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Sciatic nerve regeneration using a nerve growth factor-containing fibrin glue membrane****

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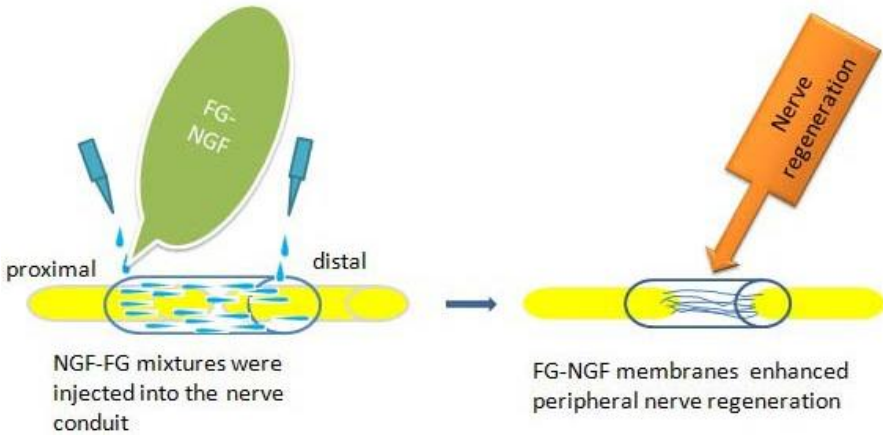
Graphical Abstract



Abstract

Our previous findings confirmed that the nerve growth factor-containing fibrin glue membrane provides a good microenvironment for peripheral nerve regeneration; however, the precise mechanism remains unclear. p75 neurotrophin receptor ($p75^{NTR}$) plays an important role in the regulation of peripheral nerve regeneration. We hypothesized that a nerve growth factor-containing fibrin glue membrane can promote neural regeneration by up-regulating $p75^{NTR}$ expression. In this study, we used a silicon nerve conduit to bridge a 15 mm-long sciatic nerve defect and injected a mixture of nerve growth factor and fibrin glue at the anastomotic site of the nerve conduit and the sciatic nerve. Through RT-PCR and western blot analysis, nerve growth factor-containing fibrin glue membrane significantly increased $p75^{NTR}$ mRNA and protein expression in the Schwann cells at the anastomotic site, in particular at 8 weeks after injection of the nerve growth factor/fibrin glue mixture. These results indicate that nerve growth factor-containing fibrin glue membrane can promote peripheral nerve regeneration by up-regulating $p75^{NTR}$ expression in Schwann cells.

Key Words

neural regeneration; nerve growth factor-containing fibrin glue membrane; p75 neurotrophin receptor; Schwann cells; peripheral nerve regeneration; fibrin glue; nerve growth factor; peripheral nerve injury; sciatic nerve; microenvironment; grants-supported paper; neuroregeneration

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Author contributions: Ma SZ designed this study and drafted the paper. All authors participated in related experiments, data collection and analysis, and approved the final version of this paper.

Conflicts of interest: None declared.

Ethical approval: The study was approved by the Animal Welfare Committee of Shandong University in China.

INTRODUCTION

Complete regeneration is usually very difficult following peripheral nerve damage, though microsurgical techniques have vastly increased the success rate of surgery to repair the injured nerve. This occurs possibly because of a lack of neurotrophic factors and extracellular matrix in the injured region, which results in a microenvironment that is not optimal for peripheral nerve regeneration[1].

Nerve growth factor (NGF) was the first neurotrophic factor identified in a class of molecules responsible for neuronal survival and differentiation[2]. It has been confirmed that administration of exogenous NGF to the end of nerves can protect neurons from death and significantly improve the recovery of neurological function after peripheral nerve injury[3-4]. However, exogenous NGF has a short half-life of around 2–4 minutes in the body. Its activity is soon lost and it is sensitive to temperature, pH and other factors[5]. Therefore, many techniques, such as particle technology[6-7], stent technology[8] and coated cells[9], have been used to acquire the maximum effect of exogenous NGF by maintaining its activity and release so it can have its desired effects in peripheral nerve regeneration for a longer period.

A previous study from our laboratory gained some insight into the influences of fibrin glue embedded with NGF on the promotion of peripheral nerve regeneration[10]. However, the precise mechanism by which a NGF-containing fibrin glue (FG) (FG-NGF) membrane can improve peripheral nerve regeneration remains unclear.

The NGF receptor, also known as p75 neurotrophin receptor ($p75^{NTR}$), binds NGF, brain-derived neurotrophic factor (BDNF), neurotrophin 4/5 (NT4/5) and neurotrophin 3 (NT3). It is known that $p75^{NTR}$/NGF signal transduction is responsible for regulating growth, survival, migration, and differentiation during the development of the central and peripheral nervous systems[11-12]. $P75^{NTR}$ is crucial for Schwann cell myelination and remyelination during development and after peripheral damage and regeneration[13-15]. The level of $p75^{NTR}$ is maintained and up-regulated until nerve regeneration re-establishes axon-Schwann cell contact and remyelination[16]. Nerve injury induces the up-regulation of $p75^{NTR}$ in motor neurons but down-regulates it in sensory neurons[17-18]. This differential regulation of $p75^{NTR}$ in peripheral nerves indicates that $p75^{NTR}$ plays an important role in the regulation of peripheral nerve regeneration. In this study, we investigated the influence of FG-NGF membrane on the expression of $p75^{NTR}$ in Schwann cells at the anastomotic site of the sciatic nerve in rats.

RESULTS

Quantitative analysis of animals

Forty-eight Wistar rats were initially included and randomly and evenly divided into a FG-NGF and a saline control group. The sciatic nerve was transected in each group, then the gap was bridged with a silicon nerve conduit and the anastomotic site of the sciatic nerve was injected with a mixture of NGF and fibrin glue (FG-NGF group) or simply physiological saline (saline control group). All 48 rats were included in the final analysis.

Macroscopic observation

After the FG-NGF mixtures were injected into the nerve conduit around the anastomotic site of the sciatic nerve of the rats, the FG-NGF mixtures immediately condensed. The entire process only took an average of 10–15 seconds. At 2 weeks after surgery, the FG-NGF membranes were partially absorbed, and the volume of residual membranes was about 81.8 ± 17.5 mm^3. At 4 weeks post-surgery, the FG-NGF membranes had not been completely absorbed, and the volume of residual membranes was about 15.6 ± 6.1 mm^3. At 8 weeks post-surgery, there was still a residual amount of membranes around the anastomotic site of the sciatic nerve, and the average volume was 3.3 ± 1.8 mm^3. At 4 and 8 weeks post-surgery, the tissue adhesion around the anastomotic site of the sciatic nerve in the saline control group was obvious, while the FG-NGF group had no obvious adhesions.

FG-NGF membranes induced expression of $p75^{NTR}$ mRNA in Schwann cells at the anastomotic site of the sciatic nerve

The level of $p75^{NTR}$ mRNA expression was determined by semi-quantitative RT-PCR. The time-dependent effects of FG-NGF membranes on $p75^{NTR}$ mRNA expression in Schwann cells at the anastomotic site of the sciatic nerve in rats were shown at 2, 4, and 8 weeks post-surgery ($P < 0.05$ or $P < 0.01$), and peaked at 8 weeks ($P < 0.01$; Figure 1).

FG-NGF membranes induced $p75^{NTR}$ protein expression in Schwann cells at the anastomotic site of the sciatic nerve

Western blot analysis showed that $p75^{NTR}$ protein expression in Schwann cells was significantly increased after FG-NGF membrane treatment compared with the

saline control group at 2, 4, and 8 weeks ($P < 0.05$ or $P < 0.01$; Figure 2) and this effect was most obvious at 8 weeks ($P < 0.01$). This is consistent with the increased $p75^{NTR}$ mRNA expression as shown by RT-PCR.

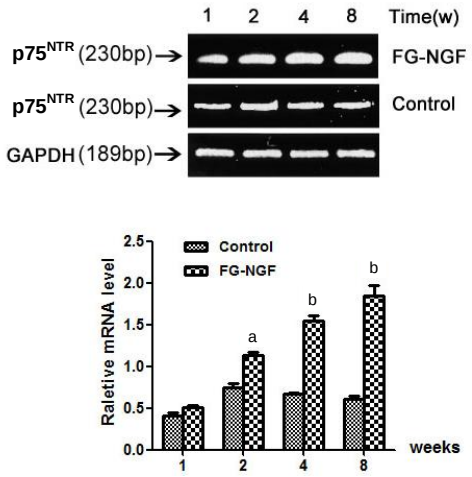

Figure 1 RT-PCR analysis of the effect of nerve growth factor-containing fibrin glue (FG-NGF) membrane on p75 neurotrophin ($p75^{NTR}$) mRNA expression in Schwann cells.

RT-PCR showed that FG-NGF significantly up-regulated $p75^{NTR}$ mRNA levels in Schwann cells compared to those of saline control cells at 2, 4, and 8 weeks (w) post-surgery ($^aP < 0.05$, $^bP < 0.01$, *vs*. the saline control group). One-way analysis of variance followed by Fisher's least significant difference test was used to compare differences between groups. $p75^{NTR}$ mRNA expression is shown as mean ± SD of three separate experiments, normalized to the expression level of GAPDH.

DISCUSSION

Schwann cells play a key role in peripheral nerve regeneration *via* the secretion of trophic support molecules[19-23]. Schwann cells can also help guide the regenerating axons by forming a type of tunnel that leads toward the target neurons, and remove axonal debris[24-25].

However, regeneration of peripheral nerve through autografts, as well as natural or engineered grafts, even with implanted Schwann cells is suboptimal. This may be due to neuronal fibrosis, death, and delayed ingrowth of regenerating axons into the distal stump[19]. Several studies have been aimed at improving neural regeneration and overcoming these problems using NGFs[23, 26-27].

NGF has properties that promote both synaptic growth and neuronal health, and it has a critical regulatory role in the development, growth, differentiation, nerve regeneration and functional recovery of the peripheral nervous neurons[26-28]. However, exogenous NGF degrades rapidly *in vivo*, and it is also sensitive to temperature, pH and other factors[29-30]. Thus, the activity of exogenous NGF in peripheral nerve regeneration is relatively short-lived.

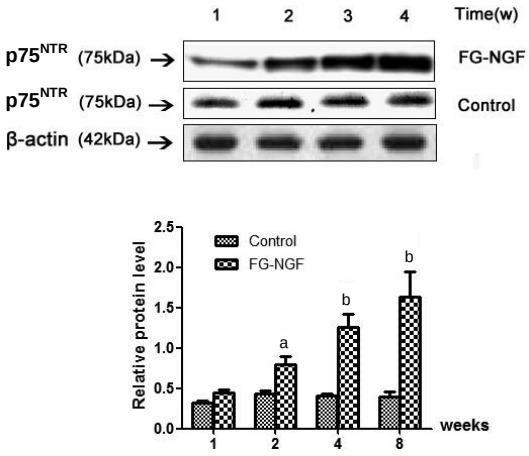

Figure 2 Protein expression of p75 neurotrophin receptor ($p75^{NTR}$) in Schwann cells after treatment with a nerve growth factor-containing fibrin glue (FG-NGF) membrane for 1, 2, 4, and 8 weeks.

$p75^{NTR}$ protein expression is shown as mean ± SD of three separate experiments. Blots obtained from Western blot analysis were probed for β-actin to normalize each lane for target protein expression level. $p75^{NTR}$ protein expression levels in Schwann cells were significantly increased compared to those of saline control cells at 2, 4, and 8 weeks (w) post-surgery ($^aP < 0.05$, $^bP < 0.01$, *vs*. the saline control group). One-way analysis of variance followed by Fisher's least significant difference test was used to compare differences between groups.

To extend the activity of exogenous NGF and improve its effects in peripheral nerve regeneration, we previously explored the technology for creating fibrin glue embedded with NGF to repair injured peripheral nerve[10]. The hypothesis is based on the fact that in local microenvironment of peripheral nerve regeneration, the extracellular matrix, including fibronectin, laminin, collagen, and other elements can promote axonal growth *via* adhesion[9, 31-32]. FG was mixed with NGF completely and evenly. Thus the mixture not only avoids degradation of NGF, but also prevents it from being easily lost[33]. Furthermore, the FG-NGF membrane promotes the sustained release of NGF as FG is gradually absorbed and NGF can exert its biological role for a longer time[10, 34]. As the membrane is used as an adhesive, it can reattach the peripheral nerve ends. It is also biodegradable and biocompatible, and is fully absorbed by the tissue[10, 35-36].

In our previous study[10], we have shown that FG-NGF membranes can be maintained for 4–8 weeks and can improve peripheral nerve regeneration in the rats[10]. However, the mechanisms by which the FG-NGF membrane exerts its effects on the repair process of peripheral nerve injury are poorly understood. To address this issue, we used rat primary cultured Schwann cells to determine the effect of the FG-NGF membrane on the expression of Schwann cell $p75^{NTR}$ at the anastomotic site of the sciatic nerve. Our results demonstrated that FG-NGF membranes significantly induced the expression of $p75^{NTR}$ in Schwann cells at both the mRNA and protein levels. Our data suggest that FG-NGF membranes can exert synergistic effects on peripheral nerve regeneration by inducing the expression of $p75^{NTR}$ in Schwann cells.

p75NTR is essential for the Schwann cell remyelination following peripheral nerve injury and myelination during development[37-43]. We designed this study to observe neural regeneration by providing exogenous NGF and FG locally to optimize the microenvironment for neural regeneration. According to the experimental results, we can infer that, after peripheral nerve injury, the local impaired Schwann cells express high expression of $p75^{NTR}$, which can bind abundant NGF. The NGF can induce the regeneration of neurons in the microenvironment supplied by FG. These results should promote further research about the potential use of this FG-NGF membrane in clinical studies. However, further studies are needed to clarify this issue.

Much indirect evidence from our previous research indicates that NGF is released continuously as FG is gradually absorbed. It was noted that in our previous study, we did not determine the release of NGF from the FG-NGF membranes around the anastomotic site of the sciatic nerve of the rats. Because it is not easy to isolate an adequate number of Schwann cells[44-45], in the present study, we used primary cell culture to obtain an adequate number of cells. Primary cell cultures of animal tissues are advantageous as specific cell types can be investigated *in vitro* away from their normal environment[46].

Primary cultured cells have advantages in accordance with having cell behavior that reflects better the *in vivo* niche, and therefore, have more preclinical and clinical adaptability[47-48]. However, other factors may affect cell behavior *in vitro*, such as the cell culture media and its supplements, the seeding density and the culture system itself, and may lead to different outcomes. Furthermore, the intrinsic biological environment around nerve regeneration *in vivo* is much more complex. Therefore, we examined whether the materials we tested *in vitro* could be successfully used *in vivo*. Further studies are necessary to elucidate these points.

MATERIALS AND METHODS

Design

A randomized, controlled animal experiment.

Time and setting

Experiments were performed at the Department of Orthopedics, Second Affiliated Hospital of Shandong University, China from June 2010 to December 2011.

Materials

A total of 48 clean, adult Wistar rats of either sex, aged 60 days, weighing 250–300 g, were purchased from the Experimental Animal Center of Shandong University in China (license No. SCXK (Lu) 20090001). The animals were housed at 23 ± 3°C, with 40–70% relative humidity, under a natural light-dark cycle, and allowed free access to food and water.

Methods

Surgical technique

The animals were anesthetized by intraperitoneal injection of 1.2 g/kg urethane. Under aseptic conditions, the left sciatic nerve was exposed. It was cut above the sciatic nerve bifurcation, which was 0.5 cm below the ischial tuberosity. Before suturing the nerve, a 17 mm silicon nerve conduit (1.55 mm inner diameter, 0.23 mm thickness) (NeuraGen®, Integra Life Sciences Corporation, USA) was used to bridge a 15 mm gap in the rat sciatic nerve. The use of silicon nerve conduits was performed as described previously[49-50]. Both the distal and proximal nerve stumps were inserted with 10–0 nylon to a depth of 1 mm into the conduits, leaving a 15 mm gap between the stumps (Figure 3). In addition, drugs were administered as follows: FG-NGF mixtures (4 mg/L) (1:1 v/v) in the FG-NGF group (FG from Guangzhou Special Technology Ltd., China; NGF from the U.S. Biological Engineering Ltd.); the saline control group received normal saline. FG-NGF mixtures and normal saline were injected into the nerve conduit from the two ends of nerve conduit. The total injection volume was 0.5 mL. The wound was subsequently closed in layers with 2-0 Dexon sutures after surgical treatment and the animals were separately housed. Tests were performed at 1, 2, 4 and 8 weeks following surgery.

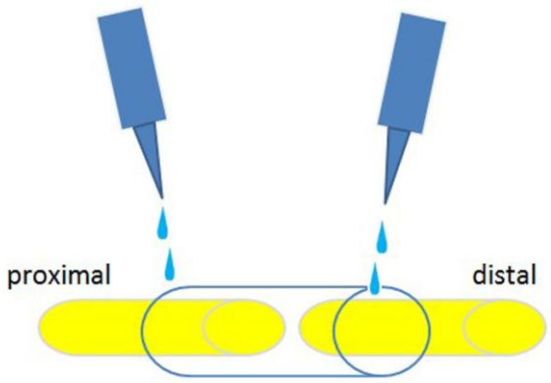

Figure 3 Surgical procedures applied during the stabilization of bacterial silicon nerve conduits at the site of the nerve injury.

Macroscopic observation

At 2, 4 and 8 weeks after surgery, animals were sacrificed to perform macroscopic observation. The changes in the FG-NGF membranes around the anastomotic site of the sciatic nerve of the rats were observed and measured. The volume of residual membranes (mm^3) was measured with calipers and calculated by the formula V = L × W × H, where L is length, W is width and H is height.

Cell culture

The animals were sacrificed at 1, 2, 4, 8 weeks for sciatic nerve extraction. The sciatic nerves in the anastomosis and in the region 1 cm distal to the anastomosis were obtained after surrounding connective tissue and epineurium were removed under a dissecting microscope. Then, the obtained nerve tissue was cut into pieces and digested with 0.25% trypsin, 0.25% collagenase and 0.25% pancreatin (40 minutes, 37°C) after repeated pipetting. The treated suspension cells were then seeded on culture plates. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco RL, Grand Island, NY, USA) containing 10% fetal bovine serum, penicillin (100 U/mL) and streptomycin (100 μg/mL) in a humidified 5% CO_2 atmosphere. Forty-eight hours later, the cells were treated with 10.5 g/L cytarabine (Ara-C) (Sigma, St. Louis, MO, USA) for 2 days.

RT-PCR analysis

After the cultures reached about 80% confluence, total RNA was isolated from the cell monolayers with a RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Reverse transcription of total cellular RNA (5 μg) was performed with a First-Strand cDNA Synthesis Kit (Amersham, Buckinghamshire, UK). According to the GeneBank sequences for $p75^{NTR}$ and GAPDH, primers were synthesized and used for PCR amplification. The primer sequences were as follows: $p75^{NTR}$ forward primer, 5′-TGA GTG CTG CAA AGC CTG CAA-3′; $p75^{NTR}$ reverse primer, 5′-TCT CAT CCT GGT AGT AGC CGT-3′; GAPDH forward primer, 5′-TGC CAC TCA GAA GAC TGT GG-3′; GAPDH reverse primer, 5′-TTC AGC TCT GGG ATG ACC TT-3′. The desired PCR products were 230 bp for $p75^{NTR}$ and 189 bp for GAPDH. The PCR amplification was performed in a total volume of 20 μL using a Taq PCR Master Mix kit (Qiagen, Hilden, Germany). PCR was performed with the following cycling protocol: an initial denaturation at 94°C for 2 minutes, followed by 30 cycles at 94°C for 45 seconds, at 60°C for 60 seconds, at 72°C for 45 seconds, with a final extension step at 72°C for 7 minutes. The PCR products were size-fractionated in a 2% agarose gel electrophoresis and visualized by staining with ethidium bromide. Photographs of gels were analyzed using the Gel-Pro Analyzer software (Version 3.1, Media Cybernetics Inc., Silver Spring, Maryland, USA).

Western blot analysis

Cells were lysed in buffer (1% sodium dodecyl sulfate, protease inhibitors), and boiled for 5 minutes. After centrifugation for 5 minutes at 20 800 × *g* at 4°C, the supernatant was collected and the concentration was quantified using the BCA assay (Pierce, Rockford, IL, USA). Protein samples (30 μg of protein per lane) were resolved by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, followed by electrotransfer onto polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). After transfer, the membranes were blocked in 5% skim milk prepared in phosphate buffered saline. The membranes were then probed with specific primary antibodies rabbit polyclonal antibody against $p75^{NTR}$ (1:1 000 dilution; Cell Signaling Technology, Beverly, MA, USA); antibody for β-actin (1:1 000 dilution) overnight at 4°C followed by horseradish peroxidase-conjugated anti-rabbit secondary antibody at a 1:50 000 dilution (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Specific bands were detected by enhanced chemiluminescence, and bands were quantified using Sigma Gel-Gel Analysis software (Sigma-Aldrich, St. Louis, MI, USA), as previously described[51].

Statistical analysis

All results were expressed as mean ± SD. Statistical analysis was performed using the software SPSS version 19.0 (SPSS, Chicago, IL, USA). The data of both RT-PCR and western blot analyses were evaluated for statistical significance by one-way analysis of variance

followed by Fisher's least significant difference test. A *P* value < 0.05 was considered statistically significant.

Research background: The obstacle for complete regeneration following peripheral nerve injury is a lack of neurotrophic factors and extracellular matrix in the injured region, both of which are necessary for regeneration.

Research frontiers: Studies have confirmed that a FG-NGF membrane can promote rat peripheral nerve regeneration, but the precise mechanism remains unclear.

Clinical significance: This study concluded the mechanism by which FG-NGF membrane promotes rat peripheral nerve regeneration, which provides experimental evidence for its clinical application in peripheral nerve regeneration.

Academic terminology: FG-NGF membrane refers to a glue membrane prepared using fibrin glue with nerve growth factor embedded (v/v; 1:1) in the environment for peripheral nerve regeneration.

Peer review: This study was the first to confirm that FG-NGF membrane promotes peripheral nerve regeneration by up-regulating $p75^{NTR}$ expression in Schwann cells and investigated the mechanism by which it promotes peripheral nerve regeneration at the anastomotic site.

REFERENCES

[1] Rajaram A, Chen XB, Schreyer DJ. Strategic design and recent fabrication techniques for bioengineered tissue scaffolds to improve peripheral nerve regeneration. Tissue Eng Part B Rev. 2012;18:454-467.

[2] Henderson CE. Role of neurotrophic factors in neuronal development. Curr Opin Neurobiol. 1996;6:64-70.

[3] Aloe L. Rita Levi-Montalcini and the discovery of nerve growth factor: past and present studies. Arch Ital Biol. 2003;141:65-83.

[4] Cheng C, Webber CA, Wang J, et al. Activated RHOA and peripheral axon regeneration. Exp Neurol. 2008;212:358-369.

[5] Edelman ER, Nugent MA, Karnovsky MJ. Perivascular and intravenous administration of basic fibroblast growth factor: vascular and solid organ deposition. Proc Natl Acad Sci U S A. 1993;90:1513-1517.

[6] Mikos AG, McIntire LV, Anderson JM, et al. Host response to tissue engineered devices. Adv Drug Deliv Rev. 1998; 33:111-139.

[7] Shakhbazau A, Kawasoe J, Hoyng SA, et al. Early regenerative effects of NGF-transduced Schwann cells in peripheral nerve repair. Mol Cell Neurosci. 2012;50: 103-112.

[8] Lundborg G, Gelberman RH, Longo FM, et al. In vivo regeneration of cut nerves encased in silicone tubes: growth across a six-millimeter gap. J Neuropathol Exp Neurol. 1982;41:412-422.

[9] Jubran M, Widenfalk J. Repair of peripheral nerve transections with fibrin sealant containing neurotrophic factors. Exp Neurol. 2003;181:204-212.

[10] Gao C, Ma S, Ji Y, et al. Siatic nerve regeneration in rats stimulated by fibrin glue containing nerve growth factor: an experimental study. Injury. 2008;39:1414-1420.

[11] Chan AA, Hertsenberg AJ, Funderburgh ML, et al. Differentiation of human embryonic stem cells into cells with corneal keratocyte phenotype. PLoS One. 2013;8: e56831.

[12] Li X, Shen Y, Di B, et al. Biological and clinical significance of p75NTR expression in laryngeal squamous epithelia and laryngocarcinoma. Acta Otolaryngol. 2012;132:314-324.

[13] Song XY, Zhou FH, Zhong JH, et al. Knockout of p75(NTR) impairs re-myelination of injured sciatic nerve in mice. J Neurochem. 2006;96:833-842.

[14] Ganeshan V, Ashton J, Schor NF. p75NTR: an enhancer of fenretinide toxicity in neuroblastoma. Cancer Chemother Pharmacol. 2013;71:777-787.

[15] Cosgaya JM, Chan JR, Shooter EM. The neurotrophin receptor p75NTR as a positive modulator of myelination. Science. 2002;298:1245-1248.

[16] Ito S, Menard M, Atkinson T, et al. Involvement of insulin-like growth factor 1 receptor signaling in the amyloid-beta peptide oligomers-induced p75 neurotrophin receptor protein expression in mouse hippocampus. J Alzheimers Dis. 2012;31:493-506.

[17] Zhang C, Helmsing S, Zagrebelsky M, et al. Suppression of p75 neurotrophin receptor surface expression with intrabodies influences Bcl-xL mRNA expression and neurite outgrowth in PC12 cells. PLoS One. 2012;7:e30684.

[18] Kiyosue T, Kawano S, Matsubara R, et al. Immunohistochemical location of the p75 neurotrophin receptor (p75NTR) in oral leukoplakia and oral squamous cell carcinoma. Int J Clin Oncol. 2013;18:154-163.

[19] Johnson EO, Zoubos AB, Soucacos PN. Regeneration and repair of peripheral nerves. Injury. 2005;36 Suppl 4: S24-29.

[20] Madduri S, Gander B. Schwann cell delivery of neurotrophic factors for peripheral nerve regeneration. J Pepher Nerv Syst. 2010;15(2):93-103.

[21] Webber CA, Christie KJ, Cheng C, et al. Schwann cells direct peripheral nerve regeneration through the Netrin-1 receptors, DCC and Unc5H2. Glia. 2011;59(10):1503-1517.

[22] Bunge RP. Expanding roles for the Schwann cell: ensheathment, myelination, trophism and regeneration. Curr Opin Neurobiol. 1993;3(5):805-809.

[23] Fansa H, Dodic T, Wolf G, et al. Tissue engineering of peripheral nerves: Epineurial grafts with application of cultured Schwann cells. Microsurgery. 2003;23(1):72-77.

[24] Miller C, Jeftinija S, Mallapragada S. Micropatterned Schwann cell-seeded biodegradable polymer substrates significantly enhance neurite alignment and outgrowth. Tissue Eng. 2001;7(6):705-715.

[25] Gomez-Sanchez JA, Gomis-Coloma C, Morenilla-Palao C, et al. Epigenetic induction of the Ink4a/Arf locus prevents Schwann cell overproliferation during nerve regeneration and after tumorigenic challenge. Brain. 2013;136(Pt 7): 2262-2278.

[26] Manni L, Rocco ML, Barbaro Paparo S, et al. Electroacupuncture and nerve growth factor: potential clinical applications. Arch Ital Biol. 2011;149(2):247-255.

[27] Hudson TW, Evans GR, Schmidt CE. Engineering strategies for peripheral nerve repair. Orthop Clin North Am. 2000;31(3):485-498.

[28] Cao J, Sun C, Zhao H, et al. The use of laminin modified linear ordered collagen scaffolds loaded with laminin-binding ciliary neurotrophic factor for sciatic nerve regeneration in rats. Biomaterials. 2011;32(16):3939-3948.

[29] Song B, Song J, Zhang S, et al. Sustained local delivery of bioactive nerve growth factor in the central nervous system via tunable diblock copolypeptide hydrogel depots. Biomaterials. 2012;33(35):9105-9116.

[30] Ching YH, Sutton TL, Pierpont YN, et al. The use of growth factors and other humoral agents to accelerate and enhance burn wound healing. Eplasty. 2011;11:e41.

[31] Evans GR. Peripheral nerve injury: a review and approach to tissue engineered constructs. Anat Rec. 2001;263(4): 396-404.

[32] Moges H, Wu X, McCoy J, et al. Effect of 810 nm light on nerve regeneration after autograft repair of severely injured rat median nerve. Lasers Surg Med. 2011;43(9): 901-906.

[33] Spicer PP, Mikos AG. Fibrin glue as a drug delivery system. J Control Release. 2010;148(1):49-55.

[34] Dong J, Cui G, Bi L, et al. The mechanical and biological studies of calcium phosphate cement-fibrin glue for bone reconstruction of rabbit femoral defects. Int J Nanomedicine. 2013;8:1317-1324.

[35] Sun T, Liu Z, Liu S, et al. The clinical study of repairing cauda equina fibres with fibrin glue after lumbar fracture and dislocation. Spinal Cord. 2010;48(8):633-637.

[36] Rafijah G, Bowen AJ, Dolores C, et al. The effects of adjuvant fibrin sealant on the surgical repair of segmental nerve defects in an animal model. J Hand Surg Am. 2013;38(5):847-855.

[37] Okumura T, Tsunoda S, Mori Y, et al. The biological role of the low-affinity p75 neurotrophin receptor in esophageal squamous cell carcinoma. Clin Cancer Res. 2006;12(17): 5096-5103.

[38] Uesugi N, Kimura Y, Yamashita T. Suppression of the p75 receptor signal attenuates the effect of ephrin-B3 and promotes axonal regeneration of the injured optic nerve. Cell Death Dis. 2013;4:e557.

[39] Roux PP, Barker PA. Neurotrophin signaling through the p75 neurotrophin receptor. Prog Neurobiol. 2002;67(3): 203-233.

[40] Mok SA, Lund K, Lapointe P, et al. A HaloTag(R) method for assessing the retrograde axonal transport of the p75 neurotrophin receptor and other proteins in compartmented cultures of rat sympathetic neurons. J Neurosci Methods. 2013;214(1):91-104.

[41] Trim N, Morgan S, Evans M, et al. Hepatic stellate cells express the low affinity nerve growth factor receptor p75 and undergo apoptosis in response to nerve growth factor stimulation. Am J Pathol. 2000;156(4):1235-1243.

[42] Montazeri F, Esmaeili A, Miroliaei M, et al. Messenger RNA expression patterns of p75 neurotrophin receptor and tropomyosin-receptor-kinase A following spinal cord injury. J Spinal Cord Med. 2013;36(3):231-236.

[43] Jedrych J, McNiff JM. Expression of p75 neurotrophin receptor in desmoplastic trichoepithelioma, infiltrative basal cell carcinoma, and microcystic adnexal carcinoma. Am J Dermatopathol. 2013;35(3):308-315.

[44] Ma J, Liu J, Wang Q, et al. The beneficial effect of ginsenoside Rg1 on Schwann cells subjected to hydrogen peroxide induced oxidative injury. Int J Biol Sci. 2013;9(6): 624-636.

[45] Zaminy A, Shokrgozar MA, Sadeghi Y, et al. Mesenchymal stem cells as an alternative for Schwann cells in rat spinal cord injury. Iran Biomed J. 2013;17(3):113-122.

[46] Musil LS. Primary cultures of embryonic chick lens cells as a model system to study lens gap junctions and fiber cell differentiation. J Membr Biol. 2012;245(7):357-368.

[47] Czekanska EM, Stoddart MJ, Richards RG, et al. In search of an osteoblast cell model for in vitro research. Eur Cell Mater. 2012;24:1-17.

[48] Severgnini M, Sherman J, Sehgal A, et al. A rapid two-step method for isolation of functional primary mouse hepatocytes: cell characterization and asialoglycoprotein receptor based assay development. Cytotechnology. 2012; 64(2):187-195.

[49] Lee JY, Giusti G, Friedrich PF, et al. The effect of collagen nerve conduits filled with collagen-glycosaminoglycan matrix on peripheral motor nerve regeneration in a rat model. J Bone Joint Surg Am. 2012;94(22):2084-2091.

[50] Liu BS, Yang YC, Shen CC. Regenerative effect of adipose tissue-derived stem cells transplantation using nerve conduit therapy on sciatic nerve injury in rats. J Tissue Eng Regen Med. 2012.

[51] Si H, Peng C, Li J, et al. RNAi-mediated knockdown of ERK1/2 inhibits cell proliferation and invasion and increases chemosensitivity to cisplatin in human osteosarcoma U2-OS cells in vitro. Int J Oncol. 2012;40(4):1291-1297.

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