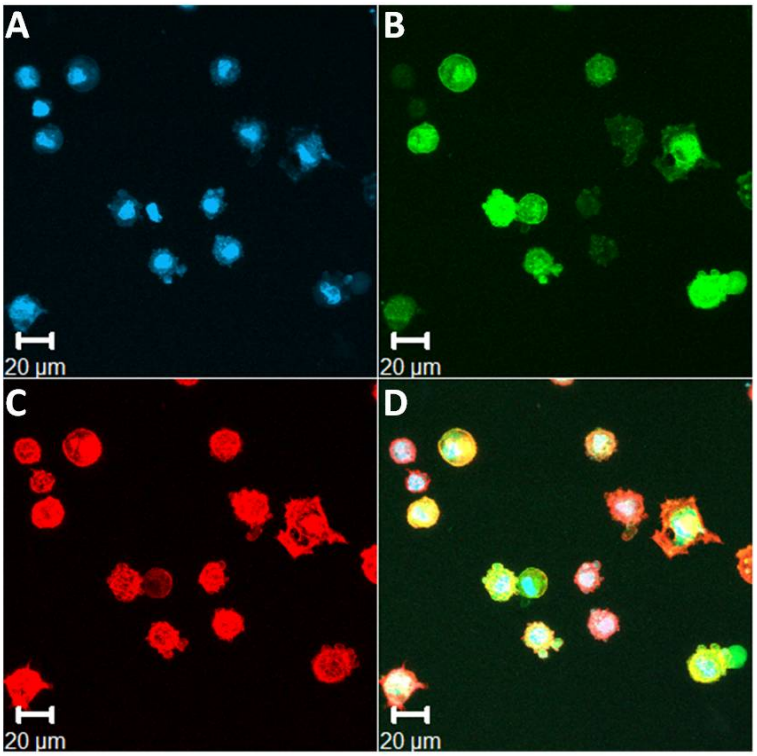


Figure S9. Representative F-actin/vinculin co-staining of hMSCs after 24 hours on the surface of a 20wt% RLP-only hydrogel with a composition of 100% RLP (1:1 (lysine : HMP) cross-linking ratio). (A) Cell nuclei were counterstained by Draq5; (B) focal adhesion sites were stained green by anti-vinculin and FITC-labeled secondary antibody; (C) F-actin filaments were stained red by TRITC-phalloidin,; and (D) merged image of triply-stained with Draq5, vinculin and TRITC-phalloidin.