

Electrophysiological Study of Sciatic Nerve Regeneration Through Tubes Seeded with Schwann Cells

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A B S T R A C T

Background & Objective: Peripheral nerve injury is a common disorder and leads to permanent neurological defects. Schwann cells have been shown to have nerve repair after being transplanted in peripheral nerve injury. The aim of this study was to determine the beneficial effect of allograft Schwann cells on electrophysiological outcome after transection of the sciatic nerve in rats.

Materials & Methods: Twenty adult male Wistar rats (200-250 g) were used in this study and left sciatic nerve was cut 10 mm in all of them and randomly divided into two groups. Then, the experimental (n=10) and control (n=10) groups received silicon tube with Schwann cells and silicon tube without Schwann cells respectively. Electrophysiological studies were performed 8 weeks after transplantation.

Results: Electrophysiological study in experimental animals showed that amplitude of nerve action potential is higher and latency is less as compared to the control group ($p < 0.05$).

Conclusion: The combination of different strategies such as silicon tube and Schwann transplantation has a more effective role in nerve repair.

Introduction

Peripheral nerve injury (PNI) is a common disorder and leads to neurological defects in most cases (Aebischer, Guenard, & Brace, 1989). In contrast to the central nervous system (CNS), the peripheral nervous system has competence to regenerate injured axon

(Yin, Kemp, Yu, Wagstaj, & Frostick, 2001) but needs favorable environment (Yin et al., 2001). Various methods such as peripheral nerve allograft (Pollard, & Fitzpatrick, 1973), fibroblast growth factor (Aebischer, Salessiotis, & Winn, 1989) and bone marrow stromal cells (Mimura, Dezawa, Kanno, Sawada, & Yamamoto, 2004) have been used in attempts to improve nerve regeneration. Artificial tubes have been used to guide nerve regeneration (Terzis, Sun,

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& Thanos, 1997; Nakamura, Inada, Fukuda, Yoshitani, Nakada, Itoi, Kanemaru, Endo, & Shimizu, 2004). Recently, vein graft was used as a tubular pathway for the growing axon in the lesion site toward the distal stump of the nerve trunk (Chiu, Janecka, Krizek, Wolff, & Lovelace, 1982; Tang, 1995). In addition, transplantation of Schwann cells into the PNI is another existing strategy. It has been shown that the effect of the trophic factors is beneficial in axonal regeneration and functional recovery following nerve injury (Zhang, Campbell, Anderson, Martini, Schachner, & Lieberman 1995; Martini, Schachner & Brushart, 1994). Neurotrophic factors from Schwann cells uphold the survival of injured neurons (Takami, Oudega, Bates, Wood, & Kleitman, 2002) and produce various growth factors such as nerve growth factor (Heumann, Korching, Bandtlow, & Thoenen, 1987), brain-derived neurotrophic factor (Meyer, Matsuoka, Wetmore, Olson, & Thoenen, 1992), ciliary neurotrophic factor (DiStefano, Friedman, Radziejewski, Alexander, Boland, Schick, Lindsay & Wiegand, 1992; Rende, Granato, Lo Monaco, Zelano, & Toesca, 1991), and glial cell line-derived neurotrophic factor (Widenfalk, Lundström, Jubran, Brené, & Olson, 2001). They also promote macrophage infiltration to the injured nerve and make arrangements a substrate for axonal growth (Zhang, Campbell, Anderson, Martini, Schachner, & Lieberman, 1995). Continuity of the Schwann cell tube with the extracellular matrix across the injury site promotes axonal regeneration (Aldskogius, Molander, Persson, & Thomander, 1987).

Therefore SCs implantation needs to be combined with other interventions to provide permissive environment to growth of axon from the lesion site. Thus, we have asked whether silicon tube Seeded with Schwann cells can regenerate axon and restore electrophysiological properties of the remyelinated axons.

Materials & Methods

Animals & Groups

All operative procedure and post-operative care of the experimental animals were carried out according to the guidelines of the Iranian Council for the Use and Care of Animals and were approved by the Animal Research Ethical Committee of Iran Medical University.

Female Wistar rats (n=20, 200-250g) were prepared from the Razi Institute animal facility. Animals were randomly divided into two groups, experimental group (n=10) that received silicon tube seeded with Schwann, and control group (n=10) that received silicon tube without Schwann cells.

Schwann Cell Cultures

Schwann cells from the sciatic nerve prepared according to a technique modified from that of Morrissey et al 1991 (Guenard, Kleitman, Morrissey, Bunge, Aebischer, 1992). Sciatic nerves were transferred on vitrogen-coated (collagen, corporation, Palo Alto, CA) into Dulbecco's Modified Eagle's Medium (DMEM; Company) stripped of their epineurium, and chopped into pieces. Then the pieces were placed into DMEM with 10% fetal calf serum (FCS, GIBCO) and penicillin / streptomycin (1000 U/ml) every five day. The cells remaining in the explants were placed in Ca²⁺ and Mg²⁺-free Hanks' Balanced Salt Solution (HBSS) containing 0.3% trypsin (Sigma, St. Louis, MO), 0.1% collagenase (Sigma), and 0.1% hyaluronidase (Sigma). Arabinoside C (1 mM) (Sigma), were used to stop proliferation of fibroblasts for 2 days. After triturating and cultured in DMEM-FCS the culture medium was replaced with mitogenic medium containing DMEM, FCS, forskoline 2µl (Sigma) and pituitary extract 10 µl. Then Schwann cells cultured in mitogenic medium at 37°C with 5% CO₂. Schwann cells were confirmed with the use of S-100 staining. Then, they were labeled with 8–9 µl of DiI (170 mg/ml in DMSO and diluted 1:10 in saline) 1, 1'-dioctadecyl-3, 3, 3', 3'-tetramethylindocarbocyanin perchlorat (DiI) from Molecular Probes (Leiden, The Netherlands; cat. No, D-282). After transplantation, the labeled cell was searched using fluorescent microscopy (Olympus Ax70).

Surgery Procedures & Transplantation

Each rat was anesthetized via intraperitoneal injection of a combination of ketamine (80 mg/kg) and xylazine (10 mg/kg). Under an operating microscope from the same rats the left sciatic nerve was exposed and was transected by means of sharp microscissors from the point of emergence from the greater sciatic foramen and a 10-mm nerve segment was removed and transferred to sterile Petri dish for Schwann cells culture. The proximal and distal nerve stumps were placed in a silicon tube as a nerve guide (11 mm long), inserted 1.5 mm into it and sutured to the silicon tube with 8-0 monofilament nylon. Subsequently, Schwann cells were injected into the middle of silicon in the gap area. The experimental group (n = 10) received Dulbecco's cell culture medium (DMEM) supplemented with Schwann cells obtained at a final density of 1×10^5 cells/µL for each animal. The control group (n = 10) received DMEM without Schwann cells.

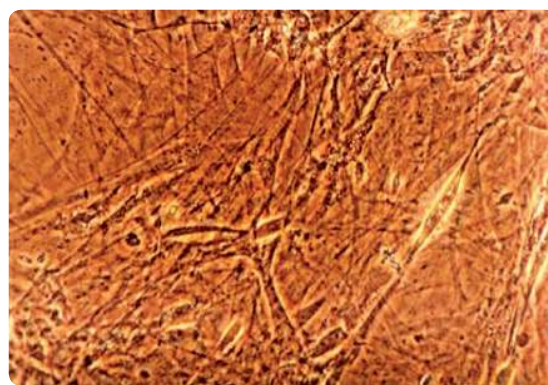
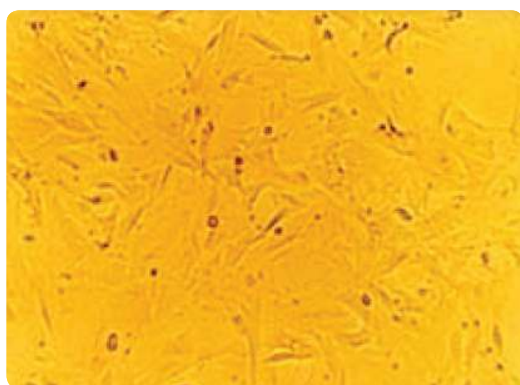
Electrophysiological study

After transplantation, electrophysiological recording was determined as reported previously (Mimura, Dezawa, Kanno, Sawada, Yamamoto, 2004). Animals anaesthetized again with intraperitoneal injection of a combination of ketamine (80 mg/kg) and xylazine (10 mg/kg), then the left sciatic nerve was exposed. Electric stimulation (duration of 0.04 ms, intensity of 2.7 mA) was applied to the proximal site of injury. Active recording cap electrode was inserted on gasrocenemius muscle and refer-

ence cap electrode inserted on knee joint. The ground electrode with stainless steel needle was inserted into the tail skin.

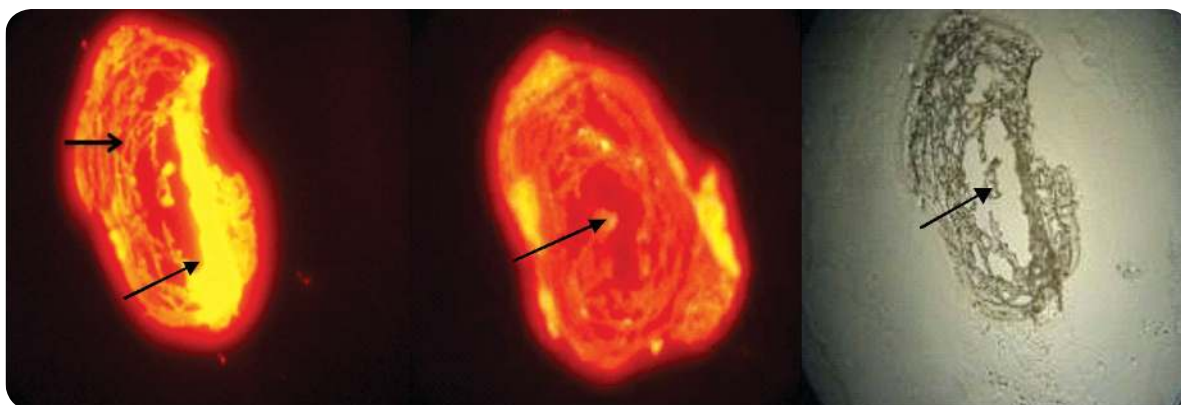
Results

Immunohistochemisry



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Figure 1: Schwann cells cultured in flask that contains DMEM & FBS (10%) in p3, after isolation of cells from sciatic nerve. Scale bar $\times 400$, olympus IX 70, Japan, BF filter (upper figure), olympus IX 70, Japan, ph1/phc filter (bottom figure)



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Figure 2: DiI labeled cells has seen with florescent microscope (Olympus Ax70) in the silicon tube two weeks after transplantation. Scale bar $\times 100$, section thickness = 70 micron. Arrows show Schwann cells adhered to wall of silicon tube and in center of tube.

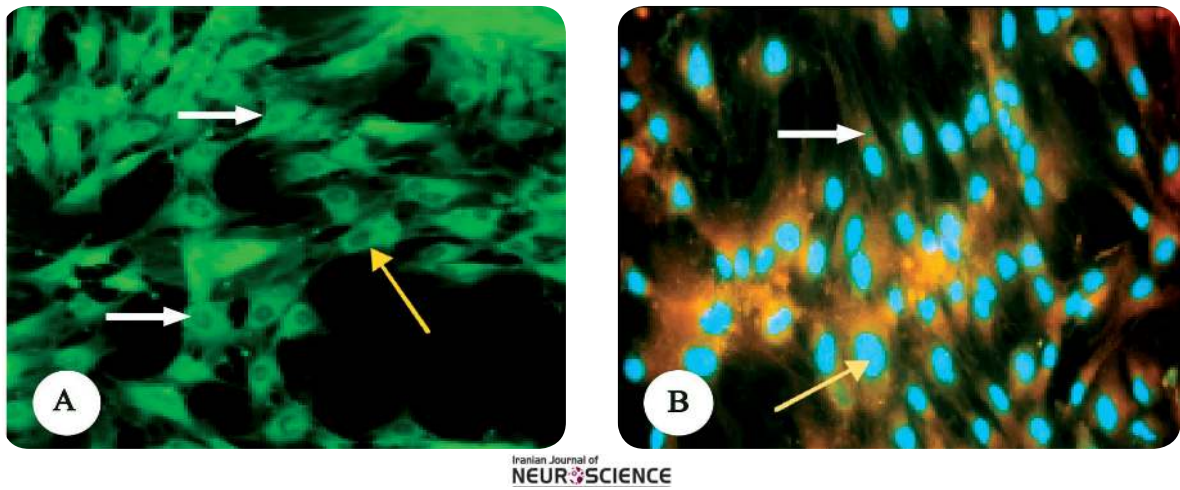


Figure 3: Schwann cells stained with S100 antibody & DAPI&DiI (A) Thick arrow show single Schwann cell in spindle shape, narrow arrow show nucleus of Schwann cell (B) thick arrow show cytoplasm of Schwann cell that is seen red and narrow arrow show nucleus that is seen blue.

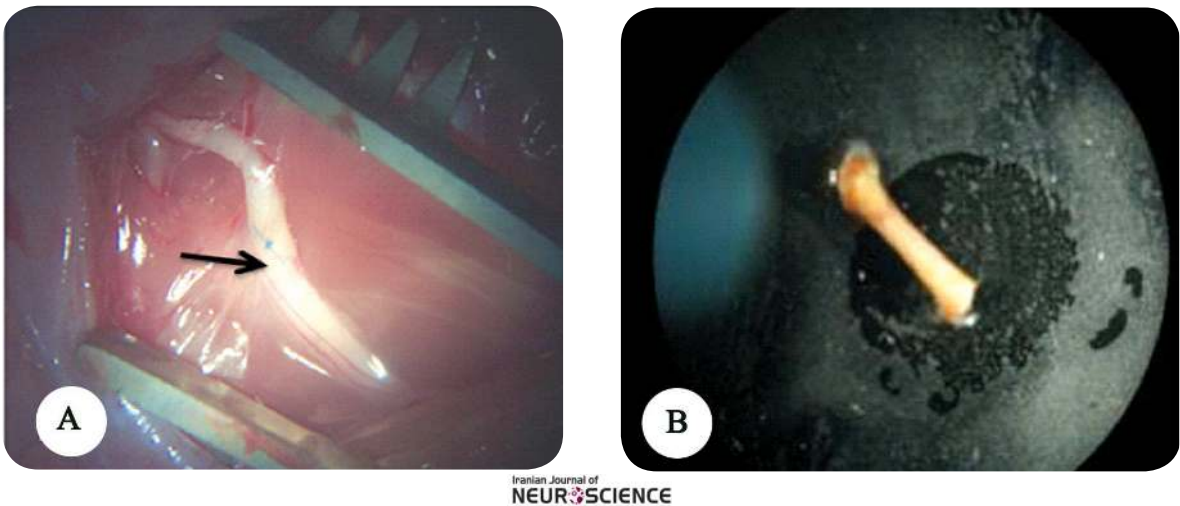


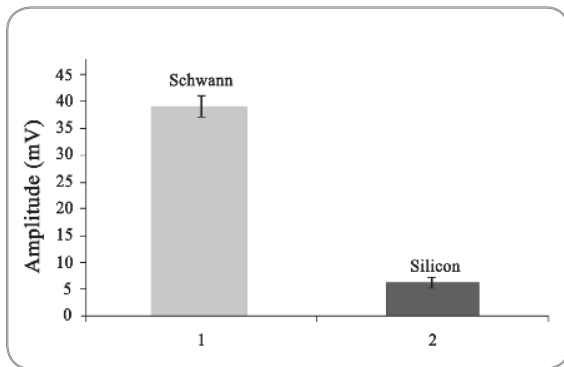
Figure 4: Sciatic nerve exposed (A) & regenerated 8 weeks after transplantation (B). As is seen in the picture (B) regenerated sciatic nerve connect proximal and distal stump and have continuity.

Groups	Amplitude (mv)	Latancy (ms)
Silicon with Schwann cells	39.04	1.64
Silicon without Schwann cells	6.28	1.96

Table 1: Electrophysiological results 8 weeks after transplantation

Electrophysiological study demonstrated that the experimental group as compared to control rats has a better recovery, amplitude and latency of regenerated sci-

atic nerve 8 weeks after the transplantation (ANOVA, $p < 0.05$).



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Figure 6: Mean amplitude (mv) bar after 8 weeks. Bar shows mean amplitude based on milivolt in two groups that are shown with numbers 1 and 2. Mean amplitude in Silicon tube with Schwann cells is 39.04 and in Silicon tube without Schwann cells is 6.28. Horizontal axis shows experimental and control groups and vertical axis is mean amplitude.

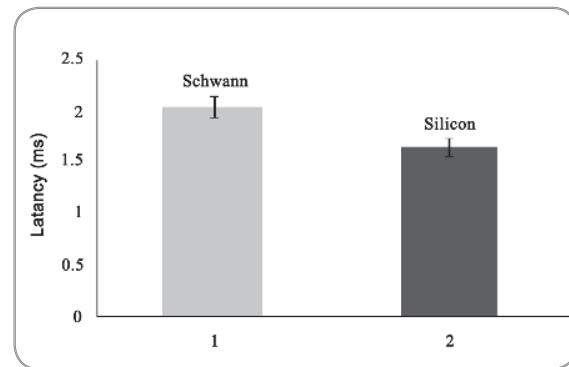
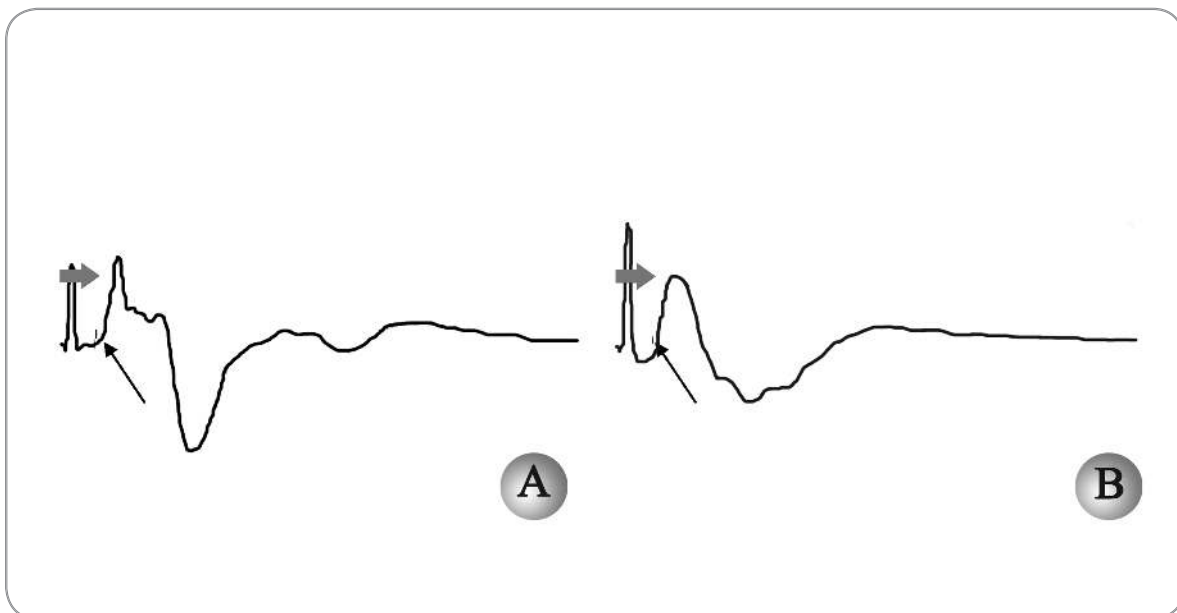


Figure 7: Mean latency (ms) bar after 8 weeks. Bar showed mean latency based on millisecond in two groups that are shown with numbers 1 and 2. Mean amplitude in Silicon tube with Schwann cells is 1.96 and in Silicon tube without Schwann cells is 1.64. Horizontal axis is experimental and control groups and vertical axis is mean amplitude.



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Figure 8: Electrophysiological graph, 8 weeks after the transplantation of Schwann cells. Compound muscle action potential in Silicon tube with Schwann cells (a) and in Silicon tube without Schwann cells (b). Shorter latency (narrow arrow) and higher amplitude (thick arrow) indicated axons regeneration.

Discussion

The present study shows that the silicon tube could conduits axonal regeneration and is a promising approach to nerve growth. After two weeks in the group Silicon tube seeded with Schwann Cells, the Schwann cells in medium proliferated and most of the cells were elongated and they had tendency to form interconnected network (Fig. 2). The purity of Schwann cells after assessing with S100 immunostaining was nearly 95% during to injection into the Silicon tube. Our finding showed that the DiI labeled cells are survived and ingrowths in the Silicon tube after two weeks of transplantation (Fig. 2).

Various methods were used to guide the regenerating axons to the distal stump, such as bone graft, metal tubes, blood vessels and fat sheaths. Our finding confirmed that the Silicon tube could provide a permissive environment for nerve repair. The Silicon tube has a good inner diameter for injection of cells and appropriate thick wall to prevent of collapsing because of absence pressure from within. In present work the increase of amplitudes and reinnervation of muscles in experimental group that received Schwann cells as compared to the control group indicated that the Schwann cells enhances nerve regeneration and improves motor performance. Schwann cells have great importance to axonal growth and are very effective during Wallerian degeneration (Ansselin, Fink & Davey, 1997). They proliferate and form the bands of Büngner which provide a conduit guiding for the regrowing axons (awcett, Keynes, 1990). Schwann cells promote locomotor recovery and axonal regeneration after transplantation into the complete transection spinal cord (Fouad, Schnell, Bunge, Schwab, Liebscher, Pearse, 2005). Further, our neurophysiological study indicated that silicon tube seeded with Schwann cells has seen the progression of nerve growth and reinnervation of muscles. Schwann cells produce ECM molecules such as laminin and collagen and express many cell adhesion molecules and receptors including L1, N cadherin, $\gamma 1$ integrins, and neural cell adhesion molecule (N-CAM) (Bolin, Verity, Silver, Shooter, Abrams, 1995; Toews, Barrett, Morell, 1998). They also synthesize neurotrophic molecules such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and ciliary neurotrophic factor (CNTF) (Bolin et al., 1995). The ECM proteins and neurotrophic factors are essential for survival of neurons and axonal regeneration (Dityatev, Schachner, 2003). The EMG demonstrated the extent of neuromuscular transmission at the neuromuscular junction

(Villiere & McLachlan, 1996). Eight weeks after transection of the sciatic nerve the values of the amplitudes were significantly higher and the values for the latencies were shorter in the Silicon tube seeded with Schwann cells group than the animals with Silicon tube without Schwann cells values. Results of this study indicate that the Schwann cells have great potential to promote regeneration of peripheral nerve injury.

There is a report that the Schwann cells seeded in semipermeable polyacrylonitrile/polyvinylchloride (PAN/PVC) guidance channels enhance peripheral nerve regeneration (Guenard, Kleitman, Morrissey, Bunge & Aebischer, 1992). Trophic factors from the Schwann cells and supporting substances from the Silicon tube are essential molecules which play crucial role in nerve growth (Marcol, Kotulska, Swiech-Sabuda, Larysz-Brysz, Golka, Gorka, Lewin-Kowalik, 2003). Extracellular matrix protein and growth factors is an important agent for the regeneration of long nerve defect (Anton, Sandrock, Matthew, 1994). Our findings were in agreement with other evidence that recommended Schwann cells transplantation with other intervention therapy such as vein or other artificial graft to conduits of the nerve is a unique strategy to promote nerve recovery (Bryan, Wang, Chakalis-Haley, 1996).

To sum up, the results of this study showed that silicon tube could be combined with cultured adult Schwann cells to bridge a sciatic nerve transection and to promote axonal regeneration across the conduit. However, additional treatments needed to provide connections between regenerating axons and target muscles.

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