

BRIDGING A 30 MM DEFECT IN THE CANINE ULNAR NERVE USING VESSEL-CONTAINING CONDUITS WITH IMPLANTATION OF BONE MARROW STROMAL CELLS

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Previously, we showed that undifferentiated bone marrow stromal cell (uBMSC) implantation and vessel insertion into a nerve conduit facilitated peripheral nerve regeneration in a rodent model. In this study, we investigated the efficacy of the uBMSC-laden vessel-containing conduit in repair of segmental nerve defects, using a canine model. Eight beagle dogs were used in this study. Thirty-millimeter ulnar nerve defects were repaired with the conduits (right forelimbs, $n = 8$) or autografts (left forelimbs, $n = 7$). In the conduit group, the ulnar artery was inserted into the L-lactide/ ϵ -caprolactone tube, which was filled with autologous uBMSCs obtained from the ilium. In the autograft group, the reversed nerve segments were sutured in situ. At 8 weeks, one dog with only nerve repair with the conduit was sacrificed and the regenerated nerve in the conduit underwent immunohistochemistry for investigation of the differentiation capability of the implanted uBMSCs. In the remaining seven dogs, the repaired nerves underwent electrophysiological examination at 12 and 24 weeks and morphometric measurements at 24 weeks. The wet weight of hypothenar muscles was measured at 24 weeks. At 8 weeks, almost 35% of the implanted uBMSCs expressed glial markers. At 12 weeks, amplitude (0.4 ± 0.4 mV) and conduction velocity (18.9 ± 14.3 m/s) were significantly lower in the conduit group than in the autograft group (3.2 ± 2.5 mV, 34.9 ± 12.1 m/s, $P < 0.05$). Although the nerve regeneration in the conduit group was inferior when compared with the autograft group at 24 weeks, there were no significant differences between both groups, regarding amplitude (10.9 ± 7.3 vs. 25.3 ± 20.1 mV; $P = 0.11$), conduction velocity (23.5 ± 8.7 vs. 31.6 ± 20.0 m/s; $P = 0.35$), myelinated axon number (7032 ± 4188 vs. 7165 ± 1814 ; $P = 0.94$), diameter (1.73 ± 0.31 vs. 2.09 ± 0.39 μ m; $P = 0.09$), or muscle weight (1.02 ± 0.40 vs. 1.19 ± 0.26 g; $P = 0.36$). In conclusion, this study showed that vessel-containing tubes with uBMSC implantation may be an option for treatment of peripheral nerve injuries. However, further investigations are needed. © 2015 Wiley Periodicals, Inc. *Microsurgery* 00:000–000, 2015.

The treatment of segmental defects of peripheral nerves remains challenging for surgeons especially when repairing long gaps in the motor or mixed nerves. Although autologous nerve grafting is considered to be the gold-standard approach in the treatment of such nerve injuries, this technique is associated with several inevitable drawbacks, including a limited source of donor nerves and donor-site morbidity. Therefore, alternative treatments are needed.

The repair of nerve defects by bridging the gap with tube-like materials (tubulization^{1,2}) has been widely performed, both experimentally and in clinical practice. One of the strategies used for improving the quality of regenerated nerves after reconstruction by tubulization is the implantation of bone marrow stromal cells (BMSCs) into the tubular lumen. Several recent studies demonstrated that undifferentiated BMSCs (uBMSCs) facilitate nerve regeneration through nerve conduits.^{3–11} Implanted

uBMSCs reportedly produce various types of growth factors and cytokines^{6,12–14} and differentiate into Schwann cell (SC)-like cells,^{7–9,11,15–17} thus resulting in the promotion of nerve regeneration within the tube.

When bridging a long nerve gap with a nonpermeable tube, BMSCs implanted in the middle of the tube are theoretically at risk of ischemia because revascularization in the tubular lumen occurs only through capillaries that extend from both ends of the tube. To address this potential problem, we developed a model of a vessel-containing tube (VCT) by inserting a vascular pedicle into a silicon tube through its longitudinal slit.^{7,18} Our previous study demonstrated the formation of a rich neo-vascular network from the vascular pedicle that was placed in a silicon tube.¹⁸ In a rodent model, successful axonal regeneration was observed across a 15 mm sciatic nerve defect that was bridged using a VCT implanted with uBMSCs (uBMSC-laden VCT), whereas no neural tissue was observed when the defect was bridged with a simple silicon tube (without vessel insertion) supplemented with uBMSCs.⁷ A vascular bundle implanted into a conduit plays important roles in the supply of nourishment to implanted uBMSCs and the provision of a scaffold for migration and adhesion of the implanted uBMSCs. The insertion of a vascular vessel into the conduit is expected to optimize the contribution of the uBMSCs to nerve regeneration.

In this study, we investigated the efficacy of the uBMSC-laden VCT in comparison with an autograft,

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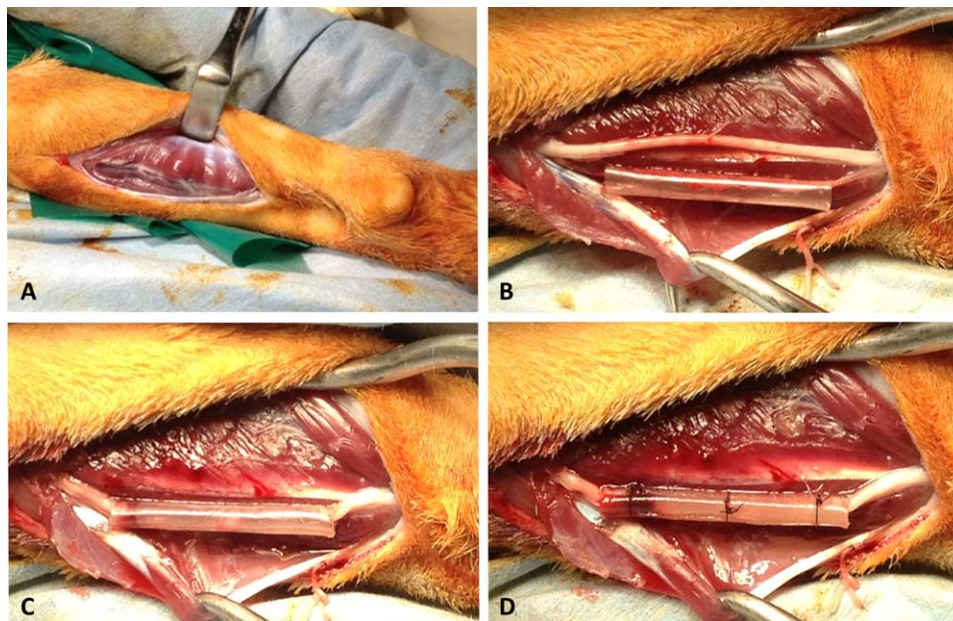


Figure 1. Surgical procedures used in the conduit group. **A:** The ulnar nerve was exposed by retracting the ulnar carpal flexor muscle medially and the digital flexors laterally. **B:** The ulnar nerve was exposed in the forearm. The accompanying vessel (ulnar artery) was inserted into an artificial nerve conduit that consisted of L-lactide and ϵ -caprolactone (P-LA-CL tube). **C:** A 25 mm segment of the nerve was removed. Each stump was secured 1.5 mm into a 33 mm P-LA-CL tube, thus creating 30 mm defects. **D:** Autologous uBMSCs were implanted into the tube.

using a canine ulnar nerve model with a 30 mm nerve defect.

MATERIALS AND METHODS

P-LA-CL Tubes

A copolymer (P-LA-CL) was synthesized by bulk ring-opening copolymerization of L-lactide/ ϵ -caprolactone at 190 °C for 5 hours in vacuum using stannous octoate as the catalyst.¹⁹ The selected mole ratio of L-lactide/ ϵ -caprolactone was 75:25, which exhibited an appropriate biodegradability.²⁰ All polymerization products were purified by precipitation from a methylene chloride solution in methanol and then dried under reduced pressure. This tube was nonporous and flexible, and its translucency facilitated the assessment of whether air was contained inside it when injecting a solution containing uBMSCs into its lumen (Fig. 1). The tube had an internal diameter of 3 mm and a wall thickness of 0.5 mm.

Animals

Eight female beagle dogs (1 year of age; 10–13 kg of body weight) were used in this study. Each animal was acclimatized before the surgical procedures, housed in a separate cage, and given standard dog food and water three times a day. All experiments were performed in accordance with the guidelines of the Animal Research Committee of Kyoto University.

Preparation and Characterization of BMSCs

Under general anesthesia (intramuscular injection of 0.08 mL/kg of medetomidine for induction, with inhalation of isoflurane (1.5–2.0%) in oxygen for maintenance) and in the prone position, the bone marrow was aspirated from the ilium using a general bone marrow biopsy technique. Briefly, a sterilized 13-gauge Jamshidi needle (Cardinal Health, McGaw Park) was used to aspirate 10 mL of bone marrow into a syringe containing 5 mL of heparinized (1,000 U/mL) saline solution.

The solution collected, which contained bone marrow, was centrifuged for 5 minutes at 300g and collected as a pellet. Marrow cells were then resuspended in 10 mL of 10% FBS–PBS (FBS: Invitrogen, Carlsbad; PBS: Invitrogen). To obtain BMSC-enriched nucleated cells, density separation (1.077 g/mL) was performed using Lymphoprep (Axis-Shield, Oslo, Norway). A suspension of marrow cells in FBS–PBS solution (10 mL) was carefully layered onto 5 mL of Lymphoprep. Separation was achieved by centrifugation at 800g for 30 minutes at room temperature. The nucleated cells collected from the PBS solution/Lymphoprep interface were then washed in PBS and transferred into 225 cm² cell culture flasks containing 50 mL of mesenchymal cell growth medium consisting of Dulbecco's Modified Eagle's Medium (Invitrogen), 10% FBS, and 1% penicillin–streptomycin. Cells were incubated at 37 °C in a humidified 5% CO₂ incubator. Nonadherent cells were removed after 48 hour.

Thereafter, the culture medium was replaced with fresh mesenchymal cell growth medium every 3 days. The cells were allowed to grow to 80–85% confluence, at which point the cultures were treated with trypsin–EDTA solution (0.25% trypsin and 0.02% EDTA; Sigma-Aldrich, St. Louis, MO) and were then harvested and diluted to 1:4 per passage for further expansion. Cells at passage 2 were used in experiments.²¹

For characterization, BMSCs at passage 2 were incubated with CD90-FITC, CD29-FITC, CD11b-PE, and CD34-PE (Sigma-Aldrich) in the dark at 4 °C for 30 minutes, followed by phenotypic analysis using flow cytometry (BD FACS Canto II; Becton Dickinson San Jose, CA) (data not shown).¹⁰

Nerve Defect Model and Experimental Design

The right forelimb of one dog and both forelimbs of the other seven dogs were subjected to surgery in this experiment (total, 15 forelimbs). The right forelimbs were reconstructed with the uBMSC-laden VCT (conduit group, $n = 8$) and the left forelimbs were bridged using autografts (autograft group, $n = 7$).

Animals were anesthetized in the lateral position, as described above, and a skin incision was made on the lower forelimb. The ulnar nerve was exposed by retracting the ulnar carpal flexor muscle medially and the digital flexors laterally (Fig. 1A). Accompanying vessels were dissected and separated from the ulnar nerve.

In the conduit group, an accompanying vessel (ulnar artery) was inserted into a 33 mm P-LA-CL tube through a longitudinal slit (Fig. 1B). A 25 mm ulnar nerve segment was removed and the proximal and distal ulnar nerve stumps were secured 1.5 mm into the tube, which created a 30 mm interstump gap in the VCT (Fig. 1C), followed by injection of 0.3 mL of mesenchymal cell growth medium containing 3×10^7 BMSCs into the tubular lumen using a 27G needle (Fig. 1D). The longitudinal slit of the tube was closed using several stitches of 5–0 nylon sutures.

In the autograft group, a 30 mm nerve segment was removed from the middle of the lower forelimb. The nerve segment was turned in the opposite direction and sutured in situ (reversed autograft).

The wound was closed with 4-0 nylon sutures in layers. Perioperative antibiotic management was carried out via postoperative subcutaneous administration of new quinolone (0.1 mL/kg) once a day for up to 7 days.

Postoperative evaluations were performed as follows. One dog in which the right ulnar nerve defect was repaired with the conduit underwent immunohistochemistry at 8 weeks. The remaining seven dogs (14 forelimbs) underwent electrophysiological studies under general anesthesia at 12 weeks. The animals were not sacrificed after the electrophysiological studies at 12 weeks. At 24

weeks, electrophysiological studies were carried out in the same manner as that described at 12 weeks, followed by histomorphometric studies of the distal regenerated nerves and the measurement of the wet weight of the reinnervated muscles.

Immunohistochemistry

To trace the transplanted cells, the BMSCs used in this experiment were prelabeled using chloromethylbenzamide dialkyl indocarbocyanine fluorescent dye (CM-DiI; Invitrogen) before implantation. At 8 weeks, the central part of the regenerated nerve in the uBMSC-laden VCT was removed. After fixation with 4% paraformaldehyde (PFA) and cryoprotection with 20% sucrose, cryostat transverse sections (thickness, 20 μ m) were prepared. After rinsing with PBS, antigen retrieval was performed using proteinase K (Sigma-Aldrich) at room temperature for 10 minutes. For blocking, donkey serum was added onto the slides, followed by incubation at room temperature for 1 hour. The primary antibody was added and sections were incubated at 4 °C for 24 hour. The primary antibodies used were a rabbit polyclonal antigial fibrillary acidic protein (GFAP) antibody (1:200, Millipore, Billerica, MA) and a rabbit polyclonal anti-S100 protein (S-100) antibody (1:500, Dako, Carpinteria, CA). Slides were then washed with PBS and incubated with the secondary antibody [donkey anti-rabbit IgG (H + L) whole antibody (1:200, CFTM488 (for GFAP), and CFTM633 (for S100) fluorescent reagents; Biotium, Richmond, CA)] at room temperature for 1 hour. After further PBS washing, cover slips were mounted onto the slides using bicarbonate-buffered glycerol (pH 8.6) and the slides were viewed using confocal microscopy (Nikon D-Eclipse C1 confocal microscope; Nikon, Tokyo, Japan). Negative controls included omission of primary or secondary antibodies on parallel sections (data not shown). To assess the phenotype of the uBMSCs injected, more than six random high-powered fields/section were photographed, taking 10 transverse sections stained with GFAP and S100, respectively. The percentage of cells in which CM-DiI was colocalized with glial markers (GFAP and S100) among CM-DiI-positive cells was calculated per section ($n = 10$ for GFAP and S100, respectively).

Electrophysiological Studies

At 12 and 24 weeks, bilateral ulnar nerves were exposed under general anesthesia and stimulated 3 cm distal to the elbow joint (S1) and 2 cm proximal to the wrist joint (S2) using a bipolar silver electrode. A pair of plate electrodes was fixed over the prominence of the muscles at the base of the most ulnar digit (hypotenar muscles) to check for the presence of compound muscle action potentials (CMAPs) in the muscles. The amplitude (peak to peak) of the CMAPs that were evoked in the

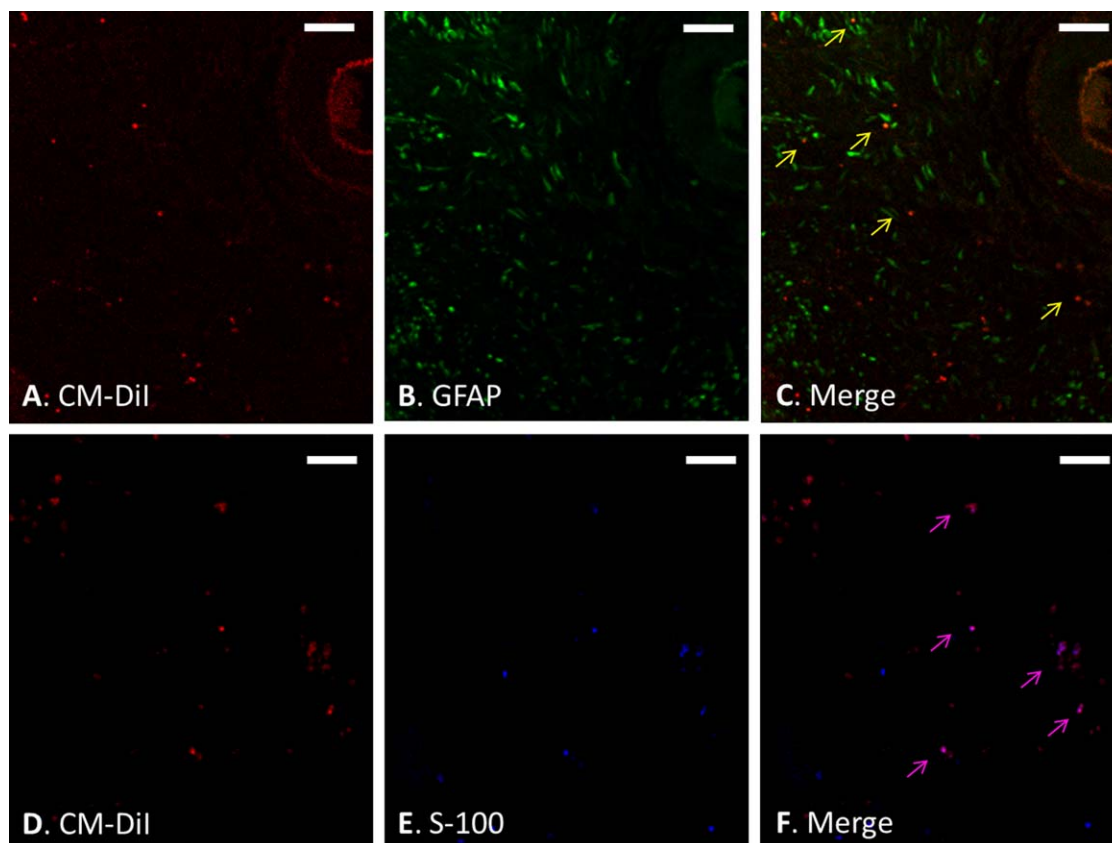


Figure 2. Expression of glial markers on implanted uBMSCs. Fluorescent microscopy revealed that uBMSCs, which were stained with CM-Dil, survived in the conduit and some of them (arrows) were immunopositive for Schwann cell markers [GFAP (C) and S-100 (F)]. Scale bar, 100 μ m.

hypothenar muscles with the supramaximal electric stimulation from S1 was measured. The distance between S1 and S2 was measured to calculate the motor nerve conduction velocity (MNCV).

Histological and Morphometric Studies

At 24 weeks, the tubes and their adjacent nerve portions were removed, fixed in 1% glutaraldehyde and 1.44% PFA, postfixed in 1% osmic acid, and embedded in epoxy resin. We prepared transverse sections (1 μ m thick) of the mid-portion of the conduit and of the regenerated nerves about 1.5 cm distal to the distal neurorrhaphy. The sections were stained with 0.5% (w/v) toluidine blue solution and examined by light microscopy (ECLIPSE 80i, Nikon, Tokyo, Japan). The counting of the total myelinated axon number and the measurement of the myelinated axon diameter were performed using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD) for morphometric analysis, as reported in our previous studies.^{7,18,22} Briefly, the entire neural area (*a*) of each specimen was calculated on an image. Six or seven fields were chosen at random so that the area analyzed would represent >20% of the entire neural area of each specimen. The number of myelinated

axons, the neural area, and the shortest diameter of each myelinated axon were calculated for each field at a final magnification of 400 $\times$. The number of myelinated axons (*b*) and neural areas (*c*) from six or seven fields were summed. The total number of myelinated axons in each specimen was estimated as $b \times (a/c)$. The mean myelinated axon diameter was expressed as the average value of the shortest diameter of all myelinated axons in the six or seven fields evaluated. Ultrathin sections of the same tissues stained with uranyl acetate and lead citrate were examined using transmission electron microscopy (TEM; Model H-7000; Hitachi High-Technologies, Tokyo, Japan).

Wet Weight of the Hypothenar Muscle

At 24 weeks, bilateral hypothenar muscles were dissected cleanly and detached from the bone at their origin and insertion, and were weighed immediately using a digital scale.

Statistical Analyses

Data are presented as means and standard deviations. Data from the electrophysiological, morphometric, and wet muscle weight measurements were analyzed using

Table 1. Summary of Results in Evaluations

	Conduit group (<i>n</i> = 7)	Autograft group (<i>n</i> = 7)	<i>P</i> values
12w			
Electrophysiological studies			
Amplitude (mV)	0.4 ± 0.4	3.2 ± 2.5	0.03
MNCV (m/s)	18.9 ± 14.3	34.9 ± 12.1	0.04
24w			
Electrophysiological studies			
Amplitude (mV)	10.9 ± 7.3	25.3 ± 20.1	0.11
MNCV (m/s)	23.5 ± 8.7	31.6 ± 20.0	0.35
Morphometric studies			
Myelinated axon number	7032 ± 4188	7165 ± 1814	0.94
Myelinated axon diameter (μm)	1.73 ± 0.31	2.09 ± 0.39	0.09
Target muscle atrophy			
Wet weight of hypothenar muscle (g)	1.02 ± 0.40	1.19 ± 0.26	0.36

the *t* test in the Excel 2010 software package (Microsoft, WA). Values of *P* < 0.05 were considered statistically significant.

RESULTS

Immunohistochemistry

CM-DiI-positive cells were observed, indicating that transplanted uBMSCs survived for 8 weeks in the VCT. 34 ± 18% and 35% ± 14% of CM-DiI-positive cells were also positive for GFAP and S100, respectively (Fig. 2), indicating that the uBMSCs were induced to differentiate into SC-like cells in the VCT.

Electrophysiological Studies

Twelve weeks after repair, M waves with a small amplitude were recorded on the hypothenar muscles in both groups, although no M wave was detected in one dog in the conduit group. The CMAP amplitude and MNCV of this dog at 12 weeks were considered as zero and were not excluded from the evaluation. Twenty-four weeks after repair, M waves were recorded in all dogs in both groups. At 12 weeks, the CMAP amplitude and MNCV in the conduit group were significantly lower than those in the autograft group (0.4 ± 0.4 mV vs. 3.2 ± 2.5 mV; *P* = 0.03 and 18.9 ± 14.3 m/s vs. 34.9 ± 12.1 m/s; *P* = 0.04, respectively). At 24 weeks, although the conduit group had a smaller CMAP amplitude and slower MNCV than the autograft group, we found no significant differences between two groups (10.9 ± 7.3 mV vs. 25.3 ± 20.1 mV; *P* = 0.11 and 23.5 ± 8.7 m/s vs. 31.6 ± 20.0 m/s; *P* = 0.35, respectively; Table 1).

Histological and Morphometric Studies

Twenty-four weeks after surgery, semithin transverse sections (toluidine blue staining) of the midportion of the regenerated nerve in the conduit group showed frag-

mented walls in the P-LA-CL tubes, indicating the degradation of the tube. Well-myelinated axons were also observed in the sections (Fig. 3). In both groups, many well-myelinated axons were found in semithin transverse sections of the regenerated nerve 1.5 cm distal to the distal neurotomy and remyelinated axons with proper myelin sheaths were observed under TEM (Fig. 4). The results of the morphometric analysis of the transverse sections of the regenerated nerve 1.5 cm distal to the distal neurotomy that was carried out 24 weeks after surgery are given in Table 1. Although morphometric measurements at 24 weeks found a smaller number and shorter diameter of myelinated axons in the conduit group than those in the autograft group, there were no significant differences between both groups (7032 ± 4188 vs. 7165 ± 1814; *P* = 0.94 and 1.73 ± 0.31 μm vs. 2.09 ± 0.39 μm; *P* = 0.09, respectively).

Wet Weight of the Hypothenar Muscle

At 24 weeks, although the wet weight of the hypothenar muscles in the conduit group (1.02 ± 0.40 g) was less than that in the autograft group (1.19 ± 0.26 g), the conduit group did not differ significantly from the autograft group (*P* = 0.36; Table 1).

DISCUSSION

This study showed that the nerve regeneration through the vessel-containing conduit supplemented with uBMSCs was inferior to that achieved through a reversed autologous nerve graft up to 24 weeks after repair. However, at 24 weeks there were no significant differences between the conduit group and the autograft group regarding electrophysiological measurements, morphometric measurements, or wet weight measurements of the reinnervated muscle.

Although reversed autografts served as controls in this study, cabled autografts (a bundle of thin sensory

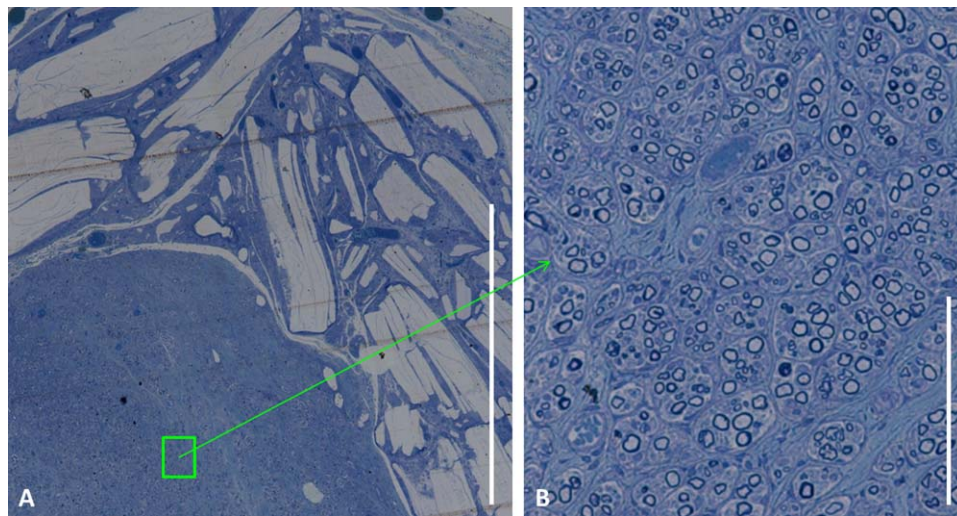


Figure 3. Midportion of the conduit. **A:** The conduit wall was degraded into fragments 24 weeks after implantation. Scale bar, 1 mm. **B:** A large number of regenerated axons were observed in the conduit. Scale bar, 100 μ m.

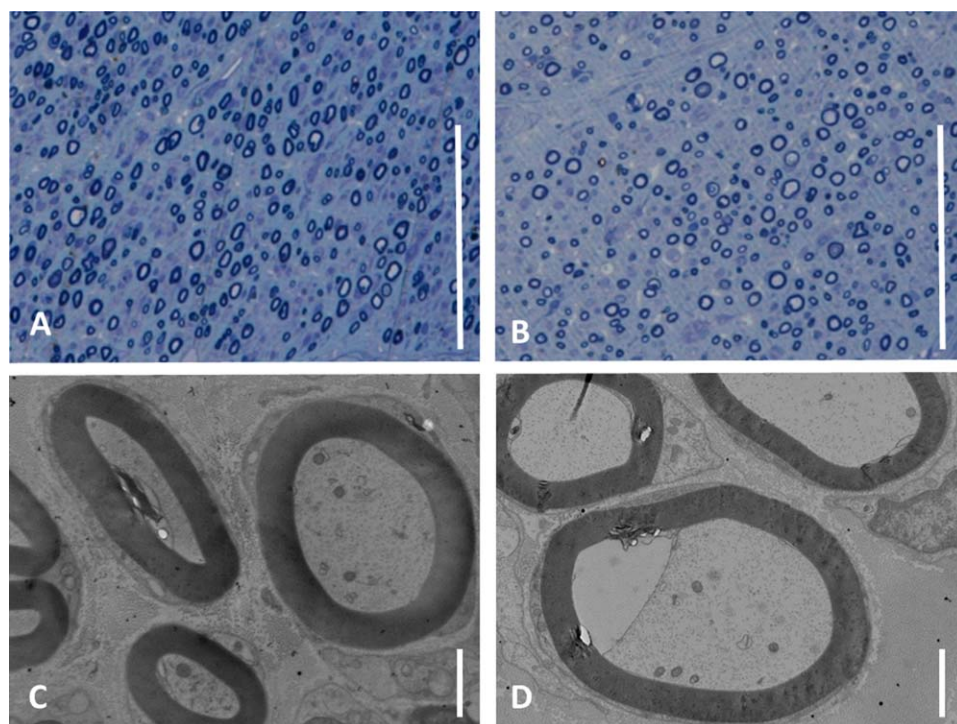


Figure 4. Distal ulnar nerves (1.5 cm distal to the conduit and the autograft). Semithin sections showed that the number of the myelinated axons in the conduit group (**A**) was similar to that in the autograft group (**B**). Scale bar, 100 μ m. The regenerated axon with proper myelin sheath was observed by electron microscopy in both groups, although the diameter of the myelinated axons in the conduit group (**C**) tended to be smaller than that in the autograft group (**D**). Scale bar, 2 μ m.

nerve) are usually used in actual clinical settings. It was reported that nerve regeneration achieved through reversed autografts was superior to that achieved via cabled autografts,²³ and that the use of motor or mixed nerve grafts, rather than sensory nerve grafts, optimized nerve regeneration across gaps in mixed nerves.²⁴ These

findings suggest that, if the uBMSC-laden VCTs had been compared with cabled nerve autografts, the differences in the outcomes of the electrophysiological, morphometric, and wet muscle weight measurements would have been smaller than those obtained via the comparison of the uBMSC-laden VCT and in situ reversed autografts.

In previous reports about peripheral nerve regeneration using canine models, nerve deficits were created in sciatic nerves,^{10,25} peroneal nerves,²⁶ tibial nerves,²⁷ and ulnar nerves.²² In this study, we used ulnar nerves because ulnar nerve palsy causes less disability in dogs, which allowed us to use both forelimbs simultaneously and to reduce the number of dogs in this study.

Because physical nerve guidance by a nerve tube may not be sufficient to achieve optimal regeneration across a long nerve gap, several modifications were investigated using canine models with more than 30 mm nerve gaps. Wang et al.²⁵ repaired 30 mm gaps in canine sciatic nerves using a chitosan tube with polyglycolic acid filaments. Okamoto et al.²⁶ reported canine peroneal nerve regeneration across a 30 mm defect through the collagen conduit packed with collagen fibers. These conduits had internal frameworks as scaffolds. Ding et al.¹⁰ bridged 50 mm gaps in canine sciatic nerves with the BMSC-laden chitosan tube inserted with poly(lactide-co-glycolic acid) filaments. The Ding's conduit had supportive cells and scaffolds in the lumen of the tube. In this study, 30 mm gaps in canine ulnar nerves were bridged with the poly L-lactide/ε-caprolactone tube with implantation of a vascular bundle and BMSCs. Our conduit is unique in that it contained vascularity and supportive cells.

It is widely accepted that cells, scaffolds, and growth factors are essential for nerve regeneration.²⁸ We believe that the vascularity of the regenerating nerve is another factor that is essential for successful nerve regeneration. Several experimental and clinical findings have indicated that new capillary formation is closely related to axonal elongation and regeneration.^{29,30} Recent research^{31,32} has revealed that the vascular endothelial growth factor (VEGF) enhances intraneural angiogenesis and nerve regeneration. However, for clinical application, the use of VEGF has several drawbacks including reduction of enzymatic activity in human bodies and the need for an exogenous carrying molecule.³² In addition, VEGF was also reported to contribute to the malignant progression of a variety of tumor cells.³³ The safety of VEGF is unclear when it is used in combination with implantation of uBMSCs, which have a potential risk of tumorigenicity.³⁴ Thus, we inserted a vascular bundle into a chamber supplemented with uBMSCs to enhance angiogenesis in the lumen of the tube.

The role of uBMSCs in the process of nerve regeneration in a VCT is not well known. Yamakawa et al.⁷ implanted male rat-derived uBMSCs into VCTs to bridge sciatic nerve defects in female rats and investigated the capacity of differentiation of rat uBMSCs in VCTs using immunohistochemistry and fluorescent *in situ* hybridization. The authors concluded that some of the implanted uBMSCs differentiated into SC-like cells in VCTs. In the

current study, we performed an immunohistochemical study on one dog to investigate if the uBMSCs implanted in VCTs differentiated into SC-like cells and, if so, to calculate the percentage of the implanted uBMSCs that differentiated to SC-like cells in the conduit. Our results revealed that about 35% of the implanted canine uBMSCs differentiated into SC-like cells in a VCT at 8 weeks, which could be one of the causes of the promotion of axonal regeneration observed in our artificial nerve conduits.

Some researchers recommend the implantation of BMSCs having differentiated closer to SC-like cells (dBMSCs) into the tubular lumen, rather than uBMSCs. We believe that uBMSCs are more suitable for incorporation into a neural scaffold. First, the process of nerve regeneration requires not only SCs, but also other types of cells, such as macrophages³⁵ or fibroblasts.³⁶ uBMSCs have a potential to differentiate into a greater variety of cells that are needed for nerve regeneration than do dBMSCs. Second, uBMSCs are reported to have a stronger ability to recognize the damaged site and to integrate into the site than do dBMSCs,^{37,38} which will improve the efficacy of cell therapy. Finally, *in-vivo* culture of uBMSCs is less time consuming, which is preferable for clinical application.

There are several possible obstacles that should be overcome before the clinical application of our conduit. It is sometimes difficult to find vascular pedicles, which are suitable for implantation, at nerve-repair sites in some clinical setting. When no artery is available around the nerve repair site, a bypass vein graft that connects neighboring arteries can be replaced with the vessels that are inserted into the tube. Subcutaneous veins can be harvested anywhere throughout the body without severe donor-site morbidity. Another obstacle is the tumorigenicity of uBMSCs. The mechanism via which BMSCs are transformed into malignant cells is related to chromosomal abnormalities. Chromosomal aberrations are less frequent in human BMSCs with a low passage number.³⁴ The human BMSCs obtained without *ex vivo* culture or after a low number of passages should be used clinically. Using a device that separates BMSCs from bone marrow aspiration efficiently,³⁹ uBMSCs can be obtained without *ex vivo* cell culture and transplanted at that spot. This may be a possible method to reduce the tumorigenicity of BMSCs. Further studies will be needed to promote the safety of the clinical application of BMSCs.

The limitations of this study included the use of a small sample of dogs. We were not allowed to perform an experiment accompanied by neuralgic pain on a large number of dogs, from the animal protection point of view. Therefore, we reduced the number of dogs used in this study, which resulted in the loss of power in the statistical analysis.

In conclusion, the implantation of undifferentiated bone marrow stromal cells and of a vascular bundle into a nerve conduit is considered to be a strategy that may lead to the production of artificial nerve conduits that can be an alternative to autologous nerve grafts. However, uBMSC-laden VCTs need some modifications, such as additional implantation of some materials as scaffolds or administration of some drugs as growth factors into the conduit.

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