Supplementary data for

Development of polyacrylamide/chitosan composite hydrogel conduit containing synergistic cues of elasticity and topographies for promoting peripheral nerve regeneration

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Table S1. Ratio of PAM/CS hydrogel solution (solution volume: 100mL).

Young's modulus(kPa)	Acrylamide (g)	N-H-methylene bisacrylamide(g)	Ammonium persulfate(μL)	chitosan(g)
2.151	23.2	0.08	200	1
4.186	23.2	0.2	200	1
5.882	23.2	0.4	200	1
8.41	23.2	0.6	200	1
10.024	23.2	0.8	200	1

Table S2. Variation of groove width of hydrogel with different elasticity after soaking.

Groove size	10(μm)	30(μm)	50(μm)	80(μm)	Young's modulus
Groove dimensions before soaking	10±0.5	30±2	50±2.5	80±4.5	8.41kPa
Groove size for soaking water for 15 days	10±2.5	35±5	55±8	100±2	2.151kPa
	10±2.5	30±4	55±5	95±3	4.186kPa
	10±2	30±4	55±5	95±3	5.882kPa
	10±2	30±3	55±3	95±2	8.41kPa
□	10±1.5	30±3	50±3	95±2	10.024kPa

Table S3. The change of ridge width of hydrogel with different elasticity after soaking.

Ridge size	4.5(μm)	10(μm)	20(μm)	65(μm)	Young's modulus
Ridge dimensions before soaking	4.5±1	10±0.5	20±1.5	65±4.5	8.41kPa
Ridge size soaked in water for 15 days	5±2	10±5	20±5	75±4	2.151kPa
	5±2	10±3	20±4	75±3	4.186kPa
	5±2	10±3	20±3	75±3	5.882kPa
	5±1	10±2	20±3	75±2	8.41kPa
□	5±1	10±2	20±3	75±2	10.024kPa

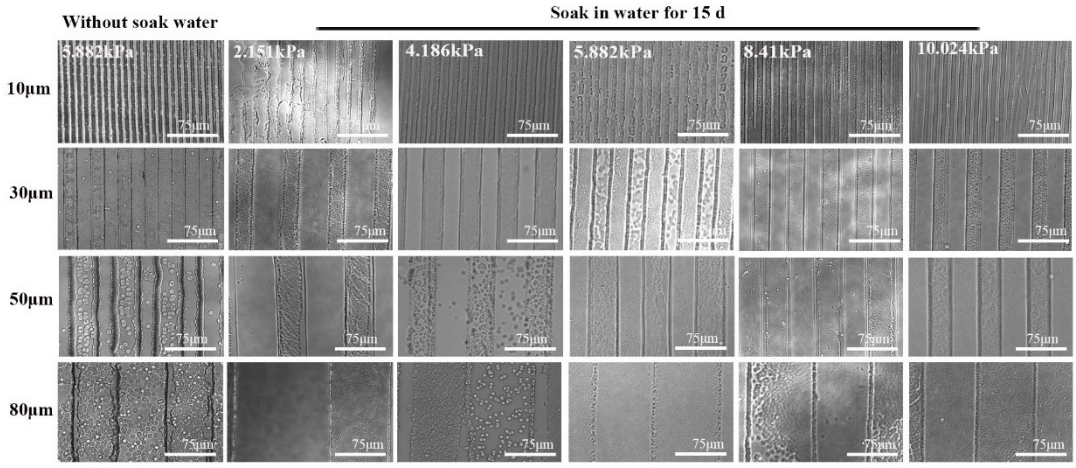

Fig. S1. Samples of different sizes were immersed in PBS for 15 d and then observed under a light microscope the erosion of the micro-pattern structure.

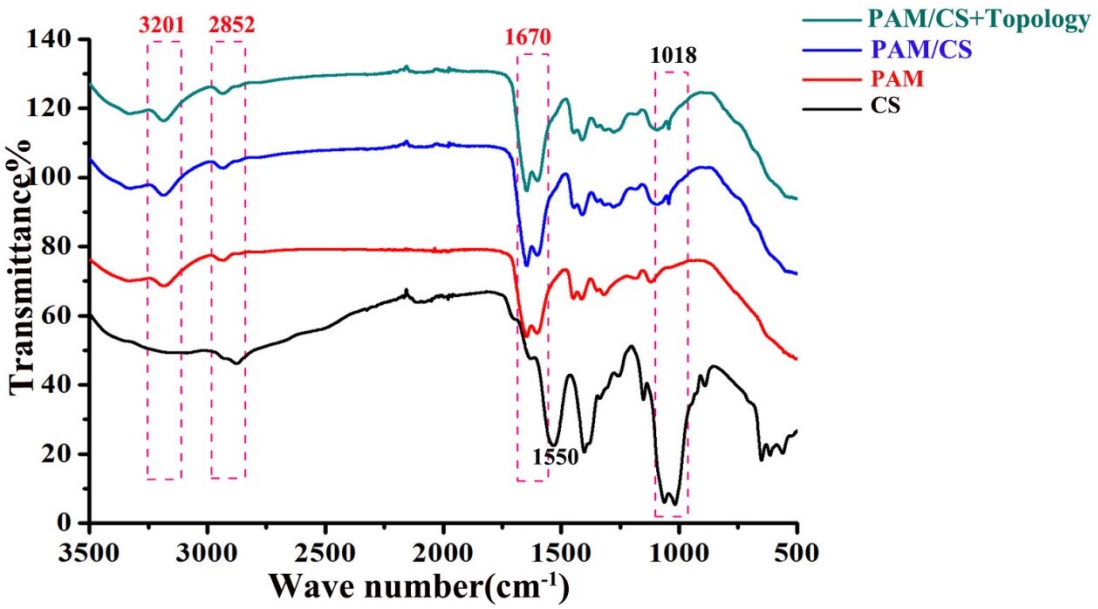

Fig. S2. ATR-FTIR spectra of chitosan, polyacrylamide, polyacrylamide/chitosan and polyacrylamide/chitosan with surface topology.

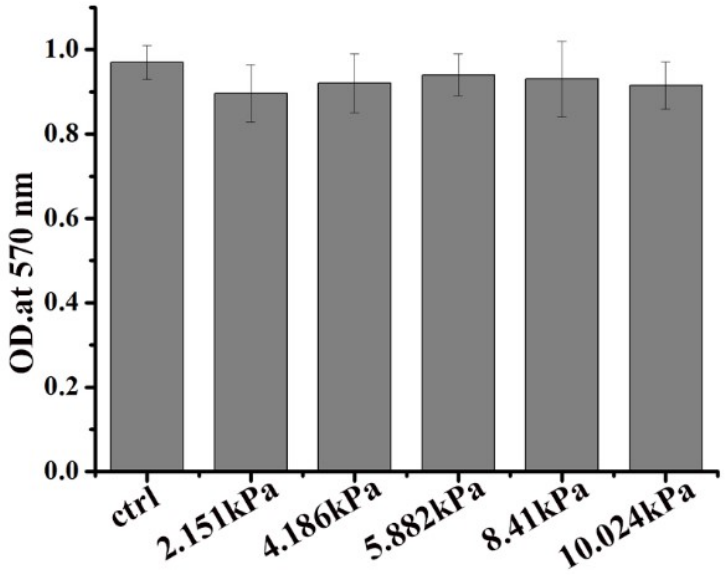

Fig. S3. In vitro cell cytocompatibility testing. The cytocompatibility of the hydrogel on L929 cells was detected by MTT at 48 h, n=4. All results are mean ± SD.

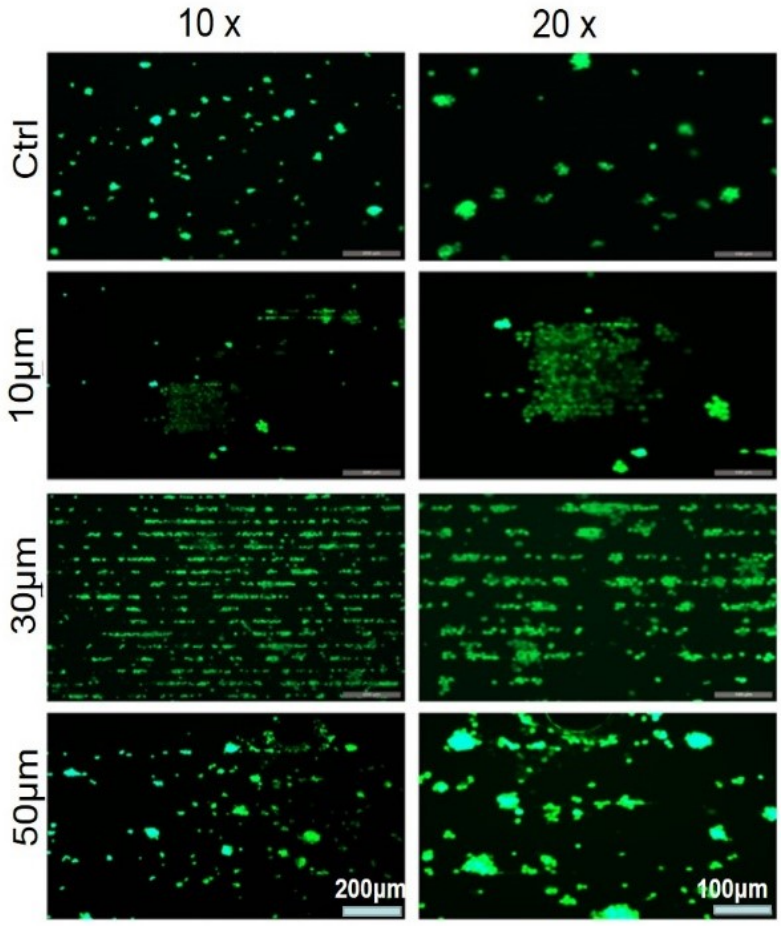

Fig. S4. Immunofluorescence staining of Schwann cells on hydrogel with elastic modulus of 5.882kPa and topological size of 10μm, 30 μm and 50μm, respectively after 1 day of culture.

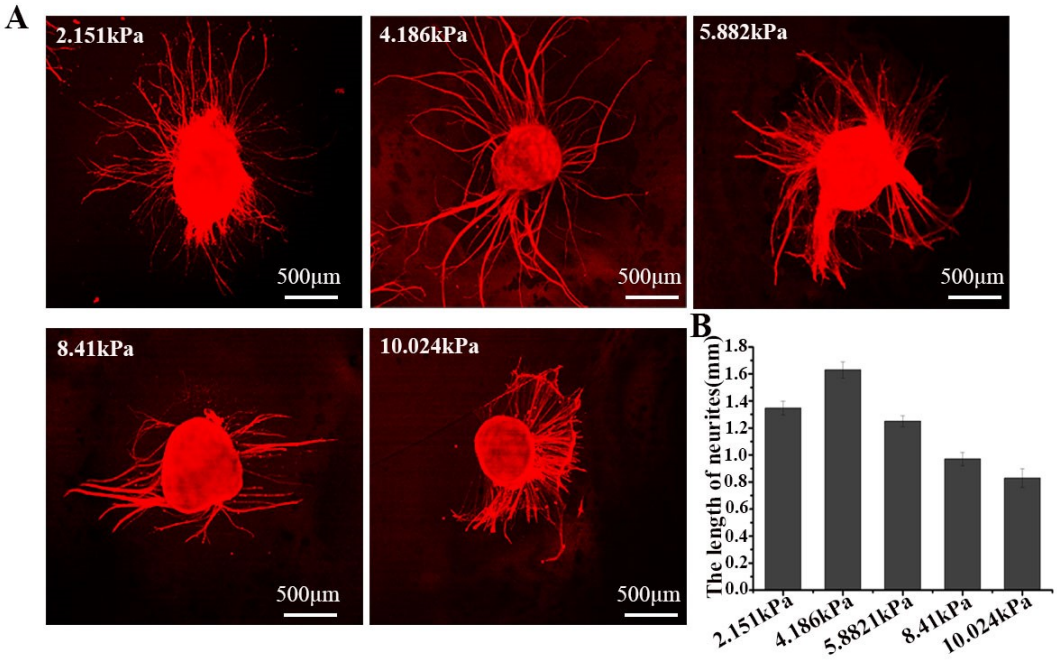

Fig. S5. Immunofluorescence staining of DRG neurons on hydrogels with different elastic modulus. (A) DRG neurons were cultured on different elastic hydrogels for 7 d. DRG neurons were stained with NF200 (red). Scale bar indicates 500 μm. (B) Statistics of nerve protrusion length on different elastic hydrogels.

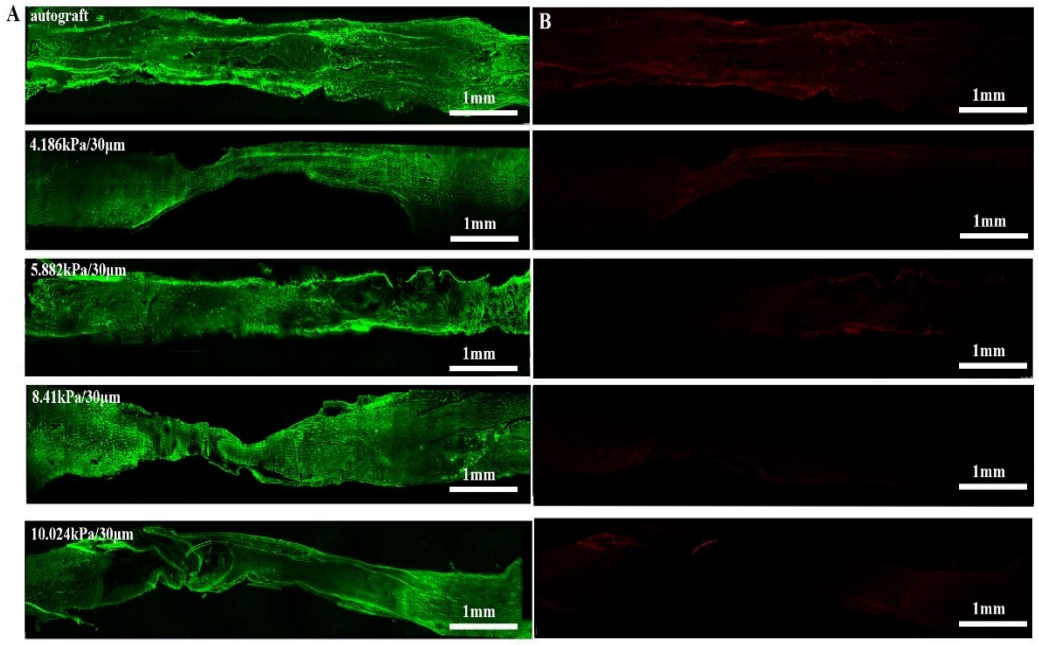

Fig. S6. Immunofluorescence staining of regenerated nerve tissue. At 2 w after surgery, representative images of nerve regeneration in the autograft group, 4.186 kPa/30μm, 5.882 kPa/30μm, 8.41 kPa/30μm and 10.024 kPa/30μm conduits. (A)Immunofluorescence staining image of longitudinal section of S100. Green color, S100. (B)Immunofluorescence staining image of longitudinal section of Neurofilaments. Red color, NF200. Scale bar indicates 1 mm.

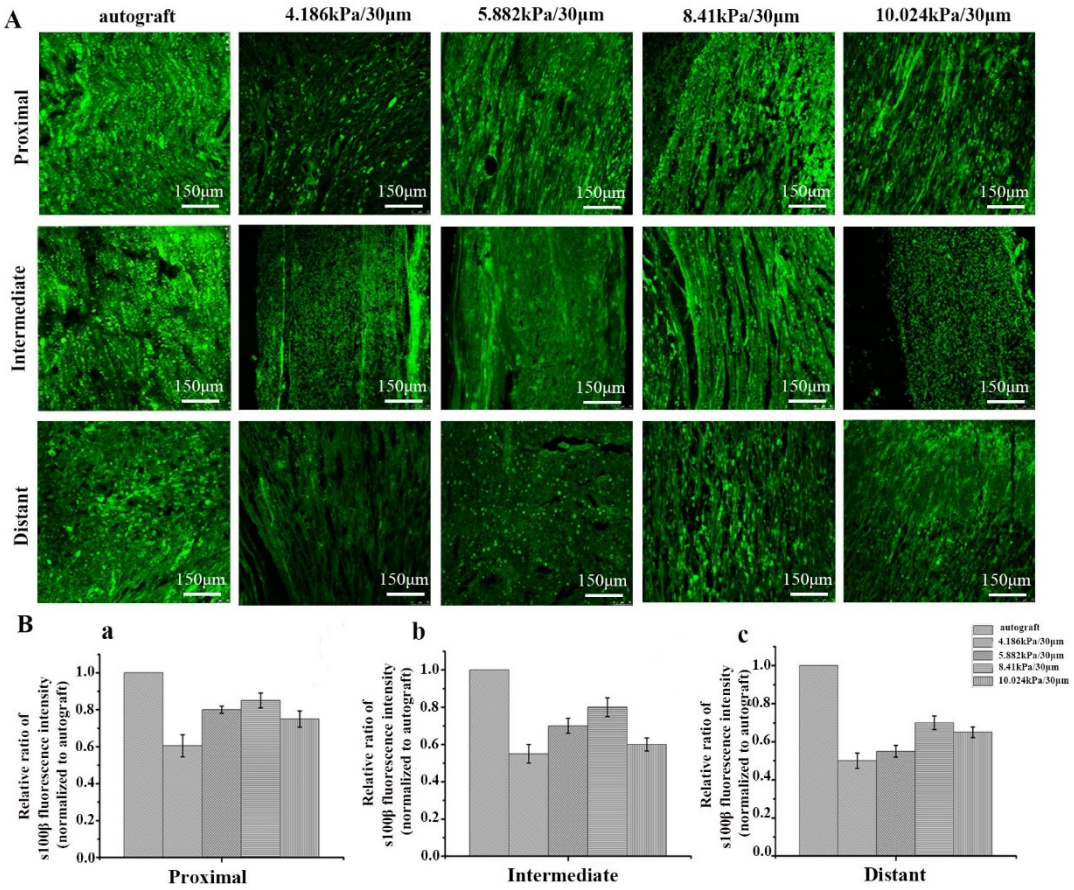

Fig. S7. The expression of the proximal, middle and distal nerves in the five groups regenerated nerves at 2 w postoperatively. (A)Immunofluorescence staining image of cross section of S100. Green color, S100. Scale bar indicates 150 μm. (B)The relative expression level of S100. Scale bar indicates 150 μm.

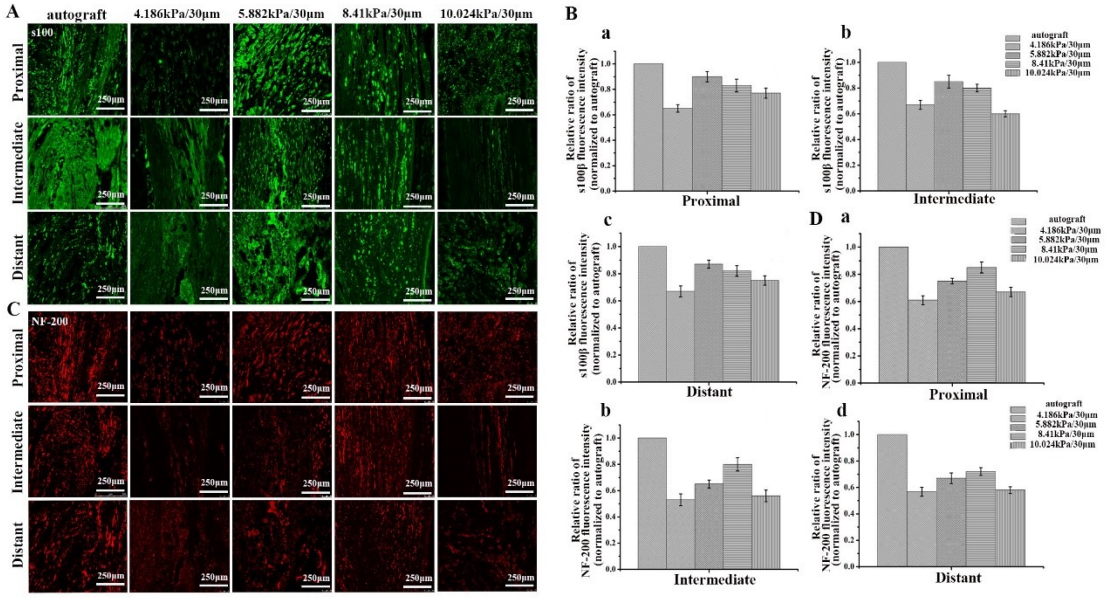

Fig. S8. The expression of the proximal, intermediate and distal nerves in the five groups regenerated nerves at 12 w postoperatively. (A)Immunofluorescence staining image of cross section of S100. Green color, S100. Scale bar = 250μm. (B)The relative expression level of S100. (C)Immunofluorescence staining image of cross-section of Neurofilaments. Red color, NF200. Scale bar= 250 μm. (D)The relative expression level of NF200. The result is the mean ± standard deviation.

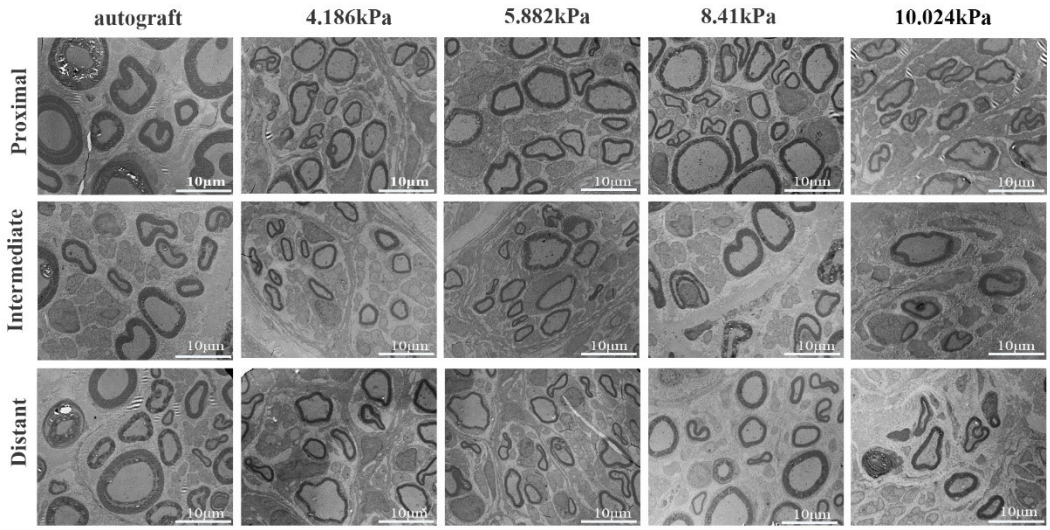

Fig. S9. The regeneration of the myelin sheath in the proximal, middle and distal cross-sections of the autograft group, 4.186 kPa/30 μm, 5.882 kPa/30 μm, 8.41 kPa/30 μm and 10.024 kPa/30 μm conduits regenerated sciatic nerve at 12 w postoperatively. Scale bar indicates 10 μm.