Supporting Information

Self-assembling peptide hydrogels functionalized with LN- and BDNF- mimicking epitopes synergistically enhance peripheral nerve regeneration

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Methods

Cell proliferation assay

The proliferation of RSCs on different hydrogels was evaluated. 50 μL of peptide solution was loaded directly into the wells of a 96-well plate, and then 100 μL of culture medium was gently added onto the peptide solution. After incubation for 15 min at 37 °C, the medium was removed and changed at least twice to equilibrate the hydrogels. RSCs were seeded on the surface of the hydrogel at a density of 8×10^3 cells/well (n = 4), and the medium was changed with fresh medium every 2 days. Cell proliferation in different groups was assessed using Cell Counting Kit-8 (CCK-8, DOJINDO, Tokyo, Japan). After culture for 1, 4, and 7 days, the samples were incubated with 10% CCK-8 working solution in cell culture medium for 3 h at 37 °C. Then, 100 μL of the supernatant was transferred to a new 96-well plate, and the absorbance was measured at 450 nm using an EnSpire Multimode Plate Reader (PerkinElmer, USA).

Table S1. Primer sequences used for quantitative RT-PCR

Gene	Primers (F=forward, R=reverse)
GAPDH	F: ATGATTCTACCCACGGCAAG
	R: CTGGAAGATGGTGATGGGTT
NGF	F: CGCTCTCCTTCACAGAGTTTT
	R: GACATTACGCTATGCACCTCAGA
BDNF	F: TCTACGAGACCAAGTGTAATCCCA
	R: CTTATGAACCGCCAGCCAAT
CNTF	F: ATGGCTTTCGCAGAGCAAAC
	R: CAACGATCAGTGCTTGCCAC
IGF-2	F: GAACAACAATAGCCGCCCAAACTC
	R: CATGTTCTGTTCCTCTCCTTGGGT
S100	F: GTTGCCCTCATTGATGTCT
	R: CTGCTCTTTGATTTCCTCC
MBP	F: TCTGGCAAGGACTCACACAC
	R: AAATCTGCTGAGGGACAGGC
NCAM	F: TTCAGTGACGACAGTTCGGAGC
	R: TGCGAAGACCTTGAGGTGGAT
PMP22	F: TGTACCACATCCGCCTTGG
	R: GAGCTGGCAGAAGAACAGGAAC
NRP2	F: CTTGCTCCCTCTTTGCTG
	R: TTCCTTGTGGTGTCTTCTG
VEGF	F: ACCATGCCAAGTGGTGAAGT
	R: GGGCTTCATCATTGCAGCAG
P0	F: AAGTCTATGGTGCTGTGGGC
	R: CCCATACCTAGTGGGCACTTT

Table S2. Physicochemical properties of self-assembling backbone and functional motifs

Sequences	Net charge at pH=7	Hydrophilic residue ratio	Description
$(RADA)_4$	0.0	50%	Backbone
G_2-IKVAV	1.0	14%	LA motif
G_2-RGIDKRHWNSQ	2.1	54%	BDNF motif
G_2-IKVAV-G_2-RGIDKRHWNSQ	3.1	40%	Dual-motif

Table S3. Estimated secondary structure fractions of different peptides

Peptides	Secondary structure fractions (%)*					
	H (r)	H (d)	S (r)	S (d)	Turn	Unrd
RAD	4.8	0.0	32.3	14.1	15.4	33.4
RAD-IKV	8.5	11.4	0.0	8.4	24.9	46.9
RAD-RGI	4.8	1.9	0.0	6.1	32.2	55.0
RAD-IKV-GG-RGI	3.7	6.3	11.4	9.2	26.4	43.1
RAD/IKV	6.5	0.0	36.4	15.8	12.3	29.0
RAD/RGI	1.9	2.7	33.8	14.1	15.9	31.7
RAD/IKV-GG-RGI	2.5	0.0	28.6	11.7	20.4	36.9
RAD/IKV/RGI	2.1	0.1	32.8	13.1	19.4	32.4

*Abbreviations: H (r), regular α-helix; H (d), distorted α-helix; S (r), regular β-strand; S (d), distorted β-strand; Turn, β-turn structure; Unrd, unordered structure. Total Sheet content = S (r) + S (d).

Table S4. Nanofiber width from molecular models and AFM measurements

Peptides	W_{the}*	W_{adj}**	W_{mea}
RAD	5.9 nm	12.1 nm	15.4 ± 2.1 nm
RAD-IKV	8.3 nm	14.5 nm	-***
RAD-RGI	10.5 nm	16.7 nm	-
RAD-IKV-GG-RGI	13.0 nm	19.2 nm	-
RAD/IKV	10.7 nm	16.9 nm	18.1 ± 1.9 nm
RAD/RGI	15.1 nm	21.3 nm	23.0 ± 1.5 nm
RAD/IKV-GG-RGI	20.1 nm	26.3 nm	29.6 ± 2.2 nm
RAD/IKV/RGI	15.1 nm	21.3 nm	21.6 ± 0.9 nm

*According to the molecular model proposed in Figure 1A.

**W_{the}: theoretical fiber width; W_{mea}: measured fiber width by AFM; W_{adj}: adjusted theoretical fiber width considering the size effect of the AFM probe tip based on the estimation $W_{adj} = W_{the} + 2\,(2R_tH - H^2)^{1/2}$, R_t = AFM tip size (~ 10 nm), H = sample height (~ 0.5 nm) [1, 2].

***No nanofiber formation was observed.

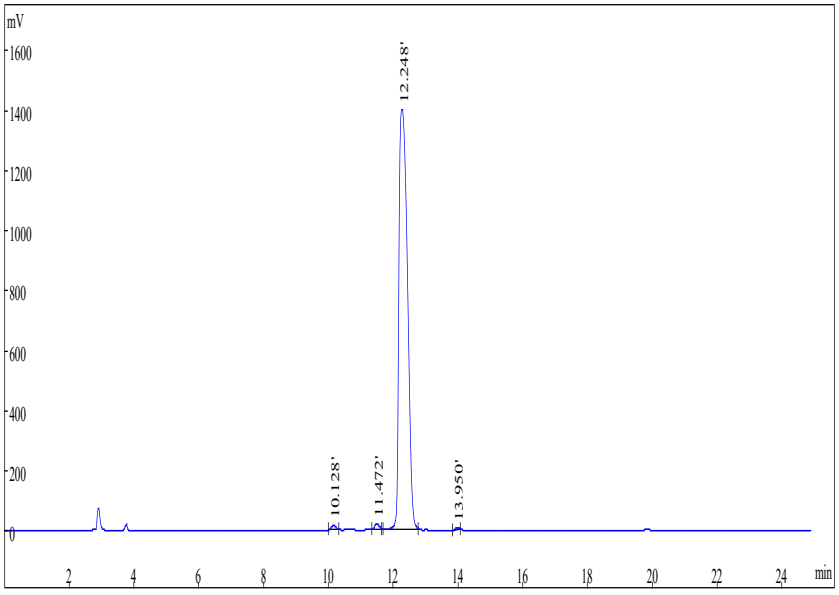

Figure S1. Analytical HPLC trace of RAD peptide.

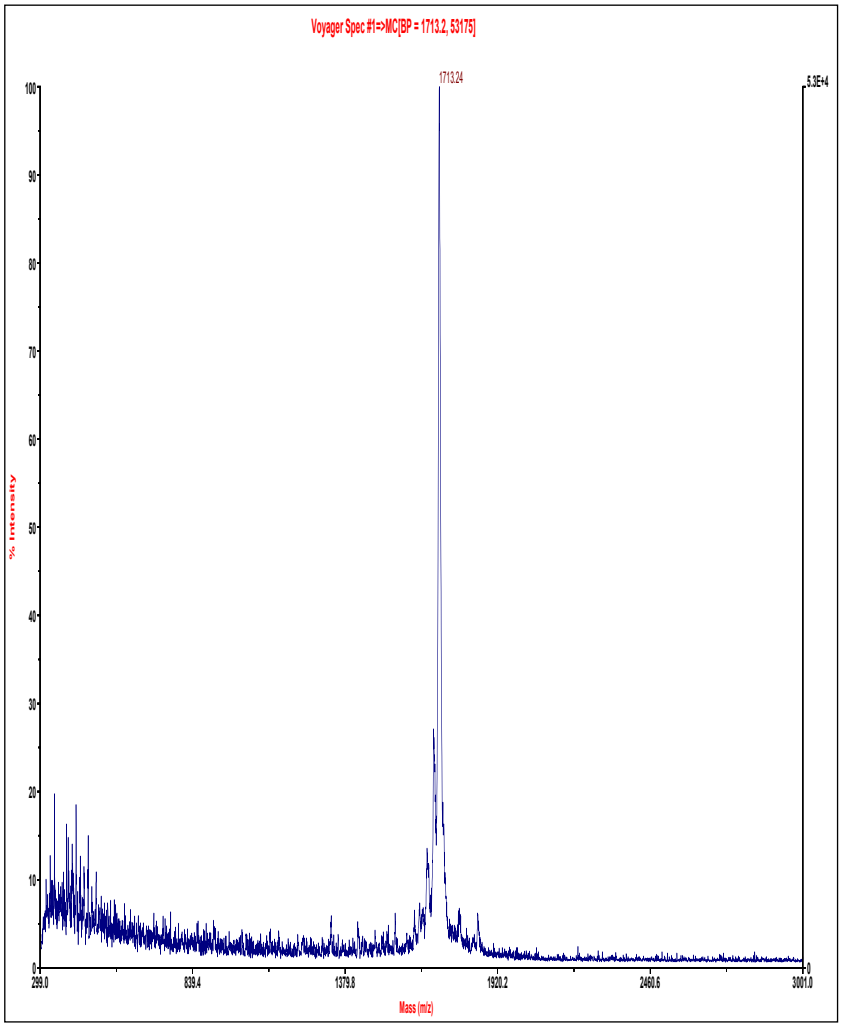

Figure S2. The electrospray ionization mass spectrometry (ESI-MS) of RAD peptide.

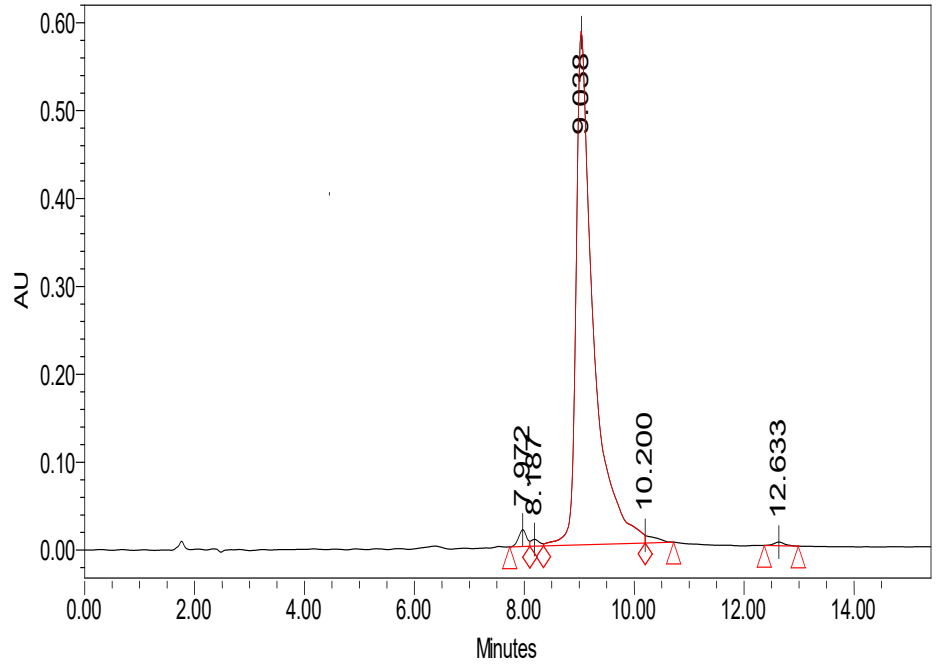

Figure S3. Analytical HPLC trace of RAD-IKV peptide.

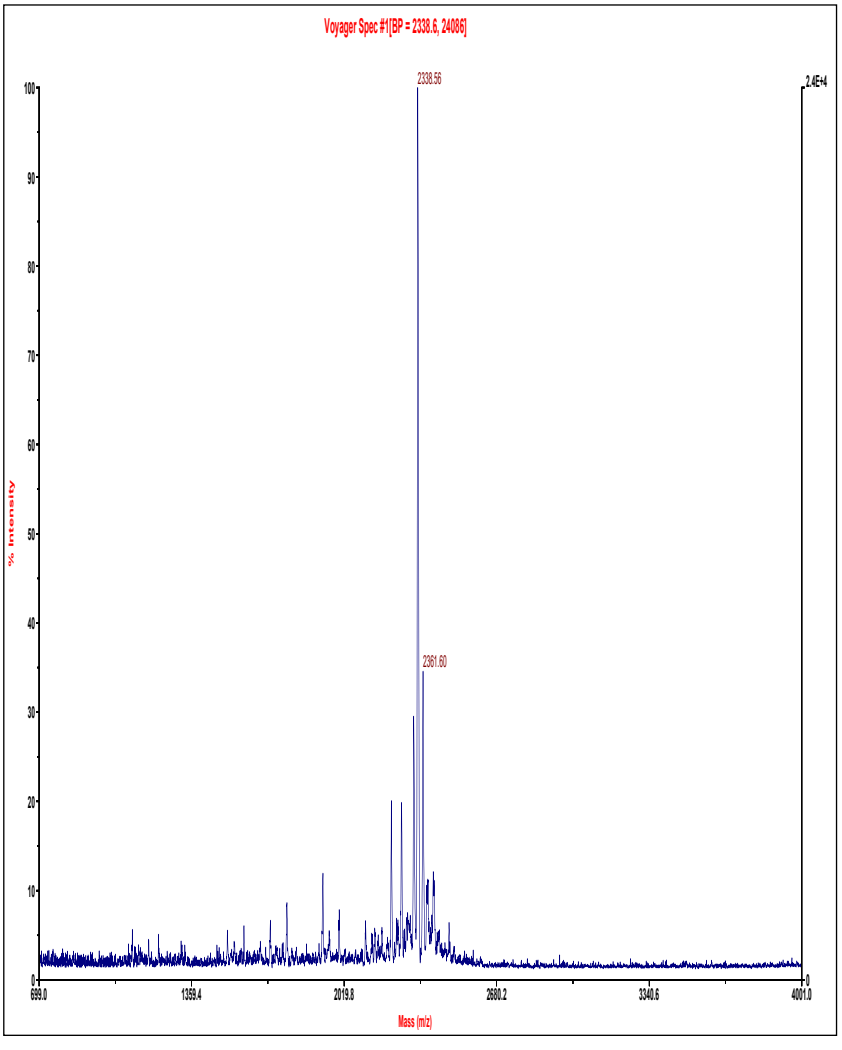

Figure S4. The electrospray ionization mass spectrometry (ESI-MS) of RAD-IKV peptide.

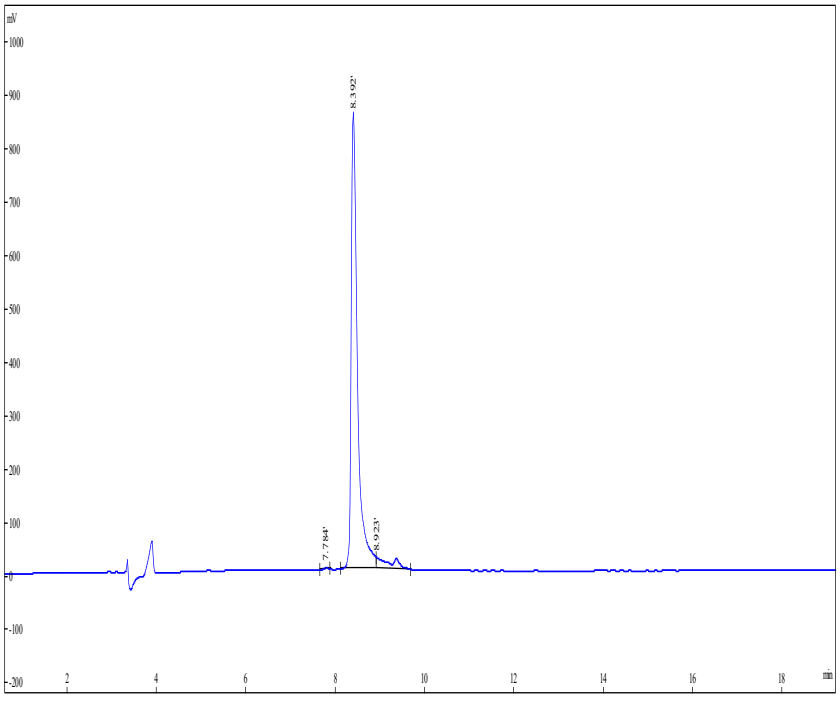

Figure S5. Analytical HPLC trace of RAD-RGI peptide.

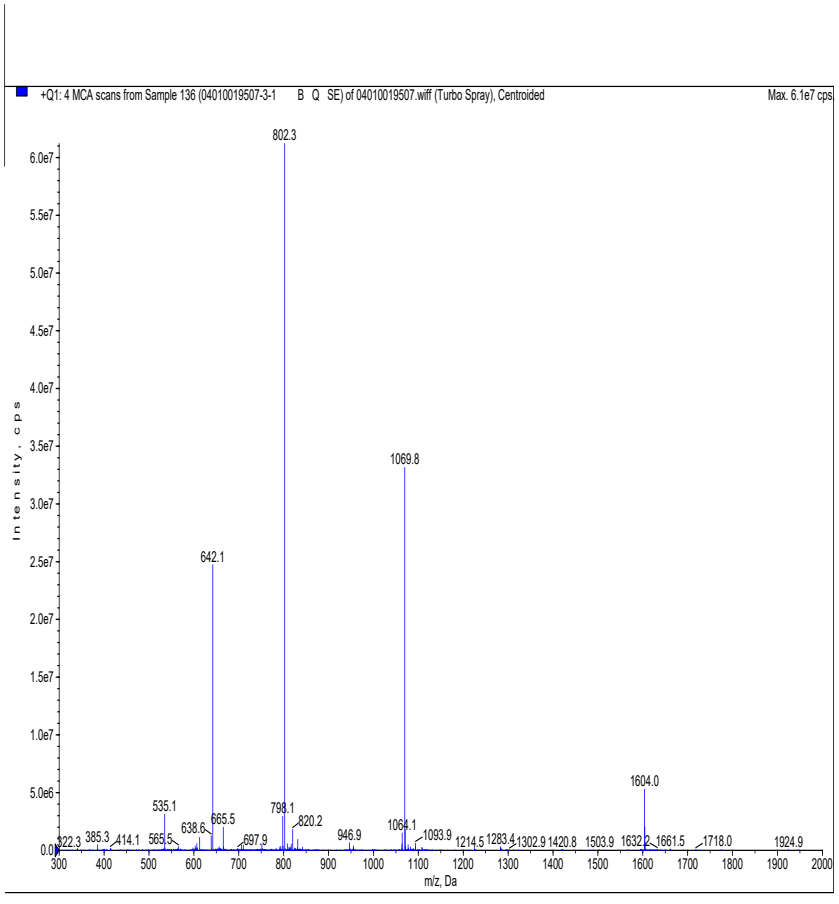

Figure S6. The electrospray ionization mass spectrometry (ESI-MS) of RAD-RGI peptide.

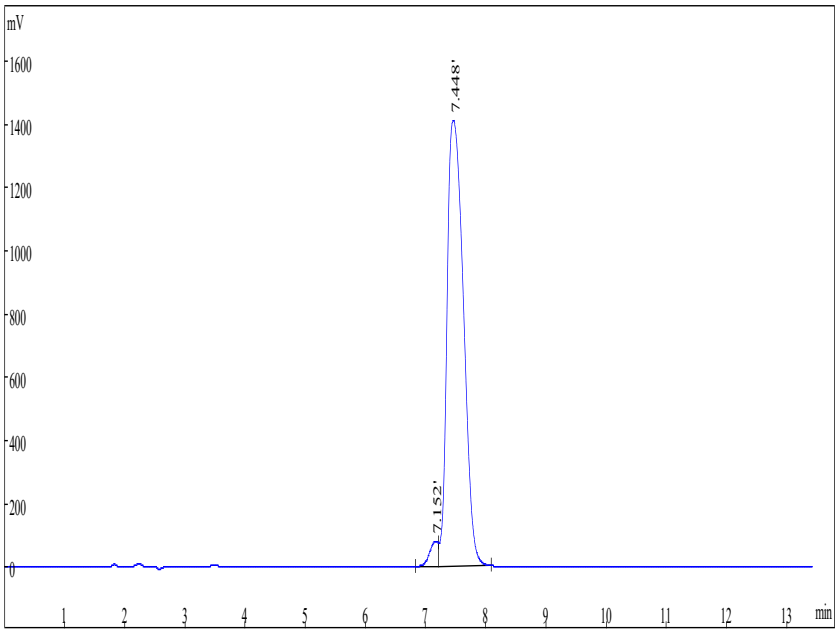

Figure S7. Analytical HPLC trace of RAD-IKV-GG-RGI peptide.

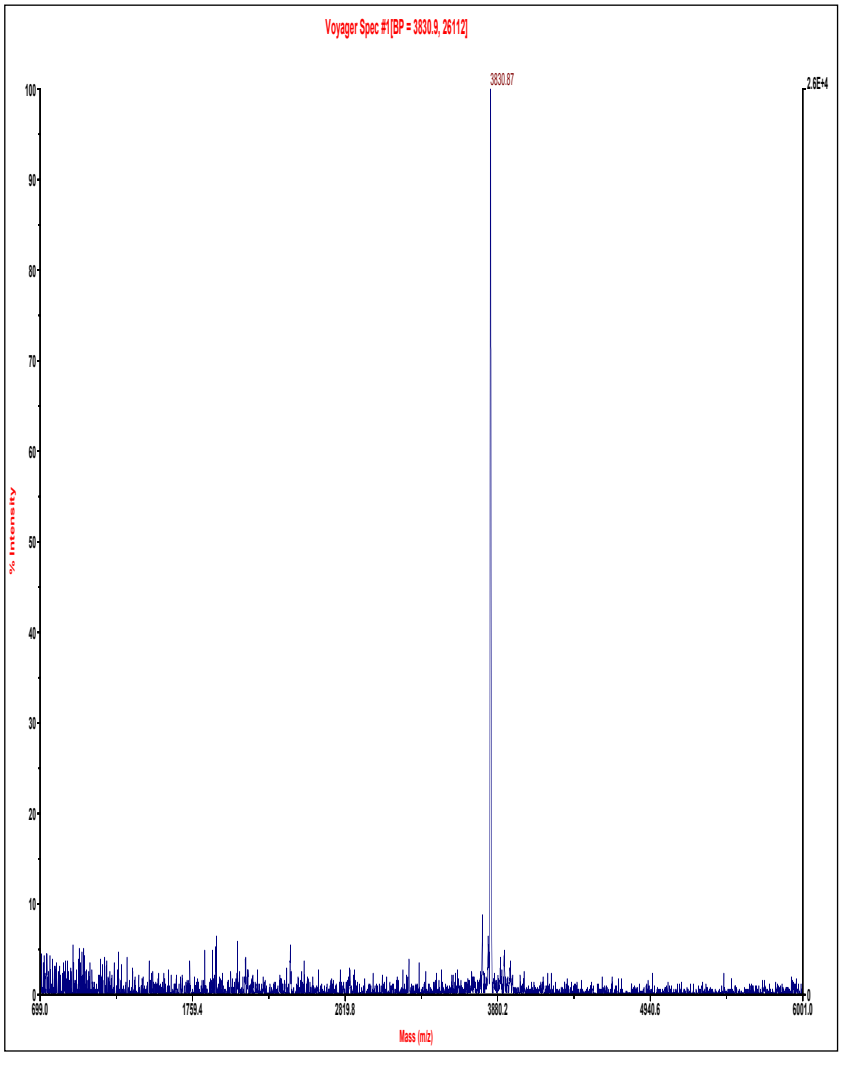

Figure S8. The electrospray ionization mass spectrometry (ESI-MS) of RAD-IKV-GG-RGI peptide.

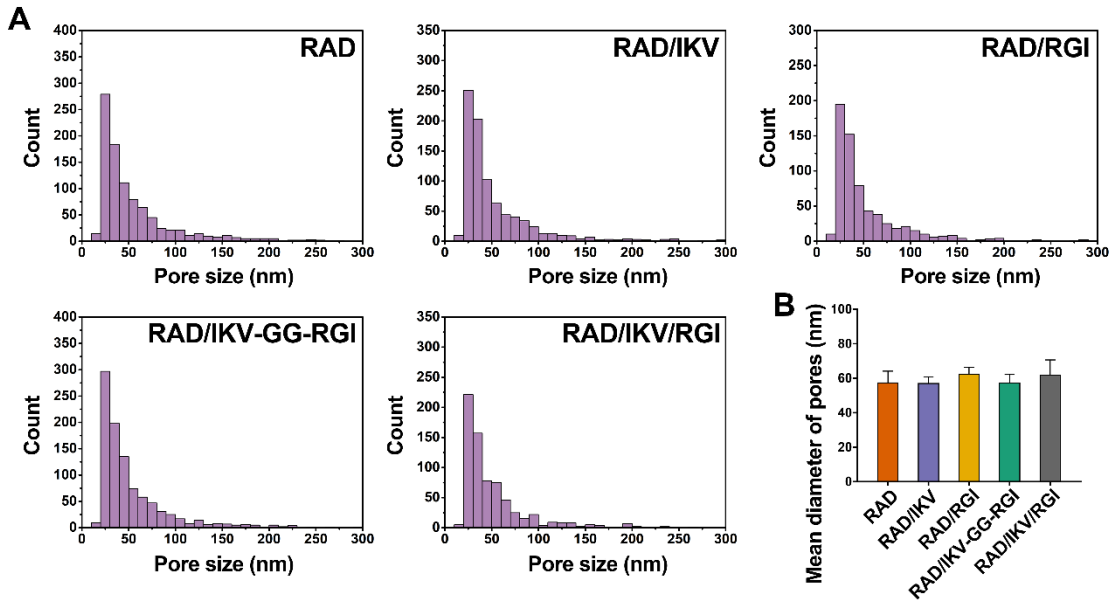

Figure S9. The size of pores in different self-assembling peptide hydrogels. (A) Typical distribution of pore sizes in different groups. (B) Analysis of the mean diameter of pores. Values are represented as mean ± SD. Statistical analysis was carried out using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test (n = 3).

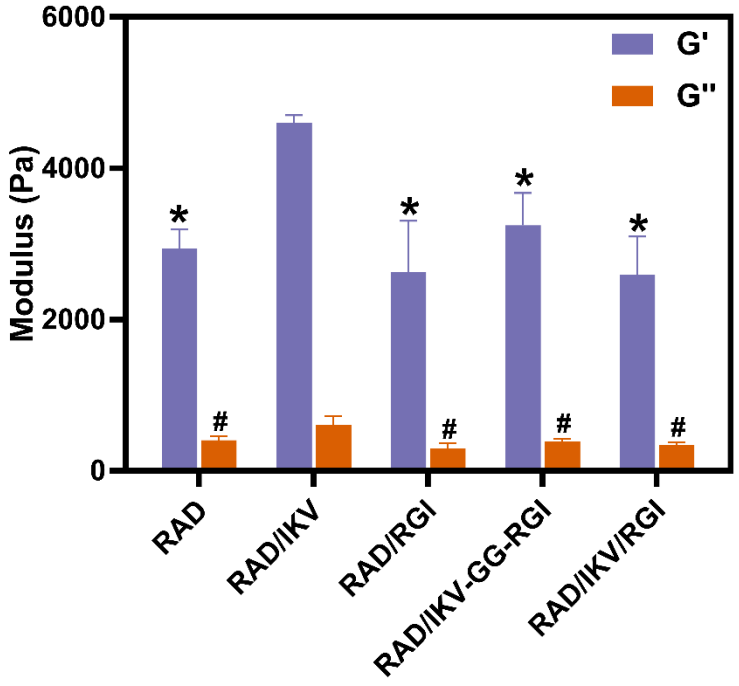

Figure S10. The average G' and G'' of self-assembling peptide hydrogels. The average G' values were respectively 2942.6 ± 250.0 Pa, 4600.0 ± 101.4 Pa, 2629.7 ± 675.1 Pa, 3246.3 ± 426.6 Pa, and 2594.8 ± 502.9 Pa. The average G'' values were respectively 406.4 ± 51.4 Pa, 606.4 ± 116.7 Pa, 298.1 ± 64.7 Pa, 386.9 ± 39.0 Pa, and 340.2 ± 35.4 Pa. Values are represented as mean ± SD. Statistical analysis was carried out using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. *$P < 0.05$, *versus* the G' of RAD/IKV hydrogel. $^{\#}P < 0.05$, *versus* the G'' of RAD/IKV hydrogel (n = 3).

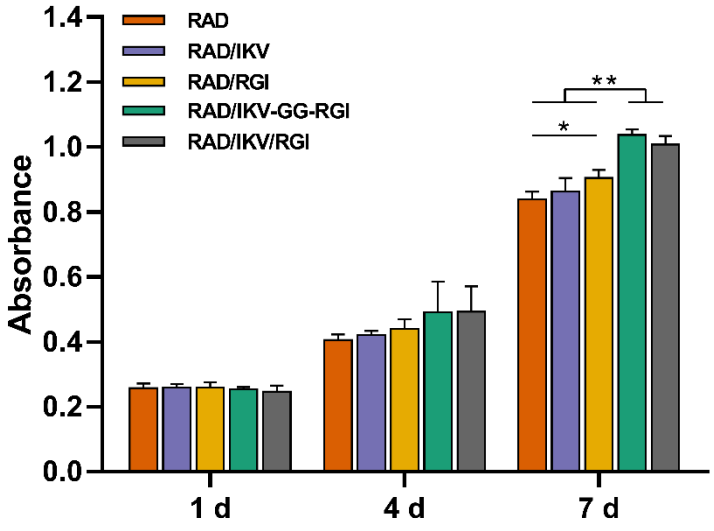

Figure S11. The proliferation of RSCs on different peptide hydrogels after 1, 4, and 7 days. Values are represented as mean ± SD. Statistical analysis was carried out using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test (equal variances) or Dunnett's T3 post hoc test (unequal variances). *$P < 0.05$, and **$P < 0.01$ (n = 4).

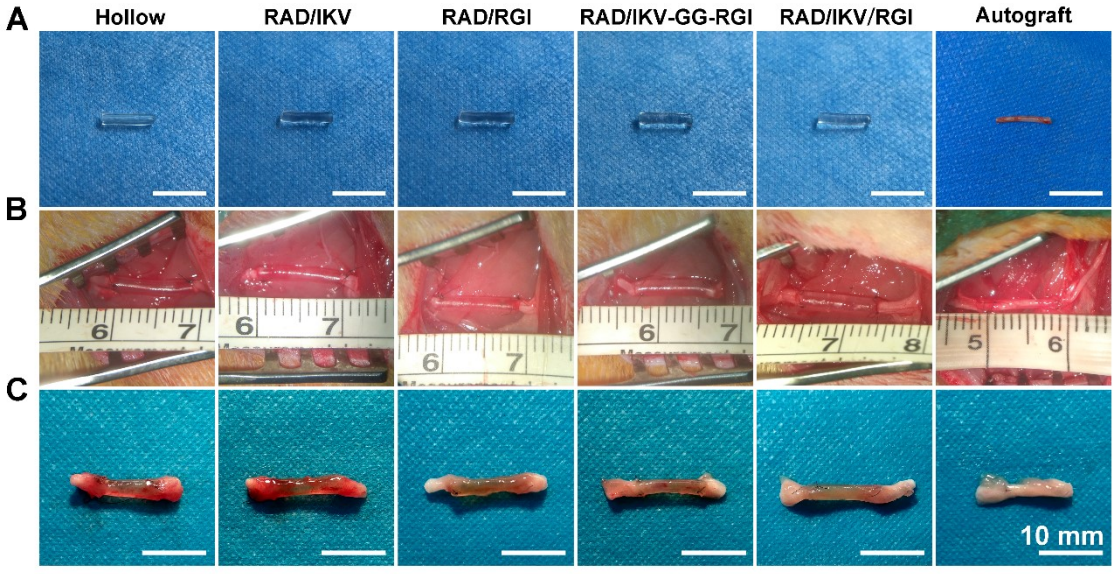

Figure S12. Nerve graft preparation (A), implantation (B) and harvest of the nerve grafts at 7 days postoperatively (C) of the different groups.

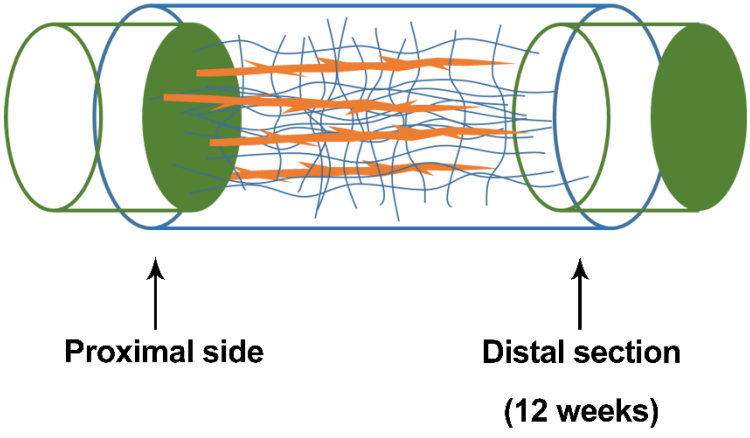

Figure S13. Scheme of transverse section of regenerated nerve at 12 weeks after surgery.

References

[1] Hong Y, Legge RL, Zhang S, Chen P. Effect of amino acid sequence and pH on nanofiber formation of self-assembling peptides EAK16-II and EAK16-IV. Biomacromolecules. 2003; 4: 1433-42.

[2] Horii A, Wang X, Gelain F, Zhang S. Biological designer self-assembling peptide nanofiber scaffolds significantly enhance osteoblast proliferation, differentiation and 3-D migration. PLoS One. 2007; 2: e190.