Peripheral Nerve Regeneration Using a Polyglycolic Acid (PGA)-Collagen Nerve Conduit Filled with Collagen Sponge: Experimental Research and Human Application

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Abstract: A novel artificial nerve conduit was developed. The conduit was made of a polyglycolic acid (PGA)—collagen tube filled with laminin—soaked collagen sponge. We evaluated peripheral nerve regeneration using a novel artificial nerve conduit on the basis of promotion of peripheral nerve regeneration. We implanted this nerve conduit across an 80 mm gap in the peroneal nerve of dogs. Histological observation 12 months after implantation showed numerous unmyelinated and myelinated nerve fibers had regenerated beyond the gap. Neurofilaments were widely observed immunohistochemically in the regenerated nerve segments. These findings indicated that newly regenerated axons had extended across the gap and connected into the distal nerve segments. Compound muscle action potentials (CMAPs) and somatosensory evoked potentials (SEPs) were recorded in all dogs. At 12 months, the CMAPs indicated complete recovery while the SEPs showed incomplete but substantial recovery. Walking patterns had returned to near-normal 12 months after implantation. Use of this nerve conduit can lead to peripheral nerve elongation and favorable functional recovery across a wider nerve gap.

Key words: artificial nerve, peripheral nerve regeneration

INTRODUCTION

Functional recovery after peripheral nerve injury continues to be a clinical challenge. Reconstruction using autografts was a clinically standard technique, although nerve autografting has some disadvantages, such as the limited supply of available nerve grafts and permanent loss of donor nerve function at the site of graft harvest. Therefore, use of artificial nerve conduits that create a favorable environment for axonal regeneration has become one of common strategies for repairing major nerve defects. Biodegradable materials that have no cytotoxicity or antigenicity are believed to be suitable for use in nerve tubes. With the experimental use of a variety of synthetic materials, such as silicone, polyglycolic acid, and polylactic acid, as nerve conduits, axonal elongation has been reported within a nerve gap of up to 50 mm, but there have been no reports on gaps greater than 50 mm.

Recently, we reported that use of a polyglycolic acid (PGA)-collagen tube filled with laminin-coated collagen fibers produced peroneal nerve regeneration in dogs, with functional recovery across an 80-mm gap. To our knowledge, this represents the most successful report to date of regeneration achieved with an artificial nerve over a gap longer than 50 mm. However, from a technical view-
Peripheral Nerve Regeneration

point it is extremely difficult to manufacture fine collagen fibers.

To overcome this problem, collagen sponge, which is easy to manufacture, was recently substituted for collagen fibers to produce a novel type of artificial nerve conduit\(^7\). Further, results from another of our studies suggested that collagen sponge may even be superior to collagen fibers as a filling material in nerve conduits used as regeneration scaffolds\(^9\). We suggested that spongiform structures might offer advantages over fibrous structures, since they (i) more space for the attachment and proliferation of cells due to their three-dimensional structure\(^9\), and (ii) an enhanced capacity for local release of various intrinsic and extrinsic soluble molecules\(^10\).

In the presence we report the regeneration of the peroneal nerve across a long gap by using a novel artificial nerve conduit, and the evaluation of the capacity of the conduit to promote nerve regeneration histologically and electrophysiologically.

**METHODS**

**Preparation of the nerve conduit**

The artificial nerve conduit was composed of a biodegradable tube with a biodegradable filling material. The tube was made of cylindrically woven PGA mesh. Its outer and inner surfaces were coated with amorphous collagen layers made by dipping the tube in 1% v/w collagen hydrochloride solution and allowing it to dry. The tube was of the same type as that used in a previous study\(^11\).

For the filling material we used atelopeptide collagen extracted from porcine skin by enzymatic treatment with pepsin. This collagen is predominantly type I (70% to 80%) and type III collagen (20% to 30%), and has very low antigenicity as a result of removal of the telopeptide. The atelo-collagen solution was homogenized and poured into the PGA mesh tube. This material was frozen once at -20°C and then freeze-dried for 3 hours. The freeze-dried material was then heated at 140°C under a pressure of 1 \(\times 10^{-1}\) Pa for 24 h to induce crosslinkings between the collagen molecules\(^12\).

The schematic view, macroscopic appearance, and ultrastructure of the conduit as observed through scanning electron microscopy (SEM) are shown in Figs. 1a, b, and c.

The artificial nerve conduits were sterilized with ethylene oxide gas before implantation. The collagen sponge was then coated with laminin by pouring 5 ml \(\times 2.0\) g/ml diluted laminin (human placental laminin, Gibco, USA) solution in 0.1 M phosphate buffered saline (PBS), pH 7.4, into the collagen sponge, just before implantation.

**Animals and surgical procedure**

Seven adult beagle dogs of arbitrary sex (8.0 to 15.0 kg) were used. The dogs were anesthetized with intramuscular ketamine hydrochloride (50 mg/kg). Under anesthesia, the left sciatic nerve and the peroneal and tibial nerve branches were exposed. The peroneal component was separated from the tibial component along the length of the sciatic nerve, and an 80-mm-long segment of the peroneal nerve was removed. The nerve conduit was implanted into the resulting 80-mm gap (Fig. 2a, b). The proximal and distal stumps of the severed nerve were inserted into the nerve conduit to a depth of 5 mm, and the conduit was secured to each end by epineural 6-0 polypropylene sutures (Prolene, Ethicon Inc., Somerville, NJ, USA). The right peroneal nerve was left intact, thus acting as a normal control in all dogs. Animal care, housing, and surgery followed the Guidelines of the Animal Experiment Committee Kyoto University (1983).

**Histological evaluation**

All 7 dogs were sacrificed about 12 months after the implantation. Each dog was deeply anesthetized with an overdose of pentobarbital and then killed. The dogs

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**Fig. 1. The nerve conduit, consisting of a PGA-collagen tube filled with collagen sponge.**

(a) Illustration; (b) macroscopic appearance; (c) scanning electron microscopic findings (\(\times 40\)).
Fig. 2. Experimental procedures. (a) Schematic view; (b) intraoperative view after implantation of the nerve conduit.

were then transcardially perfused with 21 of 0.1 M phosphate-buffered saline (PBS) as a prewash, followed by 21 of 1% glutaraldehyde in 0.1 M PBS. The sciatic and peroneal nerves on both sides were excised. Histological evaluations on both the mid-portions of the regenerated segment and the distal portion of the peroneal nerve were performed by the methods described in a previous report6). For light microscopic (LM) examination of the regenerated segment, serial sections were made and stained with toluidine blue. To investigate the microstructure of the regenerated axons, transmission electron microscopy (TEM) was also performed.

Immunohistochemistry
After 12 months implantation nerve specimens were also prepared for immunohistochemistry. The segments were post-fixed with 4% paraformaldehyde in 0.1 M PBS for 24 h, and then transferred to 30% sucrose in 0.1 M PBS for 2 days. Sections 2 μm thick were cut with a cryostat microtome. After nonspecific immunoreaction had been blocked, the sections were incubated in a solution of anti-neurofilament antibody (1: 200) (Dako Japan, Kyoto, Japan) overnight at room temperature. They were then incubated in biotinylated anti-mouse rabbit IgG solution (1: 200) for 1 h. Horseradish peroxidase-labeled secondary antibody was developed by the diaminobenzidine method. The sections were counterstained with hematoxylin and examined under light microscopy (LM).

Morphometric analysis
Cross-sections of the myelinated nerve fibers were stained with toluidine blue for analysis. These sections were taken from the mid-portions of the regenerated segment, the distal peroneal nerve, and the part of the normal right peroneal nerve corresponding to the regenerated portion. The axonal density, total area of neural tissue, and axonal diameter were evaluated by using the public domain NIH image computer program according to previous reports39. The three parameters of the mid-portions of the regenerated segment and the part of the normal right peroneal nerve corresponding to the regenerated portion were analyzed statistically using Student's t-test.

Electrophysiological recording
Electrophysiological recordings were performed every 3 months after surgery in all 7 dogs. To evaluate the recovery of the motor and sensory systems, compound muscle action potentials (CMAPs) and somatosensory evoked potentials (SEPs) were recorded. Both CMAPs and SEPs were evaluated by the latency measured from the first prominent positive peak, as an average of 50 stimulations. Measurement was taken at the same stimulation and recording sites and in the same posture to obtain reproducible results. All recordings were performed with the Nicolet Viking Quest™ System (Nicolet Biomedical, USA) under anesthesia induced by ketamine hydrochloride (50 mg/kg, im), as described previously6). To exclude individual variations and several conditions, the recovery index (RI) adopted in previous reports was employed8,13,14). For both CMAPs and SEPs the RI was defined as the ratio of the scores in the implanted side to those in the normal side at 12 months. The CMAP and SEP indices of the implanted and normal control groups were analyzed statistically using Student’s t-test.
RESULTS

General observations
Animals were observed for functional recovery until sacrifice. No serious surgical complications, such as pressure ulcers, developed in any of the dogs. The limping that was observed immediately after the original surgery disappeared gradually from about 5 months after implantation. There were no definite locomotive disturbances and no obvious differences in the range of motion between the right and left hindlimbs 10 to 12 months after implantation.

Gross view of the regenerated segment
At 12 months after surgery, a tight, cream-colored tissue cable bridged the gap in the peroneal nerve (Fig. 3) in all dogs. In all dogs, the nerve conduit had been completely absorbed, leaving no sign of any residue. Moderate adhesion was observed between the regenerated tissue and the surrounding muscles.

Histological observations
Figure 4 shows the LM findings and Fig. 5 shows the TEM findings. LM of the mid-portion of the regenerated segment 12 months after surgery revealed that numerous myelinated nerve fibers had regenerated. Regenerated axons varied in axonal diameter, although both this diameter and the thickness of the myelin sheath were smaller than those of the normal controls. LM of the distal peroneal nerve after 12 months also showed many regenerated axons, but those axons were smaller than the axons in the mid-portion of the regenerated segment. Numerous unmyelinated axons were also observed by TEM, suggesting that nerve regeneration was incomplete 12 months after implantation.

Immunohistochemistry
Neurofilaments were widely recognized immunohistochemically in the regenerated nerve segments 12 months after implantation. This indicated that myelinated and unmyelinated axons extended across the gap and into the distal nerve segments (Fig. 6).

Morphometric analysis
The results of morphometrical analysis are shown in Table 1. In the mid-portion of the regenerated segment and the distal peroneal nerve 12 months after implantation, the axonal density was higher than that of normal control nerves. In contrast, the axonal diameter and the

Fig. 3. Macroscopic view of the treated site in a dog 12 months after implantation.
A tissue cable has been interposed across the nerve defect (the portion between the two arrows).

Fig. 4. Light microscopic appearance of (a) mid-portion of the regenerated segment, and (b) the distal peroneal nerve 12 months after implantation. (c) a right normal control nerve.
(Toluidine blue stain, ×400)
total area of neural tissue were both less than those of normal nerves. Toward the distal part, the axonal diameter and the total area of neural tissue decreased, while the axonal density gradually increased.

The differences of the axonal density and the axonal diameter between the mid-portion of the regenerated segment and the part of the normal right peroneal nerve were statistically significant ($p<0.01$). The difference of the total area of neural tissue between the two groups was significant but smaller ($p=0.03$).

Figure 7 presents the distributions of the myelinated axonal diameters in (a) the mid-portion of the regenerated segment, (b) the distal peroneal nerve after 12 months, and (c) normal control nerves. The distribution of diameters of the right normal control nerves had two peaks at 6 to $8\mu$m and 12 to $14\mu$m, which is compatible with previous reports$^{15}$. Regenerated nerves showed two peaks at 2 to 4 $\mu$m and at 14 to $16\mu$m in the mid-portion, and one peak at 2 to 4 $\mu$m in the distal peroneal nerve. This contrast in peak position may indicate immaturity of the regenerated nerves$^{15}$.

**Electrophysiological evaluation**

CMAPs and SEPs were recorded every 3 months in all implanted dogs. The peak latencies of these CMAPs and SEPs recovered gradually. At 12 months, a comparison of CMAPs from the control and implanted sides indicated complete recovery (RI of CMAPs: mean=$1.03$, S.D.=$0.09$, $n=7$), while comparison of the SEPs showed incomplete but substantial recovery (RI of SEPs: mean=$1.19$, S.D.=$0.18$, $n=7$). The difference of the RI of CMAPs and SEPs was not statistically significant.

**Table 1. Morphometric analysis of nerve cross-sections 12 months after implantation, and of normal control peroneal nerves (mean±S.D.).**

<table>
<thead>
<tr>
<th></th>
<th>Mid portion of regenerated segment</th>
<th>Distal peroneal nerve</th>
<th>Normal control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of myelinated nerve fiber ($\mu$m)</td>
<td>$5.7\pm3.7$</td>
<td>$3.9\pm3.3$</td>
<td>$9.1\pm4.1$</td>
</tr>
<tr>
<td>Density of myelinated nerve fiber (counts/100×100 mm)</td>
<td>$87.4\pm11.9$</td>
<td>$98.7\pm7.9$</td>
<td>$60.7\pm4.8$</td>
</tr>
<tr>
<td>Percentage of neural tissue (%)</td>
<td>$42.4\pm4.6$</td>
<td>$31.2\pm6.6$</td>
<td>$52.5\pm2.6$</td>
</tr>
</tbody>
</table>
significant (CMAPs: $p=0.46$, SEPs: $p=0.08$). These analyses indicated that both motor and sensory nerves had at least partly regenerated over the gap, and that electrical connections to their target tissues had been re-established at 12 months.

**DISCUSSION**

Peripheral nerve regeneration with resorbable artificial conduits is a new and common-accepted experimental approach. Varieties of biodegrade materials such as PLA, poly($L$-lactide-co-6-caprolactone), poly-[bis-(ethylalanate)]-phosphazene, and copolymer of DL-lactide and epsilon-caprolactone were applied to nerve conduits with good results. Each polymers has different original characters such as handling, resorption rate, biocompatibility, and the use of neurite-promoting factors. Further studies are needed to compare the role of these polymers.

In 1996 we reported successful peripheral nerve regeneration in 25-mm gaps in the peroneal nerves of cats by using a hollow PGA-collagen tube. Horseradish peroxidase (HRP) labeling tests showed anatomical recovery of the nerve fibers, and this was the first report of successful functional recovery across a gap longer than 20 mm. The nerve conduit used was reinforced with PGA mesh in response to fears that if the outer tube were made from collagen alone it would degrade too fast to maintain sufficient space for axonal elongation, and that the ingrowth of scar tissue could prevent nerve regeneration in such a long gap. The findings of the 1996 investigation strongly indicated that the implanted PGA conduit prevented the ingrowth of fibrous scar tissue.

Several lines of evidence suggest that filled structures can both enhance nerve regeneration across a longer gap than those repairable with hollow tubes and directly support axon outgrowth. In light of this evidence, in 1999 we developed a PGA-collagen tube filled with a freeze-dried alginate gel, but the gap length that could be repaired with this type of tube was limited to less than 50 mm.

Collagen has already been reported to have several superior biological properties for peripheral nerve regeneration. We developed the new PGA-collagen tube filled with collagen fibers in 2000. Using this nerve conduit, we achieved peroneal nerve regeneration in dogs with functional recovery across a 80-mm gap that the longest gap repaired to that time. Recently a randomized prospective study using this conduit for digital nerve reconstruction in humans is discussed by Weber, Lundborg, and Meek.

However, it is very difficult to manufacture fine collagen fibers, and therefore manufacture-cost assumed to become higher than commercial base of medical product. The ease of manufacture and mass-productivity of collagen sponge make it an attractive alternative to fine fibers. Substitution of sponge for fibers as the filling material produces a novel type of artificial nerve conduit, and results from a recent investigation indicate that collagen sponge may even be functionally superior to fibers as a filling material in nerve conduits used as regeneration scaffolds. This superiority may be explained by the fact that spongiform structures offer more space for the attachment and proliferation of cells, because of their three-dimensional structure, and an enhanced capacity for local release of various intrinsic and extrinsic soluble molecules.

Laminin has been shown to improve and promote nerve regeneration. Recently, we demonstrated that the activity of laminin can be reduced by dehydrothermal (DHT) crosslinking, using PC-12 cells cultured on collagen in vitro. In the present study, we applied the laminin after DHT crosslinking, to take advantage of the spongiform structure, whereas in a previous study using collagen fibers, laminin was added before crosslinking. The sponge was used in an attempt to extend the preservation period of laminin in situ.

Implementation of these modifications resulted in a novel nerve conduit that induced superior nerve regeneration, as demonstrated in the present study by both histological and electrophysiological examination.

Histological and morphological observations at 12 months revealed that the axons in the distal part of the...
nerve were remarkably immature. The diameters of myelinated axons decreased gradually toward the distal part of the regenerated nerve; this was similar to the results from a previous report on allografts15). On the other hand, the axonal density became higher toward the distal part of the regenerated nerve. These findings indicate that multiple aberrant axons were sprouting from one proximal nerve fiber. As the distal portion of the regenerated nerve was further from the neuronal body, it is likely that the distal nerve portion was less mature with respect to axonal diameter and myelination. This observation suggests that it is important to evaluate not only the mid-potions but also the distal parts of regenerated nerve segments. In our study, a maturation gradient of regenerating axons still existed 12 months after implantation. This finding also demonstrated the significance of long-term evaluation of the quality of nerve regeneration.

Neurofilaments were widely observed immunohistochemically in the regenerated nerve segments 12 months after implantation. This indicated that myelinated and unmyelinated axons extended across the gap and into the distal nerve segments, and that nerve regeneration was undergoing at least 12 months implantation. Therefore, longer observation is necessary to confirm the results of nerve regeneration. Recently we established that regeneration using this nerve conduit at the distal nerve portion after 3 years implantation was more mature than that after 12 months implantation with respect to axonal diameter and myelination (submitted).

The superior electrophysiological recovery that results from the use of this implant was demonstrated by the RIs of both the CMAPs and SEPs. At 12 months, the RI of the CMAPs indicated complete recovery, while that of the SEPs showed incomplete but substantial recovery. These analyses demonstrated that regenerated nerves had been enhanced over the gap, and that electrical connections had been re-established.

The experimental use of promoting factors in association with nerve conduits has been widely studied29,30. Aldini reported the effectiveness of controlled release factors during polymer degradation15,31. In the present study, although no nerve-growth-promoting factors except laminin were added extrinsically to the implanted material, excellent nerve regeneration occurred across a longer gap than has been reported previously. There remains the possibility that extrinsic nerve-promoting factors applied for adequate periods and in sufficient doses would also accelerate nerve regeneration in combination with the nerve conduit utilized in the present study.

Teramachi reported that collagen sponge was superior to amorphous collagen in an artificial trachea20. Recently, we applied collagen sponge scaffolds to other organs, and reported good regeneration in the esophagus32, tracheal carina30, stomach30, and small intestine30. The results of these studies have conclusively demonstrated the clinical potential of collagen sponge as a biomaterial.

In conclusion, a PGA-collagen tube filled with laminin-soaked collagen sponge induced axonal regeneration. Morphological, electrophysiological, and functional recoveries of the regenerated nerves were observed. This newly developed nerve conduit is a promising tool for use in peripheral nerve regeneration, and provides a suitable experimental model for clinical application.

References


Part 2) Clinical Application of Nerve Conduits Consisting of a Polyglycolic Acid (PGA)-Collagen Composite Tube Filled with Collagen Sponge

Tatsuo Nakamura

Abstract: Clinical application of bioabsorbable nerve conduits began on 1st February 2002, in Japan. A total of 65 grafts were inserted in 46 patients in four hospitals until the 31th October 2002. Each conduit consisted of a polyglycolic acid tube coated with collagen and filled with collagen sponge. The conduits ranged from 0.7–13 mm in diameter and 4–94 mm in length. Indications included peripheral nerve injury, mainly common and proper digital nerves, and neural invasion by tumors. To date, no side-effects or complications have been reported in any of the treated patients. Rapid recovery of nerve function was observed in several patients. Such conduits are promising for clinical use as an alternative to conventional autografts.

Key words: peripheral nerve, regeneration, collagen, polyglycolic acid (PGA), in situ tissue engineering

INTRODUCTION

Clinical application and evaluation of bioabsorbable polyglycolic acid (PGA)-collagen nerve conduits began on 1st February 2002, in Japan. The conduits were used in four hospitals with the permission of the Ethical Committee of each hospital and with the informed consent of each patient. Although this clinical trial has only been running for 9 months, rapid and unexpected recovery of nerve function in several patients prompted this preliminary report.

Structure of the Nerve Conduit

The nerve conduits currently used clinically have the same structure as those used previously in animal experiments. From the results of these studies laminin was suggested to promote nerve regeneration in some degree. However, the our previous study indicated that the differences of the functional recovery were not statistically significant between the laminin group and non-laminin group at electrophysiologic evaluation three months after the reconstruction of the ischiadic nerves of adult cats. Furthermore there is no laminin product permitted by the Ministry of Health, Labour and Welfare in Japan to use clinically. Namely all the laminins used in the previous animal experiments were intended for research purposes and have not yet been tested for safety and efficacy in medical device. Therefore, in this clinical trial, laminin was not added to the collagen sponge.

The nerve conduit used here was composed of biodegradable polymer PGA and collagen. The PGA fiber was medical grade PGA polymer used widely in surgical sutures (Mitsui Chemical Ltd., Japan). Collagen was atelo collagen enzymatically extracted from porcine skin (Nippon Meat Packers Co. Japan). PGA woven tubes with a range of diameters were manufactured from the PGA fibers, coated with collagen solution, and dried. This procedure was repeated 10 times to form a collagen layer on the PGA surface. The inner space of the tube was filled with collagen sponge (Fig. 1). To prevent early breakdown of the collagen, it was dehydrothermally cross-linked at 140°C for 24 h. This conduit was designed to remain in the body for approximately 2 months and then absorb into the tissue. Conduits with

Fig. 1. Scanning electron micrograph of a longitudinal section of the nerve conduit (Fig. 1a) and a transverse section of the nerve conduit (Fig. 1b).

The inner space of the conduit was filled with collagen sponge.
Fig. 2. Surgical procedure.
Both nerve ends were inserted into the conduit and fixed with 9-0 sutures. In the case of partial resection of the nerve, the tube was cut longitudinally to cover the damaged part of the nerve.

Fig. 3. Intraoperative finding of the reconstructed left nervus obturatorius.
At the operation a part of the left nervus obturatorius was resected because of cancer invasion and only the nerve sheath remained at that part. The nerve conduit with a diameter of 4 mm was used to reconstruct the nerve.

Surgical Procedure
At the time of surgery, the nerve conduit was cut to an adequate length and both nerve stumps were inserted into the nerve conduit. Under a surgical microscope the nerve and conduit were fixed with 9-0, 10-0 sutures. It was very important to fix both ends so that they faced each other in the tube. If the nerve end was directed in the wrong direction, nerve connection would not be achieved in the conduit. In cases where the nerve was partially resected, the conduit was cut open longitudinally and the partially resected section of the nerve was covered. The cut line was then sutured together (Fig. 2).

Fig. 4. Intraoperative findings in a case of chronic nerve injury of finger.
The patient had had his finger amputated following an accident 8 months previously. During the operation neuromas (upper) were resected and the degenerated parts of the proper digital nerve were also removed. Implanted nerve conduits connected both ends of the nerve (lower).
Table 1. Surgical indication of nerve conduit.

<table>
<thead>
<tr>
<th>Institute</th>
<th>Indication</th>
<th>Nerve</th>
<th>Number of operated site</th>
<th>Size of conduit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kyoto Prefectural University</td>
<td>Tumor: rectal cancer</td>
<td>Retro peritoneal cavity: l.</td>
<td>1</td>
<td>25 mm, φ4 mm</td>
</tr>
<tr>
<td>of Medicine (one case, one nerve)</td>
<td></td>
<td>obturator n.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nara Medical University &amp; Inada</td>
<td>Tumor: cholesteatoma</td>
<td>Face: facial n.</td>
<td>1</td>
<td>14 mm, φ0.7 mm</td>
</tr>
<tr>
<td>Hospital (43 cases, 62 nerves)</td>
<td>Neuroma</td>
<td>Forearm: median cutaneous n.</td>
<td>1</td>
<td>84 mm, φ3 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arm: ulnar n.</td>
<td>1</td>
<td>40 mm, φ7 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hand: common digital n.</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Foot: superficial peroneal n.</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>(94 mm)</td>
</tr>
<tr>
<td>Acute Nerve Injury</td>
<td>Leg: deep peroneal n.</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Buttock: sciatic nerve</td>
<td>1</td>
<td>20 mm, φ13 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hand: common digital n.</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proper digital n.</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kyoto University Hospital</td>
<td>Tumor: cholesteatoma</td>
<td>Head: Chorda tympani n.</td>
<td>2</td>
<td>5 mm, φ1 mm</td>
</tr>
<tr>
<td>(2 cases, 2 nerves)</td>
<td>with otitis media</td>
<td></td>
<td>(both)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>(46 cases, 65 grafts)</td>
<td></td>
<td>65</td>
<td>4 mm ~94 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Ave 33 mm)</td>
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Surgical Indications of the Nerve Conduit

The surgical indications of the conduit in the four facilities were listed in Table 1.

First Clinical Case

The first clinical nerve reconstruction using the PGA-collagen conduit was performed on 1st February 2002 in the hospital of Kyoto Prefectural University of Medicine by Dr. Hagiwara and his group. The patient was a 66-year-old woman who had undergone low anterior resection of the colon for rectal cancer 3 years previously. The patient had also undergone replacement of the head of the femur and had suffered from right foot disturbance since then. Because of recurrence of rectal cancer, total pelvic exenteration including the right half of the bladder, rectal and sigmoid colon, and the invaded part of the sacral bone was performed. The left half of the bladder was free from cancer invasion and not resected. Although part of the left nervous obturatorius was resected because of cancer invasion and only the nerve sheath remained at that part. Because the patient had had disturbance in her right leg, it was necessary to maintain movement of the left leg for good quality-of-life after the operation. Thus, it was decided to use the PGA-collagen conduit for reconstruction of this nerve (Fig.3). A 25-mm long conduit with a diameter of 4 mm was used. At the end of the operation, the patient had muscle palsy of the left leg at adduction and external rotation, and could not move without the manual support of others. After surgery the patient recovered the motor function of her left leg rapidly. At discharge, 52 days after surgery, the patient was able to walk as well as before the operation.

Indication for Injury

In cases of amputation of fingers or limbs, microsurgical anastomosis is carried out clinically. In such cases, the main interest of surgeons has focused on vascular anastomosis, because this is essential for viability of the graft. Less attention has been paid to the function of the nerves. As a consequence, patients often suffer from severe cold pain in the graft and sometimes even desire re-amputation because of intractable severe pain. The PGA nerve conduits were used in patients with this type of chronic nerve injury. At implantation, several neuromas were observed at the site of the injured nerve, some of which were reconstructed with autografts. These neuromas were removed and a PGA conduit was implanted so that it bridged the fresh ends of the proximal and peripheral parts of the relevant nerve. The severe pain experienced by the patient disappeared just after the operation, indicating that the pain had probably been caused by the neuroma.

A Typical Case of Proper Digital Nerve Reconstruction

The patient was a 53-year-old man who had cut his right third finger in an accident in August 2001. The finger had been amputated and re-anastomosed by microsurgery. However, flexion contracture with traumatic anesthesia (British Classification S-O) appeared after surgery on the anastomosed finger. A second operation was therefore performed 8 months after the first (May 2002). During surgery, neuromas were observed at the site of anastomosis. These neuromas were resected with the degenerated proper digital nerves. A PGA-collagen conduit was implanted on both sides of the finger, bridging the normal cut end and the distal end of the proximal
digital nerve (Fig. 4). After the operation, the patient recovered his feeling sensation up to grade S-3 (British Classification) in the two-point discrimination test; a distance of 15 mm could be discriminated on the ring finger at 5 months.

Interestingly, much quicker functional recovery than expected was observed in many patients, suggesting that a mechanism other than direct recovery of the nerve through the reconstructed route, for example via a bypass route, had been established after the initial injury, and suppression of the nerve graft by the neuroma was taking place at the time of the second operation. When the neuroma was removed, the suppression was also removed and functional recovery through the by-pass appeared clinically.

Evaluation of Functional Recovery of the Nerve

Functional recovery of the reconstructed nerve was evaluated with conventional clinical neurological tests such as the Tinel sign, two-point discrimination test or pin-touch test (Semmes-Weinstein monofilament test). For objective evaluation, the following tests were also carried out on cases at Inada Hospital and Nara Medical University: (i) LASER Doppler micro-circulation metering; (ii) thermography of the skin; and (iii) current perception threshold (CPT) test. Among them, the CPT test could access objectively sensory function of the patent before and after the surgery in which recovery of the nerve fibers could be quantitatively evaluated distinguishing unmyelinated and myelinated fibers. The results of these objective evaluations will be published in a further report.

The recovery rate of injured peripheral nerves has been reported to be 1 mm per day. When the PGA conduit was used, recovery of the reconstructed part of the nerve occurred much faster than the expected rate. The mechanism responsible for this rapid early recovery is still unknown.

Although clinical application of this new conduit began only 9 months ago, no complications involving the PGA-collagen conduit have been reported to date. In cases of chronic nerve injury, the patients recovered from cold pain immediately after surgery. In such cases, a thermography test indicated rapid recovery of the skin temperature. Consequently, such patients with severe pain seem to be good candidates for this type of procedure.

In the future, a slowly degrading conduit must be developed for long segment reconstruction. Because the nerve conduit described in this study is absorbed by the body in only 3-4 weeks, it is not appropriate for long segment reconstruction where a longer period of time is required for nerve regeneration. In the first case at Inada hospital, the patient had suffered severe pain in the left ankle since a road traffic injury 2 years previously, and could not put his left ankle on the floor while walking. Two PGA conduits were implanted on the lateral and inner sides of the left ankle. After this operation, in which several neuromas were removed, the dysesthesia disappeared in the skin area, although loss of feeling continued on the lateral side of the ankle. Because the patient desired a further operation to rectify this loss of feeling, a second operation was performed 6 months after the first. This is the only case where re-operation was performed on a PGA implant. During the second operation the implanted PGA conduit was found to have disappeared completely and had been replaced by scar tissue. This operative finding indicated that implanted PGA-collagen conduit had been absorbed in the body at least by six months without any tissue reaction and was consistent with the clinical findings for the patient. In this case, insufficient removal of the damaged nerve segment might have been performed during the first operation, and a degenerated (damaged) nerve might have remained at the central nerve stump. This might explain why recovery of the nerve did not occur at this site. During the second operation a longer segment of the proximal stump was removed and reconstruction was performed at the fresh end. Thus, it is essential to distinguish the normal end of the nerve stump to be connected, particularly at the proximal stump where elongation of the nerve begins. Nerve autografts have been used clinically for the reconstruction of the damaged nerve defects. Cold preserved nerve allografts have been nominated as an alternative to nerve auto grafts. However the limited supply and limitation of graft size are now still unsolved issues on the material from the cadaver. Because our synthetic PGA-collagen nerve conduit can be freely manufactured according to the patients demand, they have wider indication of the nerve injuries than allo/auto-grafts. Hence it is promising not only as an alternative to conventional graft implantation but also as a potential measure to establish a new neuro-surgical operation method.

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References


