Journal of Cranio-Maxillofacial Surgery (2006) **34**, 50–56

doi:10.1016/j.jcms.2005.07.011, available online at http://www.sciencedirect.com

Nerve regeneration with the use of a poly(L-lactide-*co*-glycolic acid)-coated collagen tube filled with collagen gel

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SUMMARY. Aim: **The aim of this study was to develop a novel artificial nerve conduit and to evaluate its efficiency based on the promotion of peripheral nerve regeneration in rabbits.** Material and methods: **The nerve conduit was made of a poly (L-lactide-*co*-glycolic acid)-coated collagen tube filled with collagen gel. The conduits were implanted into a 15 mm gap in the peroneal nerves of five rabbits. On the contralateral side, the defects were bridged with collagen-filled vein grafts.** Results: **Twelve weeks post-operatively nerve regeneration was superior to the vein graft in the PLGA-coated collagen tube, both morphologically and electrophysiologically.** Conclusion: **The results indicate the superiority of the PLGA-coated collagen tube over vein grafts. Furthermore, they show that entubulation repair with this type of tube can support nerve regeneration over a nerve gap distance of at least 15 mm.** © **2005 European Association for Cranio-Maxillofacial Surgery**

Keywords: **nerve regeneration; PLGA; nerve conduit; collagen; tubulated conduit**

INTRODUCTION

Since *Collin* and *Donoff* (1984) reported on nerve regeneration using a collagen tube, other investigators have also observed that collagen tubes can promote nerve regeneration (*Archibald* et al., 1991; *Laquerriere* et al., 1993). However, previous studies have shown that collagen tubes fail to bridge nerve defects greater than 15 mm, since a long collagen tube can break and its lumen could collapse due to movement (*Ansselin* et al., 1997; *Yoshii* and *Oka*, 2001). In this study, a collagen tube was coated with poly(L-lactide-*co*-glycolic acid) (PLGA) to enhance its structural integrity and elasticity. Although PLGA has been proposed as a nerve conduit material (*Evans* et al., 2000; *Bryan* et al., 2003; *Bini* et al., 2004), the influence of a collagen/PLGA composite on nerve regeneration has not been established.

A large variety of substances have been used to fill the tubular nerve guides in an attempt to bridge larger nerve defects. *Williams* et al. (1987) noted that silicone tubes filled with dialyzed plasma resulted in a three to fivefold increase in functional restitution compared with silicone tubes filled with phosphate-buffered saline. *Madison* et al. (1985) filled silicone tubes with collagen or laminin-containing gel. It is generally accepted that pre-filling the tubular nerve guides with gel supports axonal growth (*Madison* et al., 1988; *Labrador* et al., 1998). *Rosen* et al. (1990) suggested that collagen is an ideal matrix for the addition of both the cellular and non-cellular components necessary for the repair of nerve gaps. One interpretation of these observations is that the gel plays a dual role, serving both as the extracellular matrix, exerting a strong influence over nerve regeneration, and as a strut to physically bolster the conduit lumen. This hypothesis was tested in this study by adding collagen gel to the PLGA-coated collagen tube and evaluating its efficiency to promote peripheral nerve regeneration. In view of the fact that the autogenous vein can serve as a successful tubular conduit for nerve regeneration (*Stauch* et al., 1996), this study evaluated PLGA-coated collagen tubes filled with collagen gel versus autogenous vein grafts filled with collagen gel for nerve regeneration of 15 mm rabbit peroneal nerve defects.

MATERIAL AND METHODS

Preparation of the PLGA-coated collagen tube

A bioresorbable collagen membrane for guided tissue regeneration (CollaTape®, Integra LifeSciences Corporation, Plainsboro, NJ, USA) was manually rolled around a Teflon mandrel to form a tube with a length of 20 mm and an inner diameter of 1 mm. In order to coat the outer surface of the prepared tube with PLGA, it was rotated at 20 rpm using a stirrer, and a PLGA polymer solution was sprayed over the rotating tube for 5 s with compressed nitrogen gas at a pressure of 5 bars. The PLGA polymer solution was prepared by dissolving 0.3% PLGA [75(lactide):25 (glycolide); Sigma, MO, USA] in methylene chloride (MC; Duksan, Kyoungkido, South Korea).

Grafting procedure

In this study five male New Zealand White rabbits were used, each weighing approximately 3.0 kg. Animal selection and management, surgical protocol, and preparation were approved by the Animal Care and Use Committee, Yonsei Medical Centre, Seoul, Korea. The rabbits were anaesthetized with an intramuscular injection of ketamine (5 mg/kg) and xylazine (2 mg/kg). After exposing the jugular vein, a 25 mm segment of vein was resected. Free from tension, the vein graft retracted to approximately 18 mm. In each animal, two peroneal nerves were exposed and divided, and nerve gaps of 15 mm were created bilaterally. On one side, the nerve ends were placed into the 17 mm PLGA-coated collagen tube. On the contralateral side, the nerve ends were inserted into the 17 mm vein graft with the vein positioned in reverse fashion to prevent any potential branching of the axons through the side branches of the vein during nerve regeneration. Both the proximal and distal nerve stumps were inserted to a depth of 1 mm and were each held in place with three sutures of 10-0 nylon. The final length of the nerve gap was approximately 15 mm. Once the sutures were in place, 150 µl of collagen (type I collagen, Nitta Co., Japan) was injected into both the tube and the vein. The collagen had been pH adjusted to 7.5 and maintained in liquid form at 4 °C. When injected into the vein or the tube, the collagen formed a gel at body temperature.

Electromyographic recordings

Just before the specimens were harvested, an electromyographic recording was taken with a Nicolet Viking Quest System (Nicolet Biomedical Inc., Madison, WI, USA) under general anaesthesia (5 mg/kg ketamine and 2 mg/kg xylazine i.m.). The peroneal nerve was exposed proximal to the nerve guide and stimulated with wire electrodes. During recording, the body temperature was maintained at 26–27 °C with a heating lamp. A recording needle electrode was placed in the anterior tibialis muscle to record the compound muscle action potentials (CMAP) and the earliest latency and peak-to-peak amplitude.

Examination of regenerated nerve

The animals were sacrificed 12 weeks postoperatively. The specimens were harvested at the midpoint of the guide and were fixed with 2.5% glutaraldehyde. They were then embedded in Epon resin using a standard method, cut cross-sectionally to a thickness of 0.5 µm, and stained with toluidine blue. Image analysis software (IBAS, Contron, Erching, Germany) was used by an anatomist who had no knowledge of the methods used in the study to measure the total number and diameters of the myelinated fibres. The average diameters of the myelinated fibres was obtained by measuring a minimum of 100 in each nerve. The quantitative results obtained were tested for statistical differences using the paired Student's *t* test.

RESULTS

The PLGA-coated collagen tube showed well-distributed 4 µm-sized pores (Fig. 1) and had a wall thickness of 0.2 mm (Fig. 2). A minor degree of

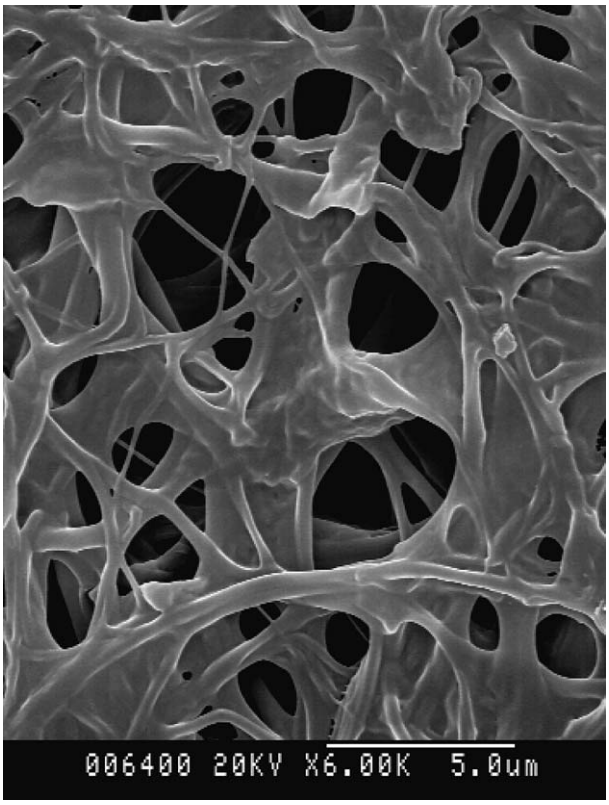

Fig. 1 – SEM micrographs of surface morphology of the PLGA-coated collagen tube.

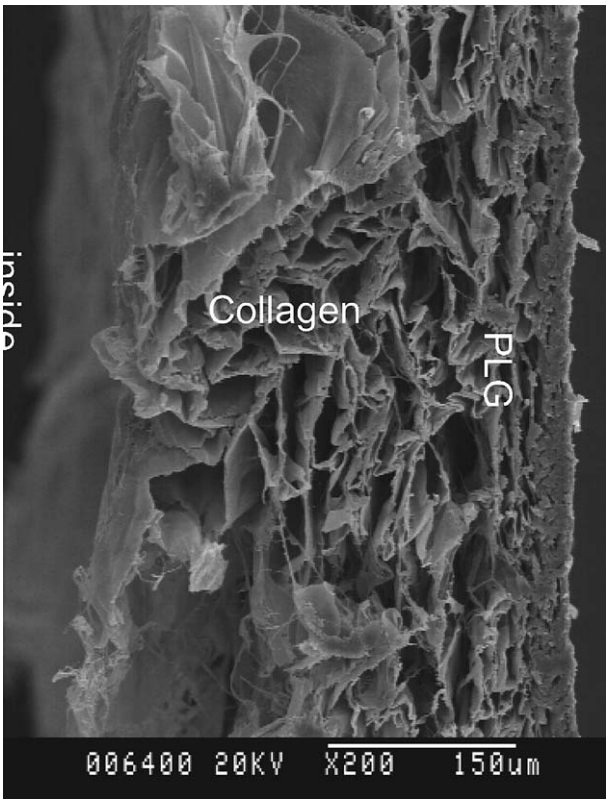

Fig. 2 – SEM micrographs of the cross-section of the PLGA-coated collagen tube.

swelling caused by water uptake was observed, but the lumina of the tubes were maintained at a constant size. The wet tubes showed an elastic modulus of 0.81 MPa, which was similar to that of nerve tissue.

After implantation to repair the defects of the rabbit peroneal nerves, the tubes were completely resorbed by 12 weeks, leaving no residue (Fig. 3). During that time, nerve regeneration was successfully accomplished. No inflammatory reactions could be identified and no neuromas were apparent. Qualitatively, no gross histological differences were found in the degrees of vascularity or extent of connective tissue between the PLGA-coated collagen tube and vein graft sides. The toluidine blue-stained semi-thin sections revealed bundles of nerve fibres encircled by perineural-like sheaths on both sides (Fig. 4).

The number and diameter of the myelinated fibres on each side at 12 weeks postoperatively are shown in Table 1. Axon formation was clearly richer on the PLGA-coated collagen tube side (Fig. 5). The number of nerve fibres on the PLGA-coated collagen tube side was significantly greater than on the vein graft side. The mean diameter of the nerve fibre on the PLGA-coated collagen tube side (5.4 μm) was greater than on the vein graft side (mean 4.8 μm). However, this difference was not statistically different.

Statistically significant differences were found when the electrophysiologic parameters (latency and amplitude of the compound muscle action potentials) of the PLGA-coated collagen tube side were compared with those of the vein graft side (Table 1). The latency was appreciably shortened and the amplitude was increased on the PLGA-coated collagen tube side (Fig. 6).

DISCUSSION

The results showed that when the PLGA-coated collagen tubes filled with collagen gel were implanted into the nerve gaps, the number of myelinated fibres was significantly higher than those of the vein grafts filled with collagen gel, suggesting an increase in the total amount of regenerated nerve tissue from the use of PLGA-coated collagen tubes. These histological findings were also consistent with the electrophysiological findings. The increase in nerve regeneration in the PLGA-coated collagen conduit might have been due to the absence of elongation and conduit collapse. The conduit itself had the necessary strength to withstand the muscular forces surrounding it, having high flexibility. In contrast, elongation was observed in the case of the vein grafts, and is believed to be due to movement of the animal.

PLGA-coated collagen conduits have several advantages. They can be bent to an angle of up to 180° and brought back to their original shape, an ability that is necessary for adaptation inside a living system. Moreover, they have a thin wall and a highly porous structure, which are important determinants for nutrient transport into the conduit (*Sharkawy* et al., 1998; *Fansa* et al., 2001). A further advantage of this type of conduit is that it can be easily fabricated to any required length and diameter. The method of fabrication in this study does not involve suturing, heating or chemical reactions for the purpose of tubulation. In addition, the fibrous structure of the conduit facilitates suturing to the proximal and distal nerve stumps. These properties make PLGA-coated collagen tubes highly suitable for use in artificial nerve scaffolds.

Previous studies have shown that regenerating axons were able to bridge tubular nerve conduits implanted into the rat sciatic nerve if the gap was 10 mm or less, but failed in most cases with 15 mm gaps (*Ceballos* et al., 1999; *Evans* et al., 2002). In an attempt to increase the efficacy of the tubes, many researchers have added neurotrophic factors or Schwann cells within the confines of the conduits (*Bryan* et al., 1992; *Bradshaw* et al., 1994; *Foidart-Dessalle* et al., 1997; *Houweling* et al., 1998; *Pu* et al., 1999). This study succeeded in repairing a 15 mm gap in the rabbit peroneal nerve using a PLGA-coated collagen tube filled with collagen gel, in the absence of these factors or cells. In order to achieve successful regeneration across longer gaps using these conduits, it might be necessary to add these factors to the

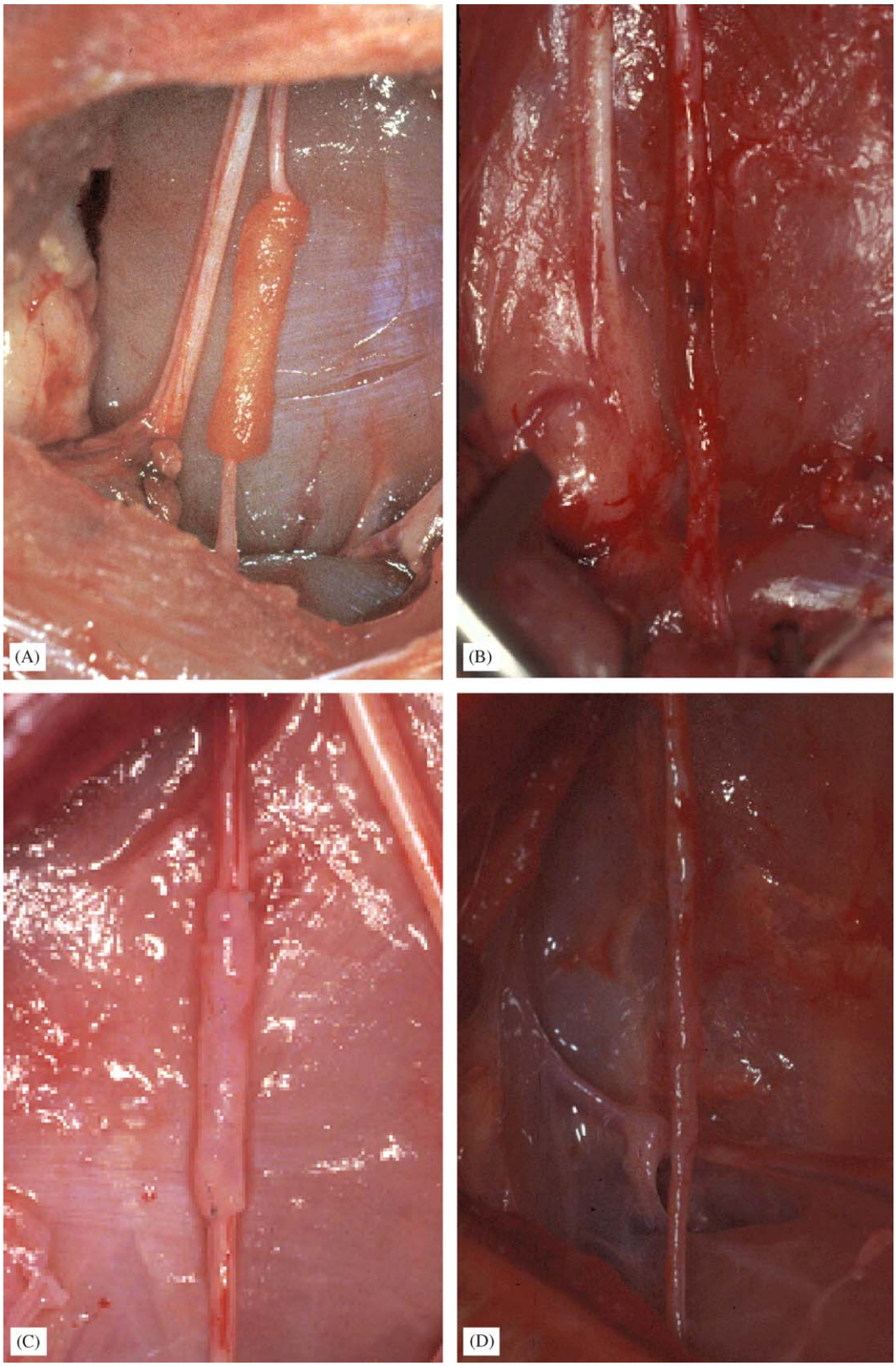

Fig. 3 – Peroneal nerve repairs. (A) PLGA-coated collagen tube graft immediately after grafting, (B) PLGA-coated collagen tube graft 12 weeks after grafting. (C) Vein graft immediately after grafting and (D) vein graft 12 weeks after grafting.

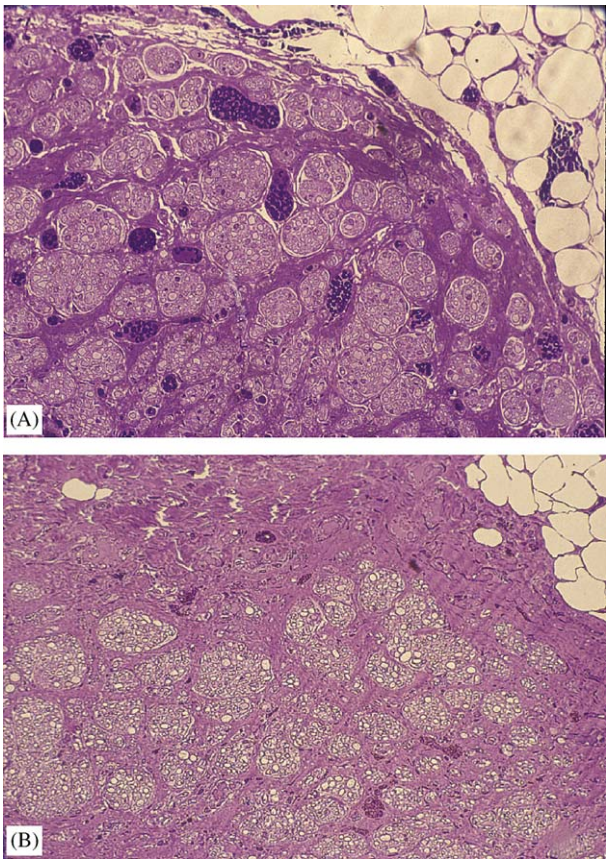

Fig. 4 – Regenerated portions of nerve. (A) PLGA-coated collagen tube side and (B) vein graft side. (Toluidine blue stain, × 200)

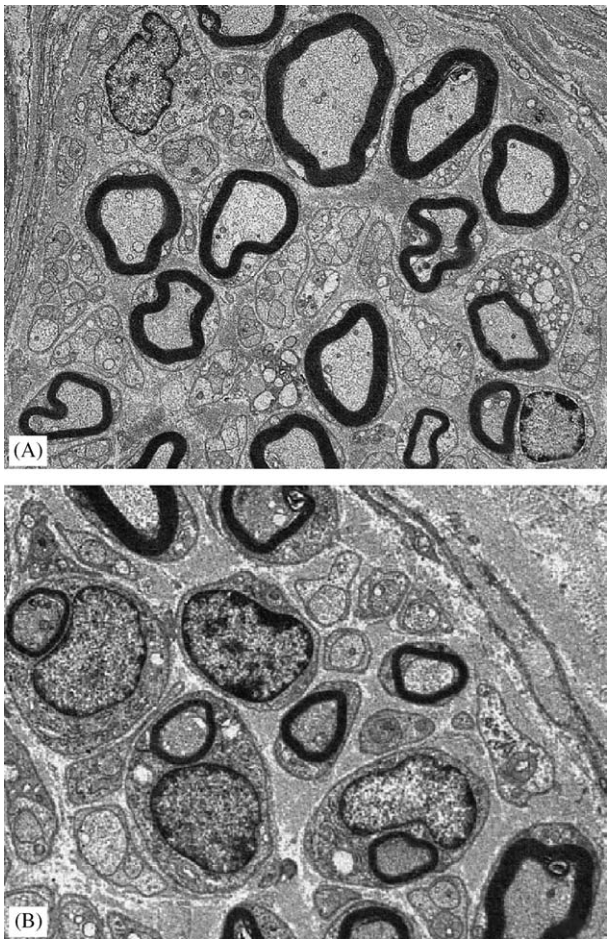

Fig. 5 – Electron micrographs of regenerated nerves 12 weeks after implantation. (A) PLGA-coated collagen tube side and (B) vein graft side (magnification X4000)

Table 1 – Total numbers and diameters of myelinated fibres and compound muscle action potentials on the PLGA-coated collagen tube and on the vein graft sides

	PLGA-coated collagen tube side ($n = 5$)	Vein graft side ($n = 5$)
Number of fibres	2724 ± 421*	1196 ± 207*
Diameter of fibres (μm)	5.4 ± 1.2	4.8 ± 0.8
Latency (ms)	1.6 ± 0.5*	2.6 ± 0.7*
Amplitude (mV)	27.7 ± 7.9*	18.9 ± 6.5*

Values shown are means ± standard deviation.
*$p < 0.05$.

PLGA-coated collagen conduit. However, the practical application of these factors depends on the carrier system used to deliver them to the site of repair, as they need to be released continuously over a sufficiently long period to induce nerve regeneration. *Rosen* et al. (1990) suggested that collagen is an ideal matrix for the addition of both the cellular and non-cellular components necessary for the repair of long nerve gaps, as collagen can act as a growth medium within a conduit. Further studies are needed on the effects of Schwann cells or neurotrophic factors added within the PLGA-coated collagen conduits utilized in the present study to determine whether they can effectively bridge longer gaps.

After nerve regeneration, it is not necessary for the guide tube to remain in the tissue. Therefore, the guide tube should eventually degrade at the site of implantation after serving its purpose. *Keilhoff* et al. (2003) reported that after 8 weeks, progressive resorption of the collagen conduits was evident. PLGA polymer is also known to degrade in a physiological environment (*Reed* and *Gliding*, 1981). In the present study, the PLGA-coated collagen tube showed complete degradation 12 weeks postoperatively, and it is conceivable that the biodegradation of the tubes was slow enough to maintain a stable support structure for the extended regeneration processes.

CONCLUSION

In this study, a PLGA-coated collagen nerve conduit filled with collagen gel promoted axonal regeneration

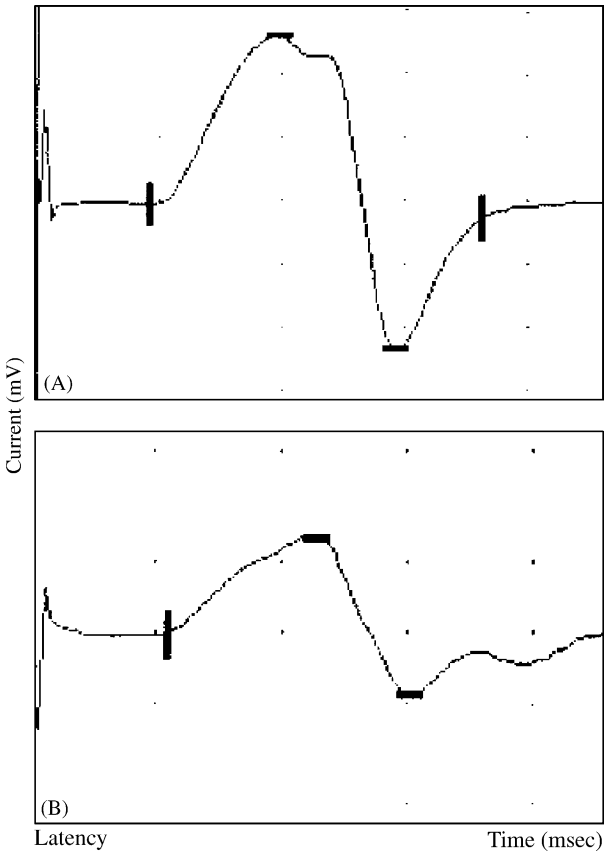

Fig. 6 – Electromyographic recordings of compound muscle action potentials (CMAP) 12 weeks after implantation. (A) PLGA-coated collagen tube side and (B) vein graft side.

over a 15-mm nerve gap with better efficacy than a collagen-filled vein graft. These results should encourage further investigation of the potential use of this type of tube in longer nerve gaps.

ACKNOWLEDGEMENTS

This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea to Choi, Byung-Ho (02-PJ1-PG1-CH07-0001).

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Paper received 26 May 2004
Accepted 13 July 2005