# Repair using a nerve connector as an alternative for Direct repair in peripheral nerve injury

**Introduction**

Nerve injuries can impose a heavy burden on patients’ quality of life. Although direct epineurial and perineurial sutures are commonly used for peripheral nerve injury, they are often accompanied by undesirable tension and their surgical outcomes can be less than satisfactory.1-4 These sutures can cause axon misalignments (such as misdirection, overlapping, and buckling), scar tissue ingrowth into the repair site, or tension-induced ischemia around the stumps, all of which can lead to poor fascicular coaptation.5 To overcome these problems, several newer methods, such as connector repair, have been considered.6

Previous studies have suggested that using a nerve conduit as a connector (covering the repair site and leaving a very short gap between the stumps) for peripheral nerve repair provides outcomes that are equivalent to, or better than, those of direct suture repair.7-9 Nerves generally regenerate along the length of the nerve conduit in a selective manner, with proximal motor nerves migrating to join distal motor nerves.10,11 The intentional gap in the connector provides an area for this selective reinnervation, thus encouraging natural regrowth while preventing the aforementioned misalignments.

The connector also provides a physical barrier to prevent the infiltration of scar tissue, allowing the diffusion of nutrients to the repair site.12,13 This barrier prevents any potential uncontrolled sprouting outside the coaptation site,12 and its length relocates the sutures away from the coaptation site, thus reducing the risk of tension and tension-induced ischemia around the stumps. The use of a connector also decreases the number of sutures required for nerve coaptation, which may reduce surgical time.6,12

The use of a nerve conduit as a connector has reportedly achieved outcomes equivalent to, or better than, those produced by direct suture repair; however, the underlying mechanisms of the conduit and the response of the axons in it remain unclear. In the present study, we investigate the peripheral nerve repair mechanisms of an artificial nerve conduit across a short gap. To do this, we performed serial in vivo imaging of transgenic mice that constitutively express yellow fluorescent protein (YFP), analyzed gene expression at the repair site, and recorded various histomorphometric measurements. We compared all conduit repair findings against their direct suture repair counterparts and against the control. Final recovery was quantified through functional assessments.

**Methods**

*Preparation of nerve conduit*

For the present study, we used a bioabsorbable polyglycolic acid (PGA) conduit filled with collagen in a honeycomb-like structure (Nerbridge, Toyobo Co. Ltd.; Fig. 1A); this product is commercially available and has been approved by the Japanese government as a medical device for clinical practice since 2013. We selected this artificial nerve for its robustness. The tube was made of PGA fiber mesh that was permeable to particles smaller than 600 KD. It had an inner diameter of 1 mm and a length of 55 ± 5 mm, and was designed to biodegrade 3 months after transplantation in vivo.14 For this study, only the outer cylinder without the inner honeycomb-shaped collagen was used,14 after being soaked in saline for 5 min.15

*Experimental animals*

We purchased 64 transgenic mice (Thy1 YFP 16) from the Jackson Laboratory (strain: B6.Cg-Tg[Thy1-YFP]16Jrs/J; 8–12 weeks old, weighing 23–28 g, 32 males and 32 females; Fig. 1B–D). In these mice, all axon fibers of the motor and sensory nerves constitutively express YFP fluorescence. Animal husbandry was conducted in accordance with the standards and regulations provided by the National Institutes of Health Guide for Care and Use of Laboratory Animals. The study was approved by the Animal Care Committee of Juntendo University. The mice were kept in groups of four in sterile cages that were maintained at 22℃ ± 2℃ and 40–60% humidity under a 12-hour light/dark cycle, and they had free access to water and 15 kGy gamma-irradiated feed (CRF-1, Oriental Yeast Co.). They were sacrificed by cervical dislocation under deep anesthesia after all grafts had been harvested.

*Experimental model*

The experimental group (n = 48) underwent a nerve transection procedure on the sciatic nerve of each hind limb. Two weeks later, this was followed by two types of repair operations: direct epineurial suture (right hind limb) and artificial nerve connector (left hind limb). The control group (n = 16) underwent the same procedures but without the nerve transection or the repair operation. Equal numbers of male and female mice were assigned to each group.

*Sciatic nerve injury model creation*

The mice in the experimental group were anesthetized via inhaled sevoflurane (5% for induction, 2% for maintenance). On each hind limb, a 15 mm skin incision was made parallel to the femur to expose the sciatic nerve. To manipulate the sciatic nerve, a microscope (MZ16FA, Leica Microsystems) was used. Once the nerve was detached from the surrounding tissue, it was transected halfway between the hip and knee joint (Fig. 1E–G). The femoral muscle was partially peeled and placed between the nerve stumps to avoid natural reconnection, and the wound was closed using 5-0 nylon. The animals were then left for 2 weeks (to allow the formation of Wallerian degeneration in the distal stump) before the repair operations were conducted.

*Repair operations*

The experimental group underwent the repair operations 2 weeks after the initial lesion-generating operation. Each mouse was placed under anesthesia, then its wounds were reopened, and the sciatic nerve stumps were exposed under a microscope (Fig. 1H and I). The Wallerian degeneration was confirmed by the disappearance of YFP expression in the distal stump (Fig. 1J and K). The ends of both stumps were debrided to the minimum extent to remove neuromas, and the two types of nerve repair (one to each hind limb) were performed as follows.

*Epineurial suture – Direct repair (DR)*

Every right hind limb was repaired using a direct epineurial suture, which was conducted with two 9-0 nylon stitches (Fig. 2A–C).

*Nerve repair using nerve connector – Connector repair (CR)*

Every left hind limb was repaired using a 3 mm artificial nerve. The artificial nerve was interposed with a stitch of 9-0 nylon on each end. Approximately 1 mm of each nerve end was inserted into the conduit, leaving 1 mm between the stumps inside the conduit (Fig. 2A, D, and E).

*Quantitative reverse transcription polymerase chain reaction (qRT-PCR)*

To analyze gene expression at the repair site, tissue between 1 mm proximal and 1 mm distal from the suture (in the case of DR) or all tissue inside the conduit (in the case of CR) was harvested and subjected to qRT-PCR at 3 days, 1 week, and 2 weeks after the repair operation (n = 24). The expression levels of a macrophage migration marker (*CD68*), an angiogenesis marker (vascular endothelial growth factor [*VEGF*]), an endothelial marker (platelet endothelial cell adhesion molecule [*PECAM1*], also known as *CD31*), Schwan cell migration markers (*SOX10* and *S100*), and neurotrophins (neurotrophin-3 [*NTF-3*], brain-derived neurotrophic factor [*BDNF*], and nerve growth factor [NGF]) were analyzed. The tissue was extracted and stored in RNAlater solution at −4°C overnight and then stored at −80°C until RNA extraction. Total RNA was extracted using an RNeasy Plus Universal Mini Kit (QIAGEN), and RNA purity and concentration were assessed using a NanoPhotometer (IMPLEN). RNA samples with a 260 nm/280 nm ratio between 1.8 and 2.0 were used for qRT-PCR. We used a high-capacity RNA-to-cDNA kit (Applied Biosystems) for reverse transcription into cDNA and TaqMan Fast Advanced Master Mix (Applied Biosystems) for qRT-PCR. All primers were purchased from Applied Biosystems, and the measured values were compared using *GAPDH* as a reference gene. An ABI 7500 FAST PCR system (Applied Biosystems) was used for thermocycling, and the conditions were 95°C for 20 s, followed by 45 cycles of 95°C for 3 s and 62°C for 30 s. The amount of each target gene relative to the *GAPDH* reference gene was determined using the comparative threshold cycle method, and the mean values were calculated.