Supporting Information

The role of an ultrasound-responsive injectable piezoelectric hydrogel in promoting nerve regeneration and alleviating neuropathic pain

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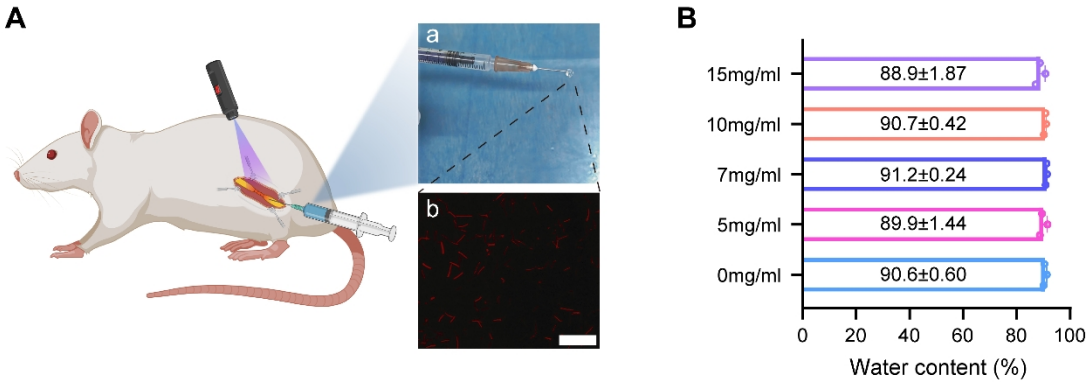


Figure S1. Characterization of the injectability and water content of GelMA and 10 mg/mL sPLLA-Gel hydrogels.(A) Schematic illustration of using piezoelectric hydrogel for peripheral nerve injury treatment. (a) Injectability test of the piezoelectric hydrogel. (b) Fluorescence image of Rhodamine B-stained sPLLA (red) (scale bar: 200 μm). (B) Water content of sPLLA-Gel hydrogels with different sPLLA concentrations.

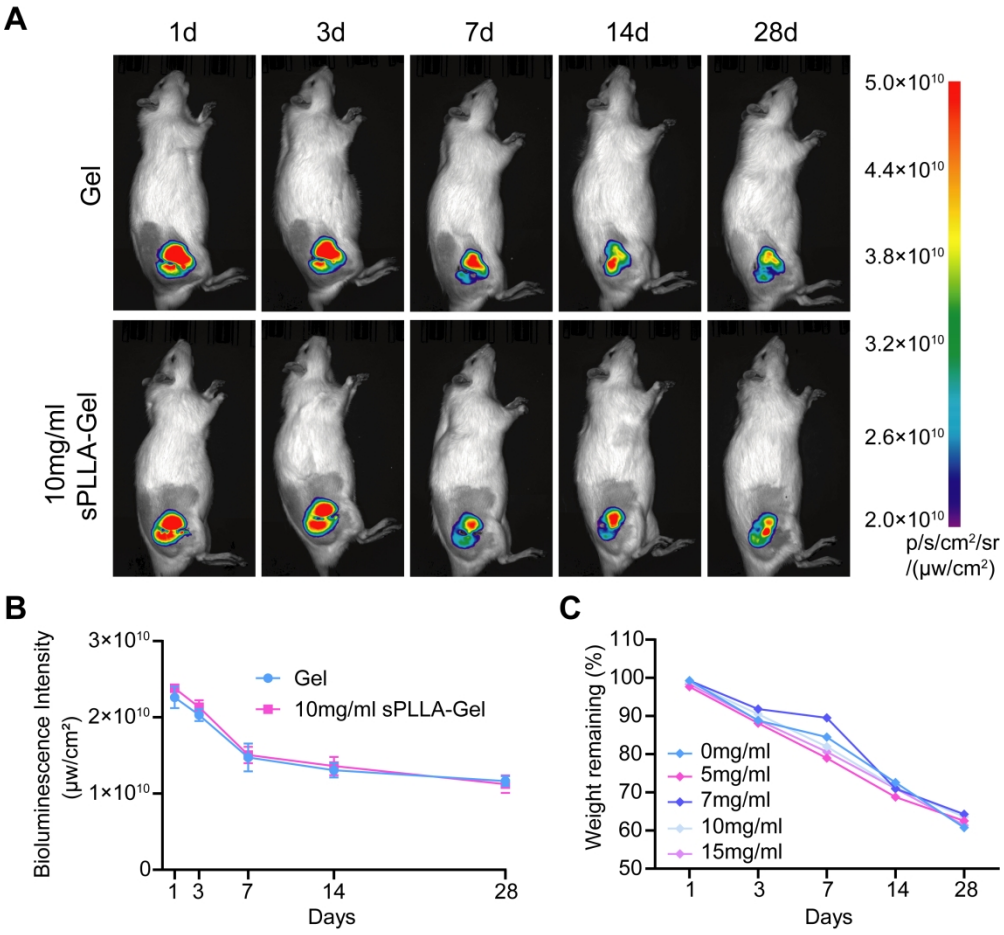
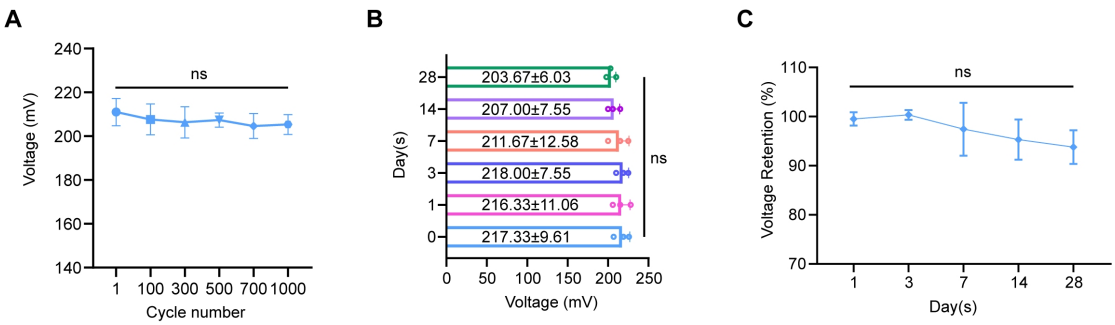


Figure S2. In vivo and in vitro degradation of GelMA and 10 mg/mL sPLLA-Gel hydrogels.(A) In vivo degradation imaging of GelMA and 10 mg/mL sPLLA-Gel in rats. (B) ROI analysis of bioluminescent signals showing no significant difference in the degradation rate and ratio between GelMA and sPLLA-Gel in vivo (n = 3). (C) Degradation profiles of sPLLA-Gel hydrogels with different sPLLA concentrations in PBS buffer containing 1 mg/mL lysozyme at 37 °C (n = 3).



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2 **Figure S3.** Stability of piezoelectric output of sPLLA-Gel under cyclic ultrasound
3 stimulation and during in vitro degradation. (A) Cyclic stability of piezoelectric
4 output of sPLLA-Gel under repeated ultrasound stimulation. (B) Output voltage of 10
5 mg/mL sPLLA-Gel hydrogel measured after lyophilization at different time points
6 during in vitro degradation (n = 3). (C) Normalized voltage retention at each time
7 point relative to day 0 (n = 3).

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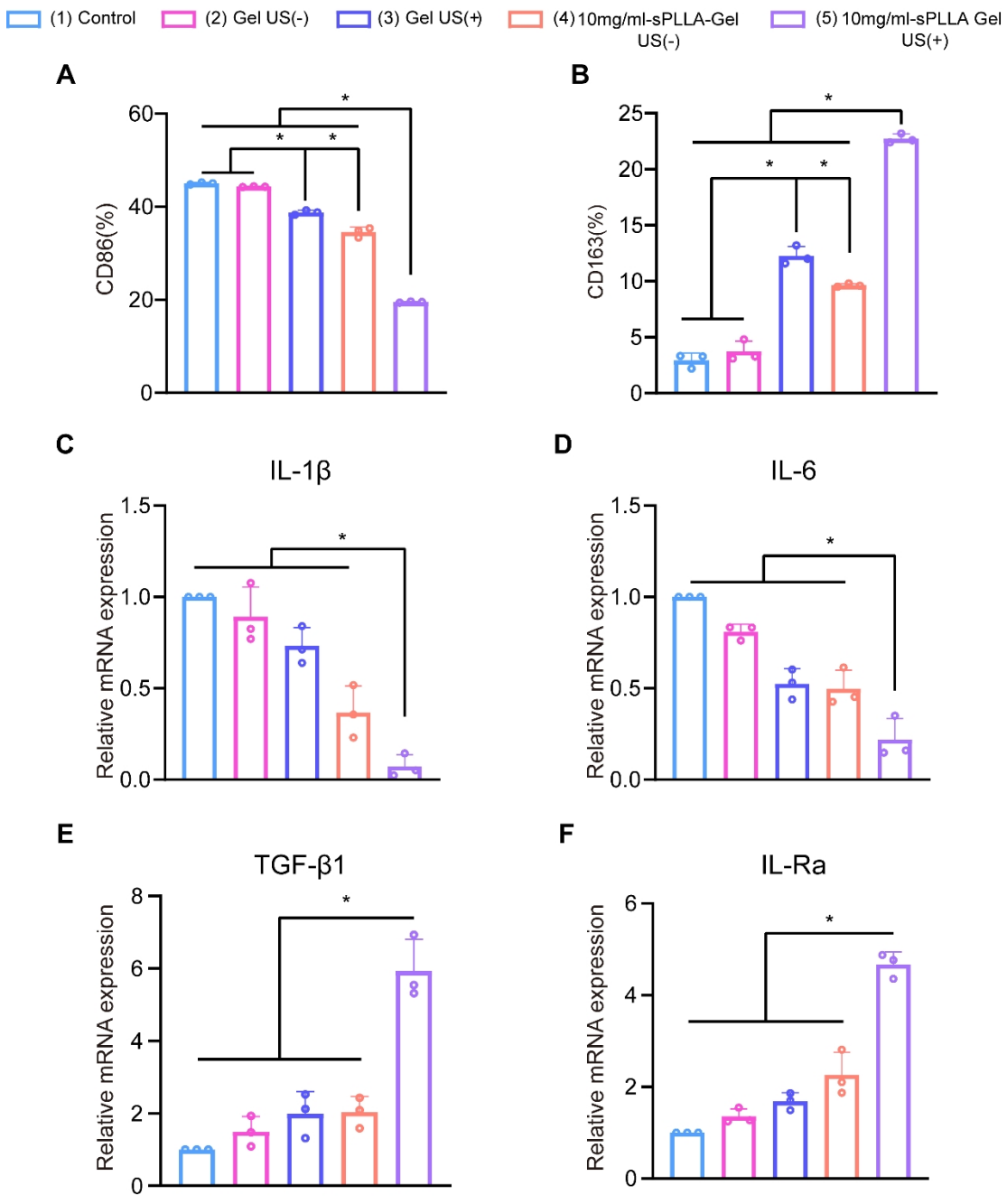

Figure S4. Quantitative analysis of FCM results for (A) CD86 and (B) CD163, and RT-qPCR detection of pro-inflammatory (IL-6 and IL-1β) (C, D) and anti-inflammatory (TGF-β1 and IL-1Ra) (E, F) gene expression in macrophages ($n = 3$). $P < 0.05$.

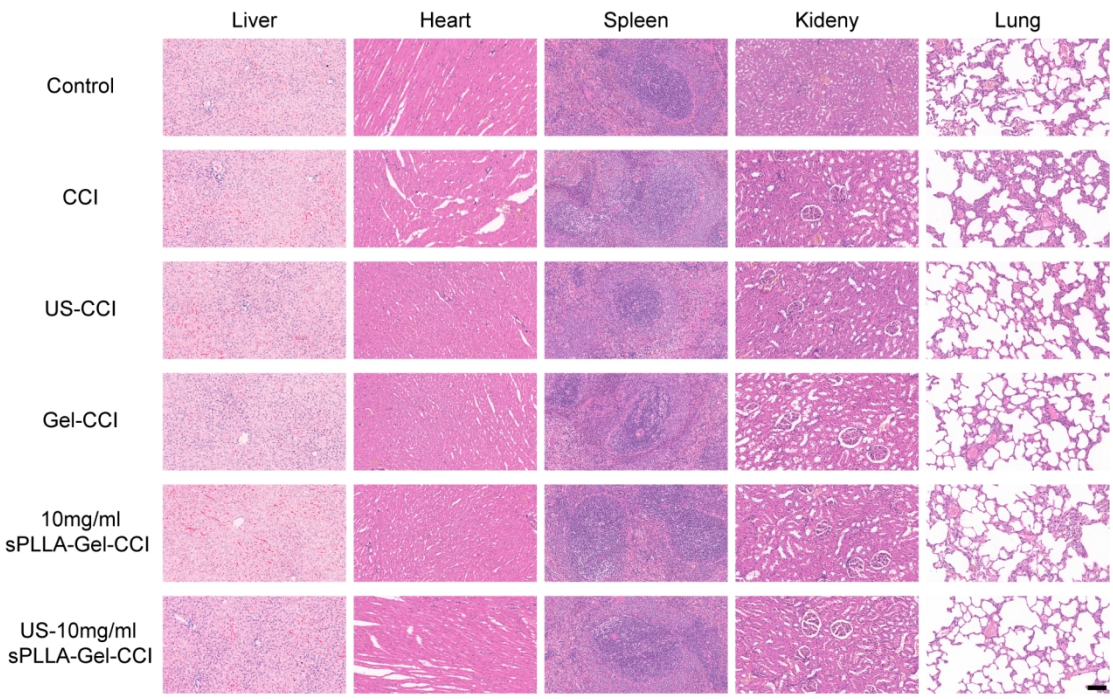

Figure S5. HE staining of major organs from rats in each group (scale bar: 100 μm).

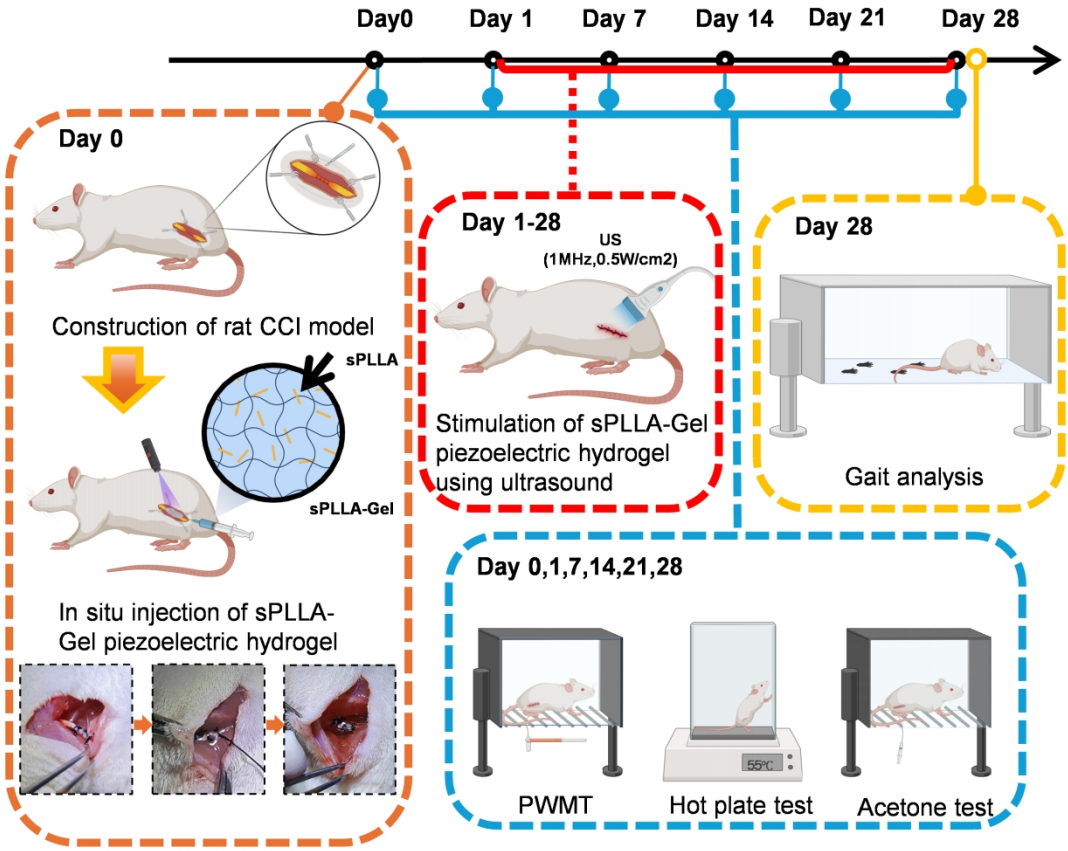

Figure S6. Schematic timeline of in vivo experiments and behavioral assessments.

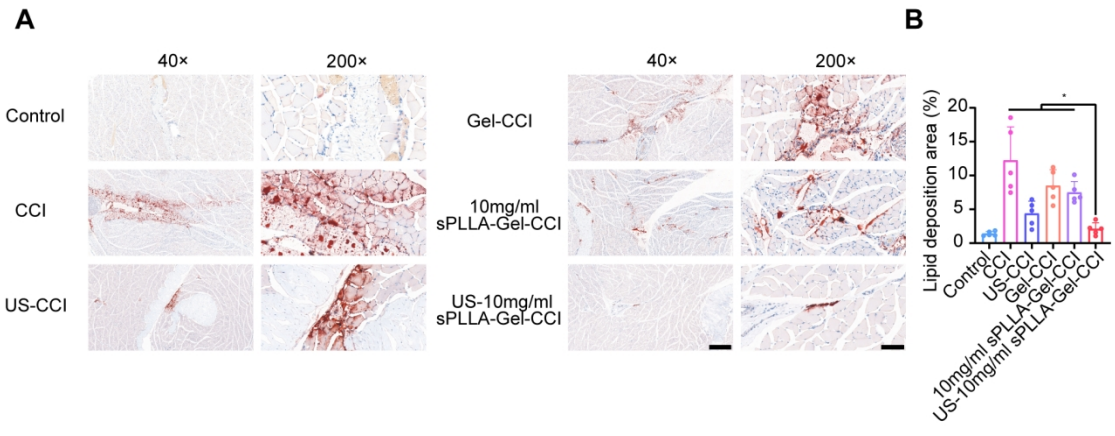

Figure S7. Oil Red O staining of the gastrocnemius muscle in rats from each group at 28 days post-surgery (A) and semi-quantitative analysis of lipid droplet area ratio (B) .(40×, scale bar: 500 μm; 200×, scale bar: 100 μm) (n = 5).

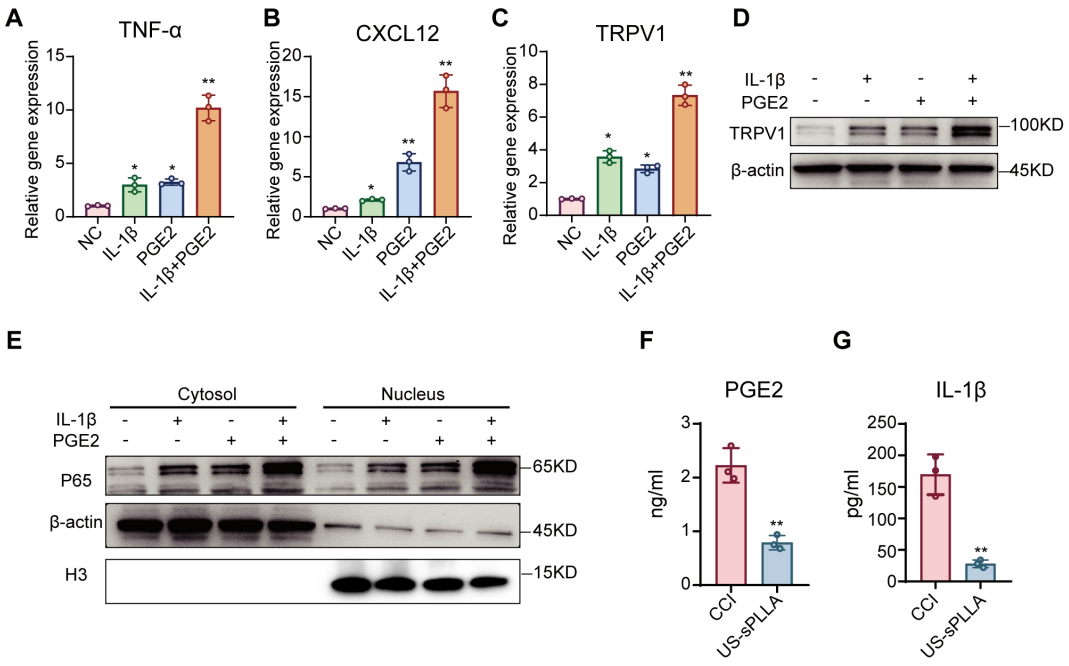

Figure S8. (A–C) Statistical results of qRT-PCR after 7 days of induction of BV2 cells with IL-1β and PGE2 (n = 3). (D) Western blot results after 7 days of induction with IL-1β and PGE2 showing that both IL-1β and PGE2 activated TRPV1 channel protein. (E) Nuclear and cytoplasmic fractionation Western blot results of BV2 cells treated with IL-1β and PGE2 demonstrating that both factors activated p65 protein and induced NF-κB nuclear translocation. (F–G) ELISA analysis of spinal cord tissues from the CCI group and the US-sPLLA group showing that the US-sPLLA hydrogel effectively reduced the production of PGE2 and IL-1β (n = 3).

Supplementary Tables

Table S1. Primers used for RT-qPCR analysis.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
IL-6	CTTGGGACTGATGCTGGTGA	TTGGGAGTGGTATCCTCTGTGA
IL-1β	TGGAGAGTGTGGATCCCAAG	GGTGCTGATGTACCAGTTGG
TGF-β1	CAGTACAGCAAGGTCCTTGC	ACGTAGTAGACGATGGGCAG
IL1Ra	CTCCAGCTGGAGGAAGTTAAC	CTGACTCAAAGCTGGTGGTG
TNF-α	AGGCACTCCCCCAAAAGATG	CTGGAAGACTCCTCCCAGGT
CXCL12	CGGTAAACCAGTCAGCCTGA	TCACATCTTGAGCCTCTTGTT
TRPV1	AGCCCCACATCTTTGCTACC	GTGTAATGCTGTCTGGCCCT