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Article

# Nerve Defect Treatment with a Capping Hydroxyethyl Cellulose/Soy Protein Isolate Sponge Conduit for Painful Neuroma Prevention

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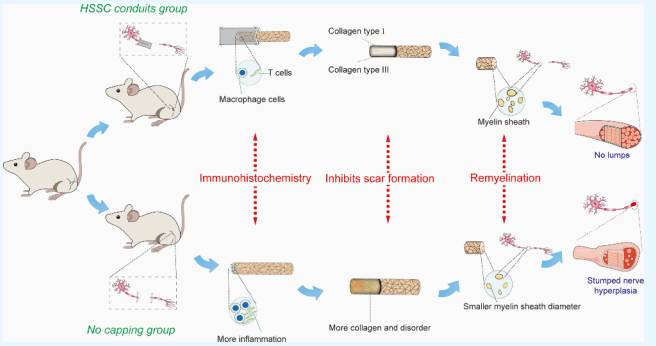
**ABSTRACT:** Painful neuroma, as one of the complications of nerve injury from disease or trauma, results in instinctive neuropathic pain that adversely affects a patient's quality of life. To intercept neuroma development, capping strategies have been performed as effective therapies. Nonetheless, the most appropriate biocompatible material to shield the nerves is an urgent clinical requirement. Herein, a compatible hydroxyethyl cellulose (HEC)/soy protein isolate (SPI) sponge capping conduit (HSSC) is used to prevent neuroma *in vivo*. Following capping on the sciatic nerve stump *in vivo*, the behavior of the rats and the structure of tissues are compared through histological assessment and autotomy scoring. The HSSCs gained a dismal autotomy score and enhanced the amelioration, where inflammatory invasions and overdeposition of collagen are defeated. The expression of myelin growth linked genes (Krox20, MPZ, and MAG) in the HSSC group at the eighth week was almost 2 times higher than that of the no capping group. The HSSC conduit served as a physical barrier to repress the infiltration of inflammation as well as provided an optimum microenvironment for facilitating nerve rejuvenation and intercepting neuroma development during nerve amelioration.




## 1. INTRODUCTION

Painful neuromas after peripheral nerve injury can cause neuroma-associated pain for patients with nerve injury. It has been estimated that more than 1.6 million people have had amputations, and 60% of painful neuromas are caused by amputations in the United States.[1] The number of amputees is increasing year by year, which is caused by accidents, gunshot wounds, burns, frostbite, electric shocks, malignancies, and other diseases.[2] More than 80% of these amputation surgeries arise from peripheral vascular disease and diabetes.[3,4] Most of these patients also suffered from neuropathic pain such as phantom limb pain during and after wound healing.[5] The pain can last for years, starting from a week after the amputation, and has a significant impact on the patient's life and work.[6]

The mechanism of painful neuroma is not yet well understood, but there are several potential explanations for painful neuroma, including sustaining irritation of the injured axons with mechanical or chemical actions, continuous stimulation of axons in painful neuroma by spontaneous discharge, and gate theory.[7] However, these mechanisms can provide only a partial explanation. Nonetheless, the damaged nerve fibers surrounded by the scar tissue are considered the most plausible cause of painful neuroma.[8] After the amputees experience peripheral nerve injury, a chaotic structure in regenerated axons and connective tissue is formed due to excessive regeneration of axons.[9] Subsequently, the peripheral nerve starts the repair process through Wallerian degeneration, and then immune cells activate the inflammatory process.[10,11]

Although surgical resection is the main treatment option for early neuroma, the dissociated severed nerve after the removal of the neuroma is prone to recurrence in the unchanged microenvironment. Therefore, the displacement of a proximal stump to muscle or bone might be used as a complementary or alternative therapeutic option for the prevention and treatment of neuroma. However, this approach may fail to play a role in digital nerves, which cause the nerve to separate from the muscle or bone again as the body moves, with only 14% success in one series. With the rapid development of tissue engineering, it is an effective way to prevent the dissociation of the nerve and cap the damaged nerve through implanting capping conduits.[12] The capping conduits cover the nerve,

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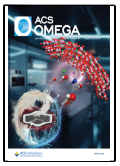

reducing the risk of the nerve sticking to the tissue and thereby leading to a relatively low incidence of neuroma. Although autologous venous nerve conduits have shown promise for the treatment of neuromas, their usage is limited by their size. The technique of silicone capping of amputated nerves might provide an alternative, although silicone is a foreign material that will not dissolve in the body.[13] So, finding a good nerve-capping conduit with good biocompatibility and biodegradation has been a clinical problem.[14]

In previous work, we found that hydroxyethyl cellulose (HEC)/soy protein isolate (SPI) composite conduits can not only connect two injured nerve ends, offering a neurotrophic environment for the Schwann cell regeneration and proliferation *in vivo*, but also result in a good repair effect in 10 mm injured sciatic nerves in rat. Notably, the degraded peptide of soy protein can be absorbed by the surrounding tissues for other cellular processes.[15,16] At the same time, the HEC/SPI materials showed good biocompatibility and did not cause inflammatory response. Thus, based on our previous work, HEC/SPI conduits were tried as a cap for the injured nerve end, which is expected to inhibit the formation of a neuroma. In this work, the experimental animal model was established by cutting off the sciatic nerve of SD rats, covering the proximal end with HEC/SPI sponge capping (HSSC) conduits and cutting off the 10 mm distal end to prevent the bridging of the proximal nerve and distal nerve. In addition, a series of histomorphological and molecular biological evaluations were conducted to explore the potential inhibitory effect of HSSC conduits on neuroma.

## 2. RESULTS AND DISCUSSION

**2.1. Fabrication of HSSC Conduits and Detection of Painful Neuroma Index.** The HSSC conduit with one sealed end is shown in Figure 1A. The HSSC conduits were obtained from hydroxyethyl cellulose and soy protein isolate via the freeze-drying method. The HSSC conduits had a 3D porous structure, and one end of the HSSC conduit was sealed with surgical sutures. To explore the potential therapeutic effect of the HSSC for painful neuroma *in vivo*, animal experiments were performed on a sciatic nerve-capped model in rats following established protocols.[17] As shown in Figure 1B, when the sciatic nerve was amputated, a 10 mm sciatic nerve defect was formed at the center of the right thigh. Thereafter, the proximal stump was capped with the HSSC conduit. Then the behavioral and morphological evaluation were carried out later.

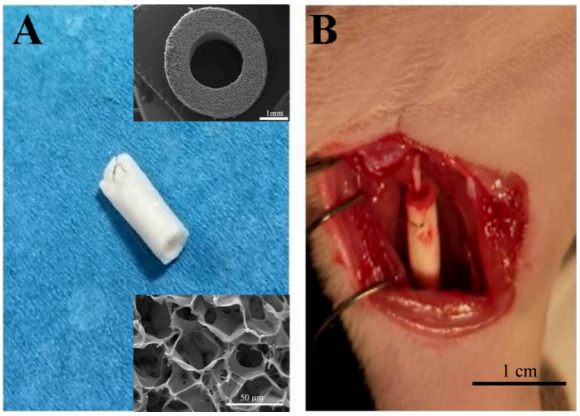


**Figure 1.** (A) Photograph of the HSSC conduit. (B) The experimental procedure of the sciatic nerve capped model in rats.

The autotomy score result is shown in Figure 2A. The HSSC capping conduit group and no capping group rats were recorded by observing the right limb of all animals. The higher the autotomy score, the more autotomy behaviors the rats had and the more incomplete the rat limbs were. If the autotomy score reached 13, then the autotomy behaviors were the most serious. The autotomy score of the no capping group and the HSSC capping conduit group were 1.33 and 0.33 at 2 weeks and 4 and 1.3 at 8 weeks, respectively. The autotomy score of the HSSC capping conduit group was lower than those of the no capping group. With the increase of time after surgery, the autotomy scores increased in both the HSSC conduit group and the no capping group. In comparison with the collagen-coated polyglycolic acid (PGA) conduit (4.05),[18] the HSSC conduit group (1.3) could decrease the autotomy scores at 8 weeks, which was close to the aligned silk fibroin (SF)/poly(L-lactic acid-*co*-ε-caprolactone) nanofibrous conduit (0.85) at 8 weeks.[19] Painful neuroma could lead to the occurrence of pain after amputation, while the autotomy score can directly reflect behavioral indicators of pain in rats. During the observation of the experiment rat behavior, the autotomy score of the HSSC conduit group was lower than that of the control group, indicating that the HSSC conduit could inhibit the production of pain.

The alpha smooth muscle actin (α-SMA) may contribute to neuroma-associated pain as either a direct cause of pain or an indirect marker of the existence of local mechanical stimuli. The potential role of α-SMA in the pathobiology of traumatic neuropathic pain might be considered as a treatment option in clinical practice.[20] RT-qPCR was performed to further demonstrate α-SMA mRNA expression in both groups (Figure 2B). The α-SMA mRNA expression in the HSSC capping conduit group (1.35 ± 0.24; 2.62 ± 0.90) was lower than that of the no capping group (1.48 ± 0.17; 4.31 ± 0.73) at the second week and the eighth week, and there was a significant difference at the eighth week. The α-SMA expression shows no significant difference in both groups at the second week. The reason may be that the expressions of α-SMA were very low at the second week. The ratio of α-SMA mRNA expression (2.62 ± 0.90) in the HSSC capping conduit group to the no capping group (4.31 ± 0.73) at the eighth week was 0.61. Because α-SMA is a biomarker of neuropathic pain, its continuous expression can cause pain in the nerves of rats, leading to autotomy.[21] Since pain leads to autotomy in rats, the result of pain marker expression (α-SMA) in the eighth week is consistent with that of the autotomy score, indicating that pain does cause autotomy in animals.[22]

Painful neuroma might develop after surgical repair, which is frequently followed by hyperalgesia, cold intolerance, as well as allodynia. It is widely known that the pathophysiology of neuroma formation depends on the presence of a chaotic admixture of neurite outgrowth and fibrous tissue, and since α-SMA is one of the markers of a myofibroblastic phenotype and is common in the muscular layer of vascular walls, upregulated α-SMA expression may increase the contractility of myofibroblasts and nonmuscle cells, and this could be a cause of pain in the region affected by a neuroma.[23,24] Herein, we created a rat nerve terminal resection model and employed the autotomy score to inspect the impacts of HSSC conduits on pain suffering. Consistent with other capping conduits, our HSSC


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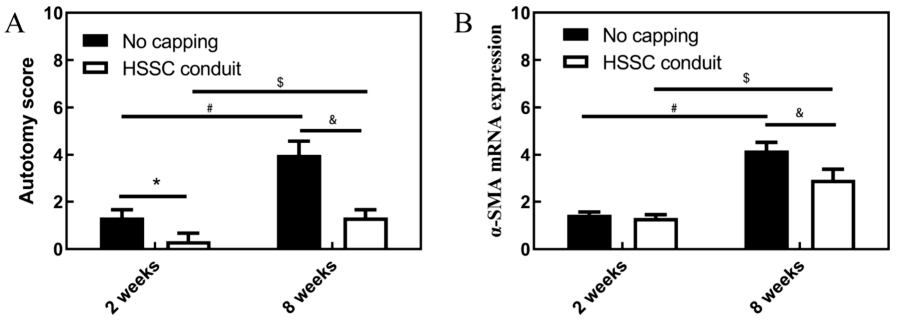


**Figure 2.** (A) Assessment of autotomy score at 2 and 8 weeks postoperatively. (B) RT-qPCR analyses of the $\alpha$-SMA gene expression.

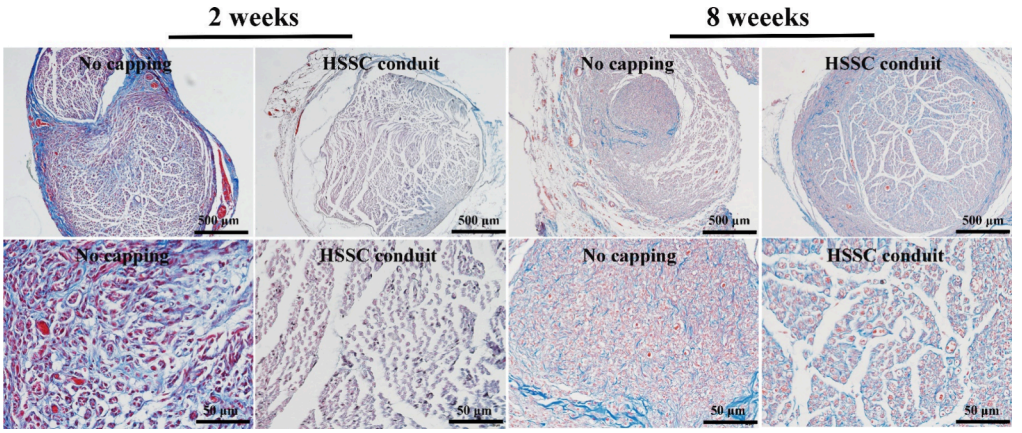


**Figure 3.** Cross sections of the proximal nerve stump with Masson's trichrome staining at both 2 and 8 weeks postoperatively.

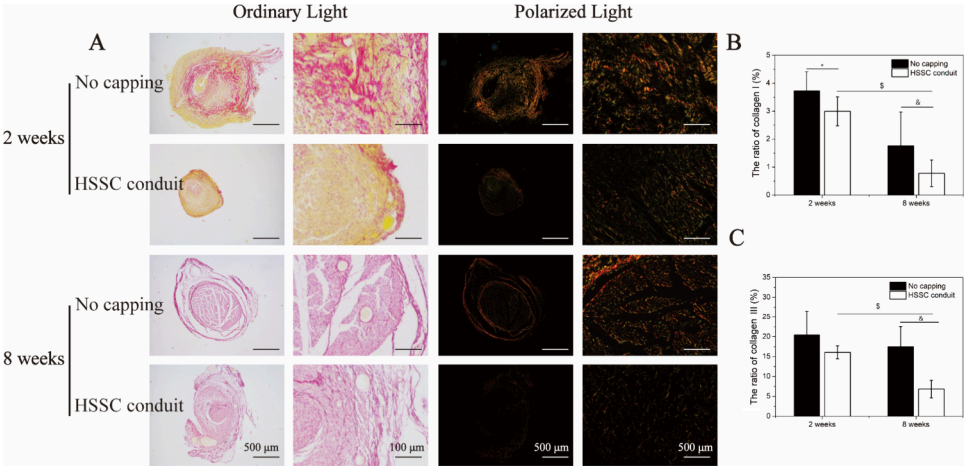


**Figure 4.** Sirius red staining and analysis of proximal nerve stumps at both 2 and 8 weeks postoperatively. (A) Ordinary and polarized light images of Sirius red staining, (B) the ratio of collagen I in different groups, and (C) the ratio of collagen III in different groups.

conduits also reduced the autotomy score and gene expression of $\alpha$-SMA.[18,25]

**2.2. Masson Staining and Sirius Red Staining.** Because of the strong link between neuroma development and fibrosis, histological samples in the two arms (HSSC capping conduit group and no capping group) were inspected. Overfibrosis was the second irritation index of neuroma-linked pain. In fibrosis development, anagenetic axons could be trapped by myofibroblasts, resulting in long-lasting mechanical induction. Masson staining exhibited much more overproliferated collagen deposition (stained with blue) in the no capping group at the second and eighth week, compared with the HSSC capping conduit group (Figure 3). The blue collagen was mixed with disorganized nerve fascicles in no capping groups, while in the HSSC conduit group, an orderly tissue configuration was found with organized nerve fibers and slightly blue-stained collagen. As reported by Yan, the aligned nanofiber conduit can reduce the blue collagen expression in the capping treatment group.[26] Since our HSSC conduit can also inhibit the development of painful neuroma, it can also reduce the formation of proliferated collagen.

The Sirius red staining can distinguish different types of collagen. The collagen type I can be dyed orange by Sirius red staining, while collagen type III can be dyed into green. As shown in Figure 4A, the content of collagen type I in no capping group content (3.73 ± 0.69%) was higher than that in


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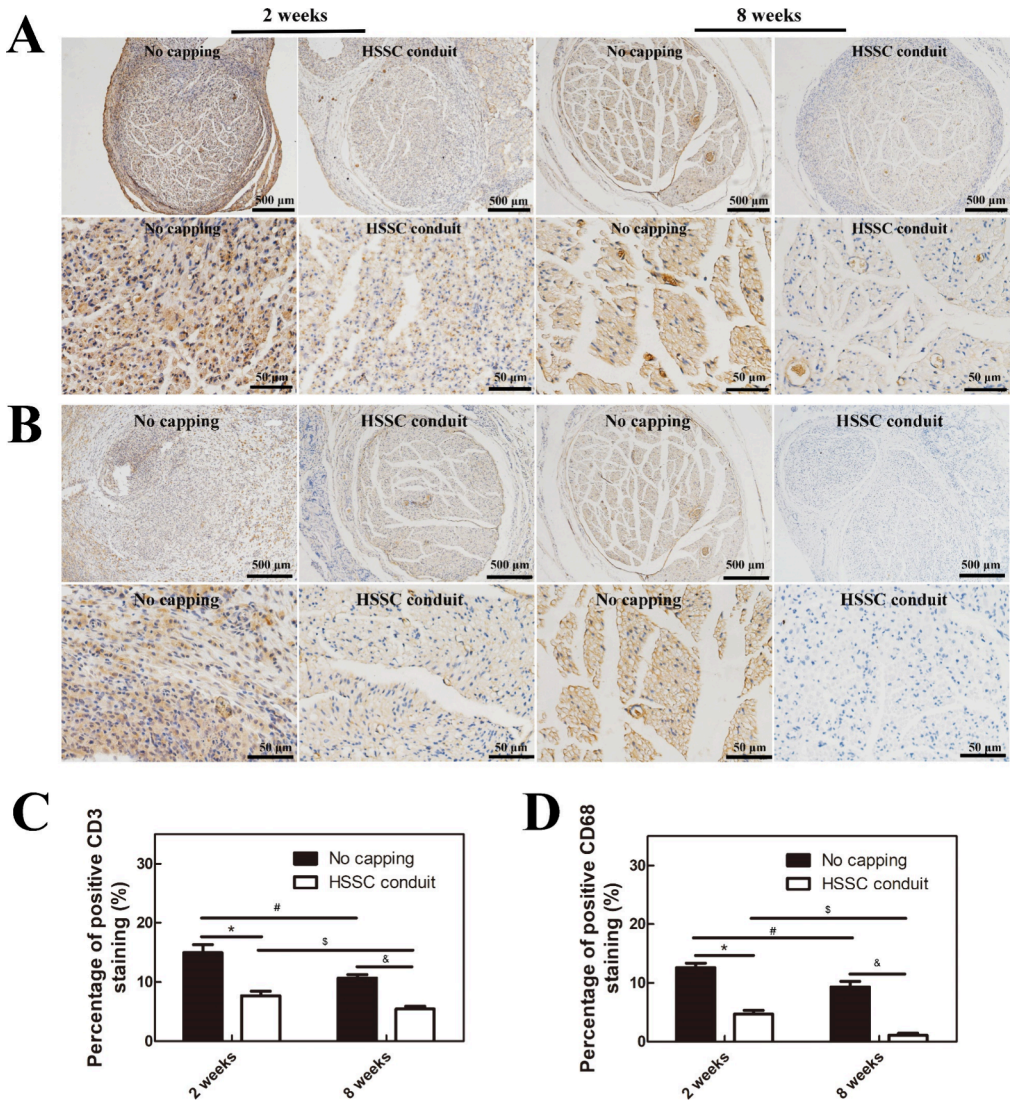


**Figure 5.** Cross sections of the proximal nerve stumps with immunohistochemical staining of CD3 (A, C) and CD68 (B, D) at 2 and 8 weeks postoperatively.

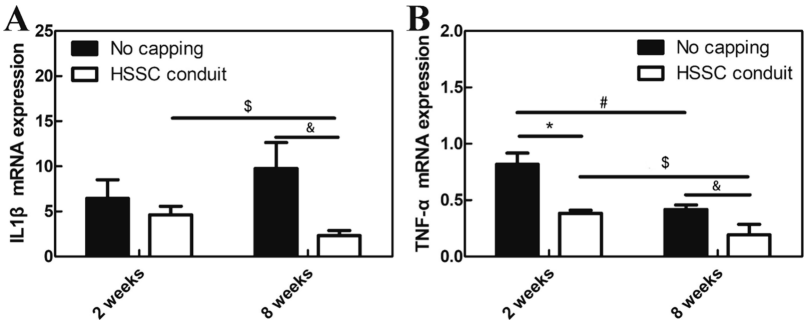


**Figure 6.** RT-qPCR analyses of the inflammatory reaction relative genes IL-$\beta$ (A) and TNF-$\alpha$ (B) in different groups.

the HSSC capping conduit group (3.0 ± 0.52%) at the second week. The content of collagen type I in both no capping and HSSC conduit groups was reduced in the eighth week, but the content in the no capping group (1.76 ± 1.21%) was higher than that in the HSSC capping conduit group (0.78 ± 0.48%) (Figure 4B). The same trend was also presented in the collagen type III. At the second week, the content of collagen type III in the no capping group (20.49 ± 5.96%) was higher than that in the HSSC capping conduit group (16.06 ± 1.23%). At the eighth week, the content of the collagen type III in the no capping group was 17.53 ± 5.03%, and that in the HSSC capping conduit group was 6.84 ± 2.22% (Figure 4C). Meanwhile, the content of collagen type III in the no capping group at the second week was similar to that at the eighth week. The Sirius red staining revealed that the collagen I, a major constituent of the fibrous connective tissue, was reduced


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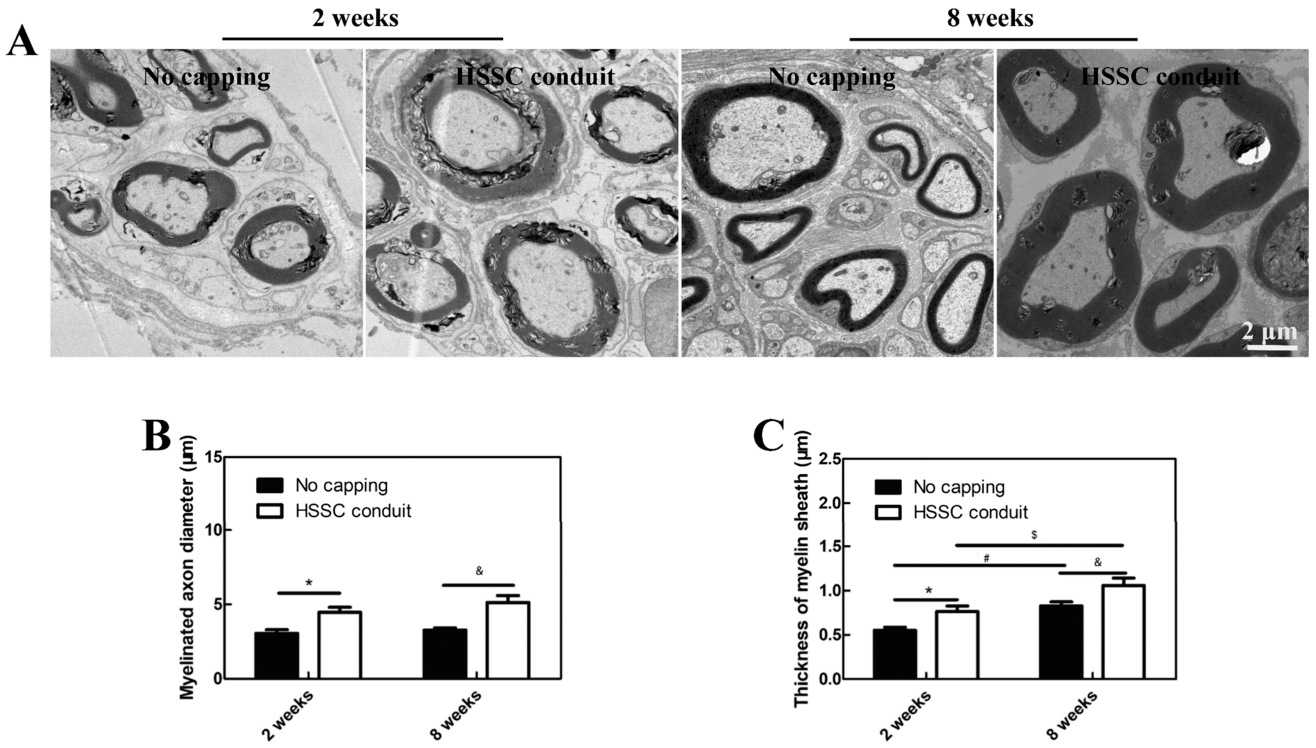


**Figure 7.** TEM assay of cross sections of the proximal nerve stump at 2 and 8 weeks. (A) Morphology of the myelin sheath in different groups. (B) Myelinated nerve fiber diameter. (C) Myelin sheath thickness.

in the HSSC conduit group. These results were consistent with the PRGD/PDLLA capping conduit, as they both can reduce the collagen I expression.[18]

**2.3. Immunological Reaction.** Immunohistochemical staining can detect inflammation in the nerve area at the terminal end. Inflammation leads to the accumulation of T cells and macrophage cells in the lesion.[27] As shown in Figure 5A, CD3-labeled T cells and CD68-labeled macrophage cells showed significant inflammation at the second week in the no capping group unlike that of the HSSC conduit group at the second week. The statistical results show that the percentage of CD3 staining was 14.93 ± 2.37% in the no capping group, and that in the HSSC conduit group was 7.7 ± 1.31% (Figure 5C). With the prolongation of treatment time, the inflammation gradually reduced. However, the positive cell ratio of CD3-labeled macrophage cells in the no capping group (10.67 ± 1.01%) was significantly higher than that in the HSSC conduit group (5.43 ± 0.83%) (Figure 5C). The same trend was presented for CD68-labeled macrophage cells. There was a significant difference observed at the eighth week. The positive cell ratio of CD68-labeled macrophage cells in the no capping group was 9.33 ± 1.62%, and that in the HSSC conduit group was 1.1 ± 0.6% (Figure 5B,D).

The differences in inflammation at the nerve ends of the two components were further demonstrated by measuring the levels of the inflammatory cytokines TNF-$\alpha$ and IL-1$\beta$. As shown in Figure 6A, the mRNA expression of IL-1$\beta$ in the no capping group increased from 6.42 ± 1.88 at 2 weeks to 9.77 ± 3.27%. However, mRNA expression of IL-1$\beta$ in the HSSC conduit group decreased from 4.70 ± 1.34 to 2.39 ± 0.46%. The mRNA expression of TNF-$\alpha$ in both groups reduced at 8 weeks, but TNF-$\alpha$ expression in the no capping group (0.79 ± 0.05; 0.44 ± 0.03) was distinctly higher than that in the HSSC conduit group (0.40 ± 0.01; 0.20 ± 0.05), respectively, at both time points (Figure 6B).

Inflammation plays an extremely important role in the process of nerve repair and regeneration. For example, the appearance of macrophages in the early stage of the hypoxic environment can secrete cytokines to promote angiogenesis, which can promote nutrient transport and thus promote myelin regeneration and axon regeneration.[11] However, inflammation plays a double-edged role in peripheral nerve injury. Moderate inflammation is essential in the regeneration process, but it is also a major source of pain. Excessive inflammatory response causes the repair of peripheral nerve injury to stay in the granulation stage. At the same time, fibroblasts are activated, so that fibroblasts continue to differentiate into myofibroblasts (myofibroblast) and continuously secrete a large amount of collagen (collage), forming the deposition of scar tissue (scar tissue).[28−31] In our previous studies, nerve conduits added with a certain amount of soy protein could effectively promote nerve regeneration and inhibit higher inflammatory response.[32,33] Because the HSSC conduit contained the soy protein, it provides a better microenvironment for nerve regeneration and protects regenerated axons from external physical stimuli.[34] All in all, the inflammatory reaction of the HSSC conduit group was significantly lower than that of the control group.

**2.4. TEM and RT-qPCR Analysis.** The myelinated axon diameter and thickness of the myelin sheath were observed by TEM (Figure 7A). As demonstrated in Figure 7B, the myelinated axon diameter of the HSSC conduit group (4.47 $\mu$m at the second week and 5.13 $\mu$m at the eighth week) was higher than that of the no capping group (3.06 $\mu$m at the second week and 3.27 $\mu$m at the eighth week). As illustrated in Figure 7C, the thickness of the myelin sheath in the HSSC conduit group was thicker than that in the no capping group at


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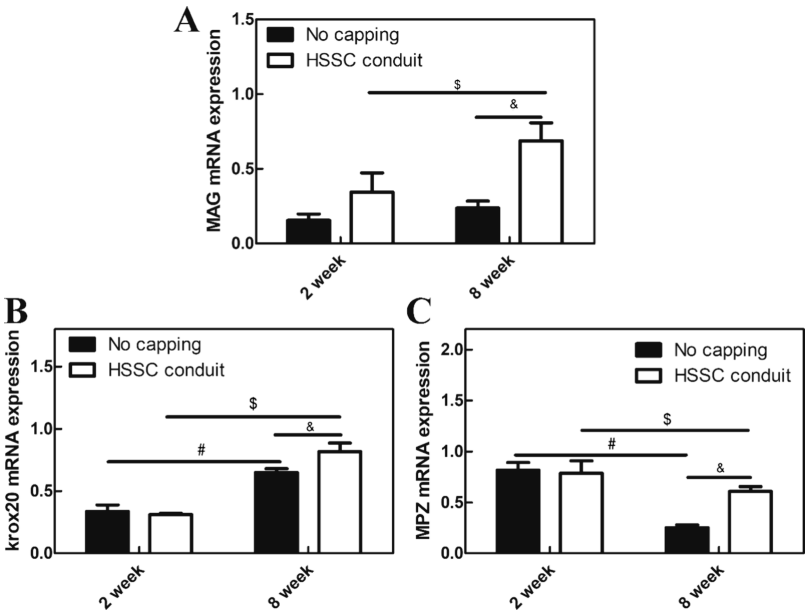


**Figure 8.** RT-qPCR analyses of the myelin maturation relative expression of genes MAG (A), Krox20 (B), and MPZ (C) in different groups.

both the second week and eighth week. HSSC conduits were able to protect the nerve fiber from irritation, and the axons were protected by myelin. Remyelination was robust in the HSSC conduit group as illustrated by the TEM cross-section imaging inspection of the reproduced myelin in the two groups. The thicker myelin sheath in the capping conduit group could be attributed to the better microenvironment provided by the capping conduits.[35] In our previous study, HEC/SPI composite biomaterials could degrade and form a variety of active peptides to promote nerve regeneration.[36,37]

To understand the precise differences between them, the myelin-linked genes MAG, Krox20, and MPZ were detected. MAG is the early biomarker of myelination; MPZ is the primary structural protein of myelin; and Krox20 is the master regulator of this process.[38−40] It can be seen from Figure 8A and 8B that the MAG and Krox20 gene expressions in the HSSC conduit group were significantly higher than that in the no capping group at the eighth week, while the MPZ was dramatically downregulated in the no capping group (Figure 8C). It could be inferred that myelination was normally activated, and the part myelination was attributed to the insufficient myelin-related protein formation. Therefore, the application of the HSSC conduit prevents painful neuroma formation by coordinating the inflammation response, collagen deposition, and demyelination process.

## 3. MATERIALS AND METHODS

**3.1. Materials.** Hydroxyethyl cellulose (viscosity, 30,000 mPa) was purchased from Shandong Head Reagent Co. Ltd. (Shandong, China). Commercial soy protein isolate (SPI), with a weight-average molecular weight (Mw) of $2.05 \times 10^5$, was obtained from DuPont Protein (Luohe, China). HEC and SPI were vacuum-dried for 24 h at 60 °C before use. Epichlorohydrin (ECH, analytical grade, liquid, 1.18 g/mL), NaOH, and acetic acid were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Other chemicals were of analytical grade agents and used without further treatment.

**3.2. Preparation of Capping HEC/SPI Composite Conduits.** The HEC/SPI composite conduit was prepared as our previous work.[41] In brief, 5 g of SPI powder was dispersed into 30 g of deionized water for 30 min. After that, 15 g of NaOH aqueous solution (5 wt %) was added to obtain a viscous aqueous solution with 10% SPI content. The 3 wt % HEC solution was obtained by dissolving 1 g of HEC powder into 49 g of deionized water. Then, the HEC and SPI solutions were mixed (with a dry weight ratio of 3:7) with the following addition of ECH (20% of the total weight of original SPI and HEC powders). Finally, the mixed solution was injected into a house-made mold (the outer layer is a 15 mm polytetrafluoroethylene tube, and a 20 mm iron core is inserted in the middle), kept in −20 °C for 12 h and −80 °C for 24 h and then freeze-dried. After that, the conduit was obtained by removal of the iron core. The freeze-dried HEC/SPI conduits were immersed in a 5% acetic acid solution, rinsed completely, and freeze-dried again to obtain the HEC/SPI conduits. The prepared HEC/SPI conduits were cut into pieces 10 mm long, with 2 mm outer diameter and 1.5 mm inner diameter. One end of the conduit was stitched together with a 5−0 suture. The HEC/SPI sponge capping (HSSC) conduits were irradiated under UV for 12 h, soaked in 75% alcohol for 12 h to achieve sterilization, and then used for nerve capping treatment.

**3.3. Animal Experiments.** All animal experiments were performed according to the "Guidelines and Regulations for the use and care of Animals of the Review Board of Hubei Medical Laboratory Animal Center", based on the Experimental Animal Management Ordinance (National Science and Technology Committee of the People's Republic of China, 1998). SD rats were from the Hubei Provincial Center for Disease Control and Prevention (China). Adult female SD rats (180−200 g) were randomly divided into a no capping group and HSSC conduits group, with 12 rats in each group. After the anesthesia of SD rats, the hair of the body was shaved at the right thigh; the epidermis was split with a scalpel; and the muscle was split to expose the sciatic nerve. The 10 mm sciatic nerve was removed from the center of the right thigh to form


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Table 1. Primers for qPCR

| Gene | Forward primers | Reverse primers |
|---|---|---|
| $\alpha$-SMA | GCTCCTCCAGAACGCAAATAT | GGGCCAGCTTCGTCATACTC |
| IL-1$\beta$ | TGACCTGTTCTTTGAGGCTGAC | CATCATCCCACGAGTCACAGAG |
| TNF-$\alpha$ | CTTCTGTCTACTGAACTTCGGGGT | ATCTGAGTGTGAGGGTCTGGGC |
| MAG | CCCTGCCTCTGTTTTGGATA | CGGGTAGTTCTTGGGGTAGG |
| Krox20 | GCTACCCAGAAGGCATCATCA | GAGTAGAGGTGGTCCAGTTCAGG |
| MPZ | GTGGTTTACACGGACAGGGAA | CCTTGGCATAGTGGAAGATTGA |
| $\beta$-actin | TGCTATGTTGCCCTAGACTTCG | GTTGGCATAGAGGTCTTTACGG |

the proximal and distal ends of the nerve and avoid nerve regeneration. The proximal nerve stump in the no capping group worked as the control group. In the HSSC group, 2 mm of the proximal nerve stump was sutured with nylon thread and HSSC conduits as the experimental group.

**3.4. Behavioral Analysis.** The autotomy score of rats was performed at the second and eighth week after the operation, and the extent of autotomy was assessed with the modified point scale.[42] One or more missing nails is scored as 1 point for a total of 5 points; 1 point for a distal toe injury is added for a total of 5 points; and 2 point for a proximal toe injury are added to one point. Finally, if the metatarsus and tarsus are missing, add 1 point each, totaling 2 points. Maximum rating: 13 points.

**3.5. RT-qPCR.** At the second as well as eighth week following implantation, we collected the nerve sections and utilized RT-qPCR to inspect the transcription changes in the $\alpha$-SMA, TNF-$\alpha$, Krox20, MPZ, and MAG genes, as well as IL-1$\beta$. We employed the Trizol reagent (Invitrogen) to purify total RNA (tRNA) from the samples by following the protocol provided by the manufacturer. Subsequently, the mRNA was converted to cDNA via reverse transcription (RT). The sequences of the utilized primers are indicated in Table 1. The qPCR was conducted on the real-time PCR system (CFX connect, BIORAD), and the program constituted 95 °C for 3 min initial denaturation: for 40 amplification cycles of 15 s at 95 °C denaturation, 20 s at 58 °C extension, and 20 s at 72 °C. We utilized a blank (mix without the template) as the negative control. Each reaction was run in triplicate. We employed the $2^{-\Delta\Delta Ct}$ approach to determine the relative expression.

**3.6. Histopathological Study.** The proximal nerves, which were covered by the HSSC conduits' or control groups' proximal nerve, were taken after 2 or 8 weeks of the operation. Then the harvested nerves were fixed with 4% (w/v) paraformaldehyde with 0.1 mol/L phosphate buffer at 4 °C for 24 h. The fixed proximal nerves were sliced, dewaxed, and soaked. Representative sections were stained with Masson trichrome, Sirius red, rabbit antirabbit CD 3 antibody (GB13014, Servicebio, China), and antirabbit CD 68 antibody (GB11067, Servicebio, China). These slice samples were observed under an optical microscope. The CD3 and CD68 expressions were measured on histological nerve samples with Image-Pro Plus 6.0. When analyzing CD3 and CD68 expression and collagen content, the proportion of positive area was statistically analyzed. Three sections and three images in each section from each animal and three animals in each group were used for statistical analysis.

**3.7. TEM Observation of a Nerve Myelin Sheath.** The apex of the proximal nerve was taken at 2 and 8 weeks after the operation and fixed with 2.5% glutaraldehyde solution. The nerve samples were washed again and dehydrated with acetone, followed by sample infiltration using a mixture of acetone and resin. The polymerization was carried out in an oven at 60 °C for 24−48 h before sectioning into 90 nm thick ultrathin sections using an ultramicrotome (Leica, EM UC7, USA). The ultrathin sections were cut and collected on copper slot grids with polyform/carbon support films and observed with TEM (TEM, HT7700, Hitachi, Japan). Images were captured and measured, and the axon diameter and myelin sheath thickness were analyzed by Image-Pro Plus 6.0 software.

**3.8. Statistical Analysis.** Differences were statistically analyzed with GraphPad Prism 5.0 software using one-way ANOVA. The difference ($^{*}P < 0.05$, comparison between the no capping group and HSSC conduit at 2 weeks; $^{\&}P < 0.05$, comparison between the no capping group and HSSC conduit at 8 weeks; $^{\#}P < 0.05$, comparison of the no capping group between 2 weeks and 8 weeks; $^{\$}P < 0.05$, comparison of HSSC conduit group between 2 weeks and 8 weeks) was considered to be statistically significant.

## 4. CONCLUSIONS

In this work, a kind of HEC/SPI sponge capping (HSSC) conduit was prepared, which exhibited good painful terminal neuroma prevention properties *in vivo*. The animal experiment results indicated that autotomy score, scar deposition, and inflammatory response were reduced in the HSSC conduit group. Moreover, the myelin thickness and axon diameter in the HSSC conduits were thicker than those of the control group, and the relative myelinization genes were also upregulated. These factors contribute to the inhibition of painful neuroma. Therefore, HSSC conduits could be a promising alternative used in capping nerve terminal ends to inhibit neuroma formation.

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### Author Contributions

#Q.D. and J.A. contributed equally to this paper. Qi Dong: Conceptualization, Data curation, Writing - reviewing and editing. Junjie Ai: Data curation. Ao Xiao: Investigation, Methodology, Writing - original draft. Ping Wu: Visualization, Investigation. Xijing Liu: Methodology. Minhao Wu: Methodology, Formal analysis. Lin Cai: Funding acquisition. Céline Huselstein: Funding acquisition. Xiaolin Feng: Supervision. Yun Chen: Methodology, Conceptualization, Supervision, Writing - reviewing and editing, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

### Notes

The authors declare no competing financial interest.

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