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Nerve capping treatment using a bioabsorbable nerve conduit with open or closed end for rat sciatic neuroma

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Highlights

- Nerve capping with nerve conduits has been introduced for treating painful neuroma.
- It remains unclear whether nerve conduits with open or closed ends are superior.
- Rat sciatic neurectomy with and without capping was performed in this study.
- Capping with closed-end nerve conduits most reduced neuropathic pain.
- Capping with closed-end nerve conduits most reduced neuroinflammation and scarring.
Abstract

Background and Aims: Nerve capping treatment using bioabsorbable nerve conduits has recently been introduced for painful amputation neuroma. However, no clinical or experimental data are available for comparing nerve conduits with open distal ends and closed distal ends. Here, we investigated the nerve conduit with open or closed distal ends as the superior capping device, using a commercially available polyglycolic acid (PGA) nerve conduit in a rat sciatic nerve amputation model.

Methods: Ninety-one rats were assigned to three groups: no-capping (n = 30), capping the resected nerve stump with open ends (n = 31), and closed-end nerve conduits (n = 30). Twelve weeks after sciatic neurectomy, with or without capping, the evaluation of neuropathic pain using the autotomy score was performed. Stump neuromas with perineural scars and neuroinflammation were evaluated histologically.

Results: The mean autotomy scores in the closed-end nerve conduit group were significantly lower than those in the no-capping group. However, the difference between
the open-end nerve conduit and the closed-end nerve conduit groups was insignificant.

Histologically, distal axonal fibers expanded radially and formed neuromas in the no- capping group while they were terminated within the PGA conduit in both capping groups. In particular, the closed-end version of the PGA nerve conduit blocked scarring from intruding through the open end and protected the nerve stump with less neuroinflammation. Nerve capping with the closed-end version of the PGA nerve conduit most effectively suppressed perineural neuroinflammation and scar formation around the resected nerve stump.

**Interpretation:** Nerve capping with the PGA nerve conduit, particularly those with closed ends, after rat sciatic neurectomy prevented amputation neuroma and relieved neuropathic pain.

**Keywords:** Amputation neuroma, nerve conduit, pain, peripheral nerve
1. Introduction

Painful neuromas of the peripheral nerve usually develop following trauma or surgery and adversely affect 2% to 60% of patients with nerve injury [1]. Amputation neuromas are formed due to spontaneous axonal regeneration at the site of injury, which creates a painful mass of neural tissue [2]. Mechanical or chemical irritation around the nerve stump locally causes severe and persistent pain, leading to the development of central neuropathic pain in the central nervous system at more proximal levels [3]. Hence, to treat painful neuroma and reduce neuropathic pain, it is necessary to cover the refreshed nerve stump and protect it from external stimuli and scarring after resection of the neuroma with or without centrocentral anastomosis [4-7]. Numerous surgical options have been proposed to minimize neuroma-induced pain, involving transposition of the resected nerve stump into the muscle or vein [8], burying it in bone [9, 10], and covering it with flaps or vascularized healthy tissue [11-13]. However, no single method is effective in the management of peripheral neuromas [3].
Recently, nerve capping treatment using several bioabsorbable nerve conduits has been introduced for painful amputation neuromas, mainly in animal studies and partly in clinical cases [14-20]. Surgical removal of the neuroma and capping the resected nerve stump with a nerve conduit could reduce the development of a symptomatic end-neuroma, protecting the nerve end from the surrounding scarring [17, 18]. In these basic animal studies, both ends of the nerve conduits, which were commercially unavailable, were open. More recently, Neurocap (Polyganics, Groningen, Netherlands) has been commercially available as a peripheral nerve capping device for the surgical treatment of neuromas in Europe and clinically provides significant pain relief [16]. Neurocap is a bioabsorbable tubular device with one open end and one closed end made from the same synthetic polymer nerve conduit as Neurolac (Polyganics, Groningen, the Netherlands), used for the treatment of peripheral nerve defects and whose ends are both open [21]. In short, Neurocap is a closed-end version of the Neurolac.

Here, it remains unclear which nerve conduit, with an open distal end or a closed distal
end, is superior as a capping device for treating painful neuroma. No experimental data is available for comparing nerve conduits with an open-distal end against those with a closed distal end. We hypothesized that a nerve conduit with a closed distal end would relieve neuroma-induced neuropathic pain when compared with a nerve conduit with an open distal end, as the closed-end could more consistently protect the nerve stump from scar invasion. This study aimed to investigate the effectiveness of nerve capping with the closed-end version of the nerve conduit, which is commercially available, using a rat sciatic nerve amputation model, focusing on neuropathic pain, histological scar formation, and neuroinflammation.

2. Materials and Methods

The study was approved by the Animal Care and Use Committee of Osaka City University Graduate School of Medicine. According to a previous systematic review, the rat sciatic nerve transection model was used to cause terminal neuromas and neuropathic
pain in the current study [22]. Ninety-one male Sprague-Dawley rats (eight weeks old), weighing approximately 250 g, were randomly divided into the following three experimental groups: 1) no-capping group, transected nerve stump without capping (30 rats), 2) open-end nerve conduit group, capping of the transected nerve stump with a nerve conduit with an open distal end (31 rats); and 3) closed-end nerve conduit group, capping of the transected nerve stump with a nerve conduit with a closed distal end (30 rats) (Figure 1). According to the Federal Animal Care Guidelines, all rats were treated and had free access to rat chow and water. Twelve weeks after sciatic neurectomy with or without capping, neuropathic pain was assessed using autotomy scores based on the state of the rat toenails. Stump neuromas were histologically evaluated with regard to perineural scar formation and neuroinflammation.

2.1 Surgical Procedure

Rats were anesthetized by subcutaneous injection of 1 mL of ketamine (50 mg/mL).
and 0.3 mL of 2% xylazine into the right dorsal back. Following the left sciatic nerve exposure, it was sharply transected at the mid-thigh level; the transected nerve was removed to prevent nerve regeneration, resulting in a 15-mm nerve defect. In the no-capping group, the proximal nerve stump was left without capping. In the capping groups (open-end nerve conduit group and closed-end nerve conduit group), the proximal nerve stump was pulled by 2 mm into the proximal end of the polyglycolic acid (PGA) nerve conduit (Nerbridge; Toyobo Co., Ltd., Osaka, Japan) and sutured with 9-0 nylon sutures in a horizontal mattress pattern on the lumen wall under a microscope (Figure 1). The distal end of the PGA nerve conduit remained open in the open-end nerve conduit group and closed in the closed-end nerve conduit group. The muscle wound beds and skin incisions were closed with a 4-0 nylon suture in all groups.

The PGA nerve conduit used in the current study has been commercially available for the treatment of peripheral nerve defects in Japan since 2013, producing clinical outcomes similar to autologous nerve grafting [23-26]. This PGA nerve conduit was subsequently
approved by the US Food and Drug Administration (FDA) in 2016 [27]. It comprises an outer collagen-coated PGA fiber mesh and an inner porous collagen spongiform matrix [28]. The size of the PGA nerve conduit (inner diameter: 2 mm; length: 6 or 8 mm) used in the current study was approximately 0.5 mm larger than the diameter of the rat sciatic nerves (approximately 1.5 mm) to prevent nerve constriction, similar to those used in a previous report [17]. The appropriate capping length of the nerve conduit was determined to be more than four times the diameter of the original nerve, as previously reported [17]. The closed end of the PGA conduit was made by 2-mm-wide fusion sealing using a tabletop heater sealer (P-200, Fujiimpulse Co., Ltd., Osaka, Japan) capable of sealing it up to 0.2-mm thickness. Therefore, 6-mm PGA nerve conduits were used in the open-end nerve conduit group, while 8-mm PGA nerve conduits were used in the closed-end nerve conduit because the length of the distal sealing end was 2 mm (Figure 1).

2.2 Evaluation of Neuropathic Pain: Autotomy Score
Neuropathic pain caused by painful sciatic neuroma was evaluated using an autotomy score [22, 29]. Autotomy is observed in animal models of neuropathic pain, such as allodynia, anesthesia Dolorosa, and phantom limb pain. The scoring scale devised by Wall et al. was used to assess the severity of neuropathic pain in the current study [29]. Briefly, one point was assigned for the removal of one or more toenails, an additional point for each distal half digit attacked, and one more point for each proximal half digit attacked. Thus, if all the digits were attacked, a maximum score of 11 was achieved. The autotomy scores of the left side of all rats, on which sciatic neurectomy with and without nerve capping was performed, were collected in each group at 4, 8, and 12 weeks after surgery.

2.3 Histological Evaluation of Neuroma

The proximal nerve stumps with and without capping with a PGA conduit, including their surrounding soft tissue, were harvested 12 weeks after surgery (Figure 1). The specimens were immersed in 4% paraformaldehyde overnight and embedded in
paraffin. Longitudinal sections of 5 μm thickness were immunohistochemically stained with anti-neurofilament protein antibody (1:200, rabbit; Millipore, Temecula, CA, USA) to evaluate the axons and with anti-sigma-1 receptor (S1R) antibody (1:400, rabbit; Proteintech, Chicago, IL, USA) to assess neuroinflammation. They were also stained with Masson’s trichrome staining and immunohistochemically stained with anti-α-smooth muscle actin (α-SMA) antibody (1:400, mouse; Sigma Aldrich, St. Louis, MO, USA) to evaluate scarring surrounding the nerve stump. The S1R- and α-SMA-positive areas were morphometrically analyzed using computer-assisted imaging, as previously described [17]. Five random sections of two representative proximal nerve stumps in each group were photographed at 200× magnification using an Olympus DP74 microscope (Olympus Corporation, Tokyo, Japan), and the percentages of areas positive for S1R and α-SMA were counted automatically using ImageJ software (National Institutes of Health, Bethesda, MD, USA) and averaged.
2.4 Statistical Analysis

All data are expressed as mean and 95% confidence interval (CI) and statistically analyzed using one-way analysis of variance (ANOVA) followed by Student’s t-test post-hoc tests. Statistical significance was set at $P < 0.05$. To determine the adequate sample size, a power analysis was performed to evaluate the autotomy score. At a two-tailed significance level of 5%, 81 rats (27 rats per group) were required to achieve 80% statistical power using one-way ANOVA based on our pilot study. Assuming that approximately 15% of rats might die in the present study, the final sample size was 96 rats (32 rats in each group). However, the final sample size decreased to 91 rats because two rats in the no-capping group, one rat in the open-end nerve conduit group, and two rats in the closed-end nerve conduit group died during the surgery.

3. Results

The autotomy scores of all three groups gradually deteriorated at 4, 8, and 12 weeks
after surgery (Figure 2). The highest mean autotomy scores were consistently observed in the no-capping group at all points, followed by the open-end nerve conduit group and the closed-end nerve conduit group. Twelve weeks after surgery, the mean autotomy scores in both capping groups (open-end nerve conduit group: 3.73, 95% CI = 2.83–4.63; closed-end nerve conduit group: 2.75, 95% CI = 1.97–3.53) were lower than those in the no-capping group (4.39, 95% CI = 3.48–5.30); in particular, the mean autotomy score in the closed-end nerve conduit group was significantly lower than that observed in the no-capping group ($p = 0.01$). Between the capping groups, the mean autotomy score in the closed-end nerve conduit group was non-significantly lower than that in the open-end nerve conduit group ($p = 0.10$).

Histologically, distal axonal fibers, immunohistochemically visualized using anti-neurofilament protein antibody, were expanded radially and formed a bulbous neuroma in the no-capping group (Figure 3). Alternatively, these axonal fibers became thinner and terminated within the nerve conduit in both the nerve capping groups. Notable
neuroinflammation, immunohistochemically visualized using an anti-S1R antibody, was observed around the bulbous end-neuroma in the no-capping group. In contrast, minimal neuroinflammation was observed around the terminated axonal fibers in both capping groups, superiorly in the closed-end nerve conduit group. Morphometric analysis showed that the percentage of areas positive for anti-S1R antibody in the closed-end nerve conduit group was the lowest, followed by those in the open-end nerve conduit group and those in the no-capping group. The percentage of areas positive for anti-S1R antibody in the closed-end nerve conduit group was significantly lower than in the open-end nerve conduit group (p = 0.01).

The fibrotic scar tissue, stained with Masson’s trichrome and immunohistochemically stained with anti-α-SMA antibody, was widely found surrounding the randomly sprouting distal nerve end in the no-capping group. In contrast, it was slightly observed around the tapering distal nerve end in both capping groups (Figure 4). However, scar formation was intruded from the distal open end of the nerve conduit forward to the distal nerve end in the
open-end nerve conduit group. Morphometric analysis revealed significantly lower
percentages of areas positive for anti-α-SMA antibody in both capping groups compared
with the no-capping group. However, the difference between the open-end nerve conduit
and the closed-end nerve conduit groups was insignificant.

These histological results indicate that nerve capping with the closed-end version of
the PGA nerve conduit most effectively suppresses perineural neuroinflammation and scar
formation around the resected nerve stump.

4. Discussion

In the current study, nerve capping with a closed-end PGA nerve conduit prevented
amputation neuroma and reduced neuropathic pain after rat sciatic neurectomy. Although
we could not show the significant superiority of the closed-end version of the nerve conduit
to the open-end version of the nerve conduit completely, especially in the autotomy scores,
the closed-end version of the PGA nerve conduit effectively blocked scarring from
intruding through the open-end and protected the nerve stump with less neuroinflammation.

The current study has two strengths: (1) application of the commercially available PGA nerve conduit for treating neuroma, which the US FDA has approved for the treatment of peripheral nerve defects in clinical practice, and (2) demonstrating the efficacy of closing the end of the PGA nerve conduit for nerve capping treatment.

Neuroma-induced pain is caused by radially extended axonal fibers from the resected nerve stump to the injured and scarred skin with mechanical and chemical irritation [3, 17]. Here, covering the resected nerve stump is necessary for pain relief to protect the nerve stump from external stimuli and scar adhesion. Vein and silicone rubber caps have been used as tubular tools for nerve capping. However, vein capping requires sacrificing an intact vein and risks easy venous lumen collapse [30]. Silicone tubes often need to be removed because silicone is a non-bioabsorbable hard material with a foreign body reaction [31]. To overcome these limitations, bioabsorbable nerve conduits have been used for capping neuromas. Even in a limited number of previous clinical reports, nerve capping
treatment with nerve conduits for the neuromas in the face, neck, finger, ankle, and foot had successful outcomes when using the pain visual analog scale and patient-reported outcome measures [14-16].

Many basic animal studies have revealed the effectiveness of nerve capping with a nerve conduit for treating amputation neuromas [17-20]. To our knowledge, all of the nerve conduits used in those studies were not clinically available and kept both the proximal and distal ends open. In addition, there have been no published articles reporting animal data regarding Neurocap with one open end and one closed end, which is commercially available for treating neuroma. However, the results of ongoing unpublished animal studies are only available on the Neurocap website. The current study is the first animal study using a commercially available nerve conduit demonstrating which nerve conduit, with open or closed distal ends, is suitable as a capping device for treating painful neuroma.

In our previous report using a bioabsorbable nerve conduit composed of poly-L-lactide and polycaprolactone (PLA/PCL) copolymer as a capping device for treating rat sciatic
neuroma, neuropathic pain assessed with autotomy scores was significantly reduced in the capping group compared with the no-capping group, even if the distal end of the nerve conduit remained open. In contrast, neuropathic pain was not significantly reduced in the open-end nerve conduit group compared to the no-capping group in the current study. This difference could be due to the material properties of the nerve conduits used. The PGA conduit used in the current study was soft and degraded relatively quickly, being completely dissolved by 12 weeks post-implantation [23, 24, 26, 27]. However, the PLA/PCL copolymer nerve conduit had a higher tubular rigidity and longer degradation time (approximately 18 months) than the PGA conduit [17, 32, 33]. As described in previous studies, functional recovery and axonal regeneration following the PLA/PCL nerve conduit (Neurolac) were superior to those following the PGA nerve conduit to reconstruct a 10 mm rat sciatic nerve defect [34]. Therefore, the protective effect of the capping might be diminished by the collapse of the PGA conduit associated with earlier absorption. In contrast, the PLA/PCL copolymer nerve conduit remained structurally stable and could protect the nerve stump until 12 weeks after the surgery. Despite the weakness of
the tubular structure of the PGA, the closed-end version of the PGA nerve conduit could statistically reduce neuropathic pain by blocking scar formation intruding through the distal end in the current study.

The current study has some limitations. First, the closed-end version of the PGA nerve conduit is not commercially available. The second was the short-term follow-up period. Future studies will be needed to evaluate the long-term efficacy of both open- and closed-end versions of the PGA nerve conduits to treat painful neuromas. In conclusion, nerve capping with the PGA nerve conduit, particularly those with a closed-end, prevented amputation neuroma and reduced neuropathic pain after rat sciatic neurectomy. The PGA nerve conduit with a closed-end is a promising nerve capping device suitable for treating painful amputation neuroma.

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**Declaration of Competing Interest**

The authors declare that there is no conflict of interest.

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evaluation of treatment using polyglycolic acid-collagen tube for chronic neuropathic pain


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Figure legends

**Figure 1.** Intraoperative gross findings in a rat sciatic nerve transection model. (A) No-capping group; (B) open-end nerve conduit group, capping the nerve stump with a nerve conduit with both ends opened; (C) closed-end nerve conduit group, capping it with a nerve conduit with one open end and one closed end. Schematic nerve capping with nerve conduit with open and closed ends (D). The polyglycolic acid nerve conduit (Nerbridge) with both ends opened (E) and one open end and one closed end (F).
Figure 1

A

B

C

D

E

F
Figure 2. Autotomy scores during the time course of the experiment. Data are expressed as mean and 95% confidence interval. *$P < 0.05$ versus the no-capping group.
Figure 2

- No-capping (n=30)
- Open-end nerve conduit (n=31)
- Closed-end nerve conduit (n=30)

\[ * p < .05 \text{ vs No-capping} \]
**Figure 3.** Histological images of longitudinal sections of the proximal nerve stump with and without nerve conduit immunohistochemically stained with anti-neurofilament protein antibody (A–C) and anti-sigma-1 receptor antibody (D–I) at 12 weeks after surgery in the no-capping group (A, D, and G), the open-end nerve conduit group (B, E, and H), and the closed-end nerve conduit group (C, F, and I). Regions marked with rectangles in panels D–F are magnified higher in panels G–I.; scale bar = 500 µm (A–F) and 50 µm (G–I).

Quantitative analysis of the percentages of positive areas for anti-sigma-1 receptor antibodies (J). Data are expressed as mean and 95% confidence interval. *P < 0.05.
Figure 4. Histological images of longitudinal sections of the proximal nerve stump with and without nerve conduit stained with Masson’s trichrome (A–F) and immunohistochemically stained with anti-α- smooth muscle actin antibody (G–I) at 12 weeks after surgery in the no-capping group (A, D, and G), the open-end nerve conduit group (B, E, and H), and the closed-end nerve conduit group (C, F, and I). Regions marked with rectangles in panels A–C are higher magnified in panels D–F. The same areas as D–F stained with Masson’s trichrome are depicted in panels G–I stained with anti-α-smooth muscle actin antibody; scale bar = 500 μm (A–C) and 100 μm (D–I). Quantitative analysis of the percentages of positive areas for anti-α-smooth muscle actin antibody (J). Data are expressed as mean and 95% confidence interval. *P < 0.05.