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Femoral nerve regeneration and its accuracy under different injury mechanisms

Aikeremujiang·Muheremu[1, 2], Qiang Ao[3], Yu Wang[2], Peng Cao[4], Jiang Peng[2, *]

1 Medical Center, Tsinghua University, Beijing, China
2 Institute of Orthopaedics, General Hospital of People's Liberation Army, Beijing, China
3 Department of Tissue Engineering, China Medical University, Shenyang, Liaoning, China
4 Department of Orthopedics, Fifth Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang Uygur Autonomous Region, China

***Correspondence to:**
Jiang Peng, M.D., Ph.D., pengjdxx@126.com.

orcid:
0000-0002-1309-1971 (Qiang Ao)

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Abstract

Surgical accuracy has greatly improved with the advent of microsurgical techniques. However, complete functional recovery after peripheral nerve injury has not been achieved to date. The mechanisms hindering accurate regeneration of damaged axons after peripheral nerve injury are in urgent need of exploration. The present study was designed to explore the mechanisms of peripheral nerve regeneration after different types of injury. Femoral nerves of rats were injured by crushing or freezing. At 2, 3, 6, and 12 weeks after injury, axons were retrogradely labeled using 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (Dil) and True Blue, and motor and sensory axons that had regenerated at the site of injury were counted. The number and percentage of Dil-labeled neurons in the anterior horn of the spinal cord increased over time. No significant differences were found in the number of labeled neurons between the freeze and crush injury groups at any time point. Our results confirmed that the accuracy of peripheral nerve regeneration increased with time, after both crush and freeze injury, and indicated that axonal regeneration accuracy was still satisfactory after freezing, despite the prolonged damage.

Key Words: *nerve regeneration; peripheral nerve injury; chemotactic regeneration; retrograde labeling; selective nerve regeneration; functional recovery; NSFC grant; neural regeneration*

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Introduction

Five percent of patients in trauma centers are affected by peripheral nerve injury (Belkas et al., 2004; Taylor et al., 2008). Since most of these patients are of prime working age, such injuries pose a serious economic burden to society (Whitlock et al., 2009). Restoration of function after peripheral nerve injury remains one of the toughest challenges in surgery. Despite the development of microsurgical equipment, with which it is now possible to suture the damaged nerves accurately within a short time after injury, complete functional recovery after peripheral nerve damage has not yet been achieved (Eser et al., 2009). Various methods have been attempted (Vetter et al., 2010; Bosse et al., 2012; Seidel et al., 2013), but none have obtained complete success, mainly because the mechanisms underlying selective peripheral nerve regeneration remain poorly understood (Calvo et al., 2012; Jesuraj et al., 2012).

To achieve full recovery from nerve injury, high accuracy nerve innervation is just as important as robust axonal regrowth (Ruiter et al., 2011). Axons regenerated from proximal motor nerve stumps should grow into the motor nerve pathways in the distal stump, and *vice versa*. However, little is known about the mechanism underlying the misdirection of regenerating axons. To achieve accurate regeneration and full functional recovery, more research must be carried out to explore how the regeneration accuracy of axons changes after injury. Novel experimental approaches, such as retrograde labeling (Hoke et al., 2006), have provided us with new tools to observe this process. We have used this approach in the present study to investigate the regeneration of axons after crushing or freezing, two common types of peripheral nerve injury, with the aim of providing new insight into chemotactic regeneration.

Materials and Methods

Animals

A total of 92 healthy male Sprague-Dawley rats, aged 8 weeks and weighing 250 ± 20 g, were provided by the Experimental Animal Center of the Chinese PLA General Hospital. The study was approved by the same institute's Animal Ethics Committee in China.

Establishment of animal models

Animals were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.3 mL/100 g) and placed on an operating table in the supine position. A surgical incision was

made in the right groin to expose the femoral nerve. Animals were divided into three groups. In the control group (n = 40), the femoral nerve was transected 5 mm proximal to the bifurcation point (Brushart et al., 1993). In the crush injury group (n = 40), the nerve was transected 5 mm proximal to the bifurcation point, and the femoral nerve trunk was crushed using microforceps (WA3010; Head Biotechnology, Beijing, China) for 5 seconds (Brushart et al., 1993). In the freeze lesion group (n = 12), the femoral nerve was transected 7 mm proximal to the bifurcation point, and a cotton swab soaked in liquid nitrogen (Air Products, Beijing, China; stored in the Orthopedic Research Institute of the Chinese PLA General Hospital was used to freeze 5 mm of nerve trunk from 2 mm proximal to the bifurcation point. The incision was closed in layers after successful injury modeling.

Retrograde labeling

A 2% solution of True Blue (Sigma, St. Louis, MO, USA) was prepared in distilled water, and a 5% solution of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (Dil; Sigma) was prepared in dehydrated alcohol. Both solutions were stored at 4°C until use.

Retrograde labeling was performed 2, 3, 6, and 12 weeks postoperatively (Ruiter et al., 2011). At each time point, 10 animals from the crush injury group, 10 from the freeze lesion group, and three from the control group were used. Animals were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.3 mL/100 g), and placed on the operating table in the supine position. An incision was made in the right groin, to expose the right femoral nerve, its muscle branch, and saphenous nerve branches. The muscle branch was cut where it grows into the quadriceps femoris muscle, and the same length of saphenous nerve was cut to prepare for the next step. A 10 μL microsyringe was used to inject 5 μL of Dil or True Blue into aseptic plastic chambers. The nerve stumps of the muscle branches were inserted into the chamber filled with Dil, and the saphenous branch into the chamber of True Blue. The incision was closed in layers and animals were returned to their home cages with food and water *ad libitum* to recover for 3 days.

Observation of the spinal cord

Three days after retrograde labeling (Hoke et al., 2006), all animals were anesthetized with 10% chloral hydrate (0.3 mL/100 g). The dorsal spinal canal was opened and the spinal cord was exposed. The L_{2-4} segment was carefully removed, placed on a freezing microtome (CE1900; Leica, Germany) at −25°C until frozen solid, then embedded, and sagittal sections were cut using the microtome until the spinal gray matter was reached. Three sections (15 μm thickness) were taken and immediately observed and photographed under a fluorescence microscope (IX70; Olympus, Tokyo, Japan). Blue and red fluorescence was used to visualize True Blue and Dil, respectively, in the ventral horn of the spinal cord. Blue, red, and purple (colabeled) neurons were counted using Image-Pro Plus 6.0 image analysis software (Media Cybernetics, USA).

Statistical analysis

All data are presented as the mean ± SD. Data were analyzed by one-way analysis of variance and the least significant difference *post-hoc* test, using SPSS 17.0 software (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

Only single-labeled Dil-positive (red) neurons were seen in the anterior horn of the spinal cord in the control group. In the crush and freeze injury groups, the percentage of Dil single-labeled neurons in the anterior ventral horn increased over time, with significant increases at each time point after 2 weeks, after both crush and freeze injuries ($P < 0.01$). No significant differences between the crush and freeze injury groups were observed in Dil single-labeled cell counts at any time point (**Tables 1**, **2**, **Figure 1**).

Discussion

Peripheral nerve injury is common in the trauma center and battlefield. The present study was designed to investigate regeneration after two types of common peripheral nerve damage: crush injury and freeze lesion. A series of pathophysiological changes is known to occur after peripheral nerve injury, in the neurons and axons at the injury site, as well as at target organs, and in the central nervous system (Lieberman, 1971). Nerve fibers at the proximal nerve stump begin to grow, while at the distal stump, axons break down, myelinolysis takes place and a new medullary sheath begins to form, providing a pathway to the growing axons from the proximal stump (Abdullah et al., 2013; Muheremu et al., 2013). Pathological changes after freeze injury include damage to the endoneurium, epineurium, and Schwann cells, at the injury site (Gaudet et al., 2011; Scheib et al., 2013). The perineurium remains intact. However, axon regrowth is impeded by the scar tissue created by the ruptured endoneurium and Schwann cells (Dubový et al., 2013; Suganuma et al., 2013; Gordon 2014), which impairs reinnervation to the distal target organs. Crush injury may also cause perineurial injury, leading to axonal misrouting.

With the development of microsurgical techniques, the timely and accurate suturing of peripheral nerve stumps is now possible, and considerable axonal regeneration can be achieved through part of the lesion. However, to date, it has not been possible to restore the function of the target organs to their pre-injury level. In the process of peripheral nerve regeneration, axonal misdirection may result in functional deficiency of original target organs (Madison et al., 2007; Vetter et al., 2010; Corriden et al., 2012; Yuan et al., 2013; Megan et al., 2014).

The theory of chemotactic nerve regeneration, proposed by Cajal (1928), assumes that nerve stumps tend to grow towards their original nerve pathways. Even before the 20th century, Bailey et al. (1993) found that nerve fibers regrowing from the proximal nerve stump tended to follow their original pathways, and called this phenomenon neurotropism,

Table 1 Number of labeled neurons in the anterior horn of rat spinal cords after surgery for crush injury or freeze lesioning

Group		Time after injury (weeks)			
		2	3	6	12
Crush injury	Dil^+ (Red)	86.4±7.2	124.6±14.9	185.4±18.2	322.7±24.2
	True $Blue^+$ (Blue)	84.2±7.4	78.2±6.6	62.2±8.4	47.3±8.4
	Dil^+/True $Blue^+$ (Purple)	32.6±5.6	26.9±4.4	18.9±4.7	8.9±2.4
Freeze injury	Dil^+ (Red)	61.2±5.2	114.2±11.8	176.5±16.3	276.4±19.6
	True $Blue^+$ (Blue)	58.4±4.3	65.3±5.2	53.7±6.3	30.5±5.1
	Dil^+/True $Blue^+$ (Purple)	62.2±8.4	23.4±2.4	16.7±3.7	7.5±1.8

Number of prechlorate (Dil) single-labeled neurons increased with time, whereas the number of True Blue single-labeled and Dil/True Blue double-labeled neurons decreased. Significant increases were observed in Dil single-labeled neurons at each time point from 2 weeks postoperatively ($P < 0.01$). No differences were observed between crush and freeze groups in the number of Dil single-labeled neurons at each time point ($P > 0.05$). Data are expressed as the mean ± SD (one-way analysis of variance and the least significant difference test).

Table 2 Percentage of prechlorate (Dil) single-labeled neurons in the anterior horn of rat spinal cords after surgery for crush and freeze injuries

Group	Time after injury (weeks)			
	2	3	6	12
Crush injury	43.6±7.6	61.8±11.4	75.2±12.1	83.4±6.0
Freeze injury	44.2±3.7	61.6±7.6	74.4±11.4	77.7±9.2

Percentage of Dil single-labeled neurons increased with time. Significant increases were observed at each time point after 2 weeks postoperatively ($P < 0.01$). Data are expressed as the mean ± SD (one-way analysis of variance and the least significant difference test).

until Cajal (1928) renamed it chemotaxis. Cajal assumed chemotactic nerve growth to have histological, topographical, and target organ specificity. Although the theory of chemotaxis in nerve regeneration has been widely accepted, there is still much dispute about its specific characteristics and mechanisms. Furthermore, the phenomenon was denied by Weiss and Tailor with their famous experiment using Y tubes made from autologous blood vessels (Weiss et al., 1943, 1944), which discouraged research on chemotaxis for several decades. Lundborg (1986, 2000), Politis (1982, 1983, 1985), Seckel (1984), Mackinnon (1986), Bruneli (1987), Brushart (1993), and Chiu et al. (1982, 1988, 1990) demonstrated the existence of selective nerve regeneration and explained its chemotactic characteristics. It was presumed that chemotactic regeneration of axons after peripheral nerve injury promoted the accuracy of nerve reinnervation and the functional recovery of target organs (Jesuraj et al., 2012; Zhou et al., 2014).

However, although chemotaxis is now a generally accepted notion, there is still no consensus on its characteristics. Politis (1985) used femoral nerve retrograde labeling to show that axons grown from proximal motor nerve stumps tend to follow distal motor nerve pathways, and proximal sensory nerve stumps tend to follow distal sensory pathways. He suggested that the specific factors produced by distal nerve stumps facilitate the selective growth of proximal nerve axons. Madison et al. (2007) proposed the "pruning hypothesis", explaining that axons following the wrong pathway will be pruned back, gradually increasing the accuracy of nerve innervation.

Our results from the present study show that the type of neurons in the anterior ventral horn at 2 weeks is random, but that accuracy increased significantly with time. After injury, nearly 80% of regenerated motor axons grew into their original pathways. The gradual increase of the proportion of Dil-labeled neurons and the gradual decrease of True Blue labeled and True Blue + Dil labeled neurons in the anterior ventral horn supports the pruning hypothesis, indicating that misdirected nerve fibers gradually shrink back in the inappropriate microenvironment. Hoke et al. (2006) also suggested that chemotactic nerve growth may be affected by different Schwann cell phenotypes. In their research, they found that Schwann cells of motor and sensory nerves have different phenotypes and produce different neurotrophic factors. Tsubokawa et al. (1999) proposed that Schwann cells strongly promote the growth of sensory nerves, but have little effect on motor axons. In our experiment, although massive Schwann cell death occurs after the freeze lesion, axonal regeneration accuracy remains equal to that of the crush injury group, contrary to Tsubokawa's theory. Our results suggest that perineural suture may lead to more accurate reinnervation than epineural suture (Bonini et al., 2013; de Ruiter et al., 2014); and that relatively long nerve gaps may be bridged by tubes that simulate the stereochemical structure of nerves (Nectow et al., 2011; Xin et al., 2011; Kim et al., 2013).

Novel experimental methods can be powerful tools (Vyas et al., 2010; Lee et al., 2012; Pujic et al., 2013). In the present study, we made some improvements to the conventional retrograde labeling technique. Since we found that it was impossible to inject Dil and True Blue into the sephanous nerve and muscle branch of the femoral nerve with the smallest available microinjector (10 μL), because of the extremely small diameter of these branches, we used small plastic chambers filled with each drug, and put the nerve stumps into those chambers. Viewing sagittal sections of

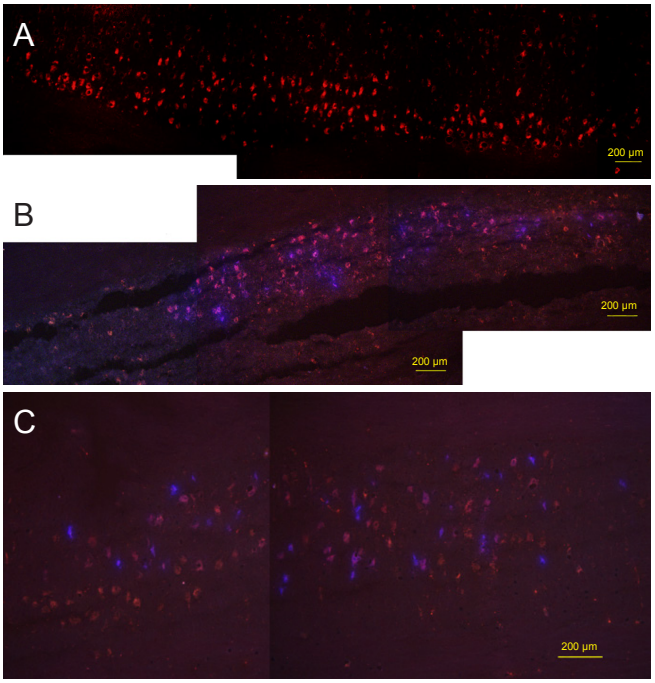

Figure 1 Retrograde labeling of neurons using Dil and True Blue, in the anterior horn of rat spinal cord 12 weeks after injury (immunofluorescence staining and fluorescence microscopy).
(A) Only Dil-labeled neurons were visible in the control group. (B, C) Double-labeled neurons in the crush (B) and freeze (C) injury groups. Red, Dil^+; blue, True $Blue^+$; purple, Dil^+/True $Blue^+$ double-labeled. Scale bars: 200 μm.

the spinal cord under a fluorescence microscope revealed neurons stained with the different dyes and confirmed that our method produced successful retrograde transport. This method can therefore be applied in other retrograde labeling studies of small-diameter nerves. Another modification we made is to eliminate the internal fixation step before removing the spinal cord. Instead we removed the spinal cords while the animals were under deep anesthesia and immediately put the cords into a freezing microtome precooled to −25°C, and embedded them once they became frozen. This modified procedure will not only protect the technician from the harmful effects of chemical fixatives such as paraformaldehyde, but will also help avoid false-positive fluorescence, which might occur with the use of an internal fixative.

Functional recovery after peripheral nerve injury cannot be further improved by traditional methods; there is therefore an urgent need to explore novel methods (Summa et al., 2010; Ladak et al., 2011; Gu et al., 2011; Fregnan et al., 2012; Napoli et al., 2012; Wang et al., 2012; Franz et al., 2013; Marquardt et al., 2013; Chan et al., 2014; Guaiquil et al., 2014; Xu et al., 2014). The results from the present study provide valuable information about selective peripheral regeneration mechanisms, and will be useful in the search for novel methods for the treatment of peripheral nerve injury.

However, as 50% of the muscle branch femoral nerve fibers were sensory, using this retrograde labeling technique, motor nerve axons that wrongly grew into sensory nerve pathways of the distal muscle branch were also considered to be correctly innervated (Politis et al, 1985). Future studies should be designed to avoid such interferences and find more accurate ways to evaluate correct nerve regeneration.

In summary, the accuracy of peripheral reinnervation rises gradually over time and may be the result of pruning at the injury site. Moreover, nerve stumps can regenerate with high accuracy over a relatively long distance if the integrity of perineurium is preserved.

Author contributions: *Aikeremujiang•Muheremu carried out the research, collected data, and wrote the paper. QA analyzed and interpreted the data. YW and PC provided technical or material support. JP designed the study, obtained funding and coordinated the research process. All authors approved the final version of the paper.*
Conflicts of interest: *None declared.*

References

Abdullah M, O'Daly A, Vyas A, Rohde C, Brushart TM (2013) Adult motor axons preferentially reinnervate predegenerated muscle nerve. Exp Neurol 249:1-7.
Bailey SB, Eichler ME, Villadiego A., Rich KM (1993) The influence of fibronectin and laminin during Schwann cell migration and peripheral nerve regeneration through silicon chambers. J Neurocytol 22:176-184.
Belkas JS, Shoichet MS, Midha R (2004) Peripheral nerve regeneration through guidance tubes. Neurol Res 26:151-160.
Bonini SA, Ferrari-Toninelli G, Montinaro M, Memo M (2013) Notch signalling in adult neurons: A potential target for microtubule stabilization. Ther Adv Neurol Disord 6:375-385.
Bosse F (2012) Extrinsic cellular and molecular mediators of peripheral axonal regeneration. Cell Tissue Res 349:5-14.
Brunelli G, Fontana G, Jager C, Bartolaininelli P, Franchini A (1987) Chemotactic arrangement of axons inside and distal to a venous graft. J Reconstr Microsurg 3:87.
Brushart TME. Motor axons preferentially reinnervate motor pathways (1993) J Neurosci 13:2730-2738.
Cajal RS (1928) Experiments dealing with the transplantation of nerves and their products designed to prove especially an attractive or neurotrophic action on nerve sprouts. Degeneration and Regeneration of the Nervous System. New York: Hafner Press, USA.
Calvo M, Bennett DL (2012) The mechanisms of microgliosis and pain following peripheral nerve injury. Exp Neurol 234:271-282.
Chan KM, Gordon T, Zochodne DW, Power HA (2014) Improving peripheral nerve regeneration: from molecular mechanisms to potential therapeutic targets. Exp Neurol 261:826-835.
Chiu DT Strauch B (1990) A prospective clinical evaluation of autogenous vain grafts used as a nerve conduit for distal sensory nerve defects of 3 cm or less. Plast Reconstr Surg 86:928-934.
Chiu DT, Janecka I, Krizek TJ, Wolff M, Lovelace RE (1982) Autogenous vein graft as a conduit for nerve regeneration. Surgery 91:226-232.
Chiu DT, Lovelace RE, Yu LT, Wolff M, Stengel S, Middleton L, Janecka IP, Krizek TJ (1988) Comparative electrophysiologic vein grafts as nerve conduits: an experimental study. J Reconstr Microsurg 4:303-310.
Corriden R, Insel PA (2012) New insights regarding the regulation of chemotaxis by nucleotides, adenosine, and their receptors. Purinergic Signal 8:587-598.
de Ruiter GC, Spinner RJ, Verhaagen J, Malessy MJ (2014) Misdirection and guidance of regenerating axons after experimental nerve injury and repair: a review. J Neurosurg 120:493-501.
Dubový P, Jančálek R, Kubek T (2013) Role of inflammation and cytokines in peripheral nerve regeneration. Int Rev Neurobiol 108:173-206.
Eser F, Aktekin LA, Bodur H, Cigdem A (2009) Etiological factors of traumatic peripheral nerve injuries. Neurol India 57:434-437.

Franz CK, Singh B, Martinez JA, Zochodne DW, Midha R (2013) Brief transvertebral electrical stimulation of the spinal cord improves the specificity of femoral nerve reinnervation. Neurorehabil Neural Repair 27:260-268.
Fregnan F, Muratori L, Simões AR, Giacobini-Robecchi MG, Raimondo S (2012) Role of inflammatory cytokines in peripheral nerve injury. Neural Regen Res 7:2259.
Gaudet AD, Popovich PG, Ramer MS (2011) Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. J Neuroinflammation 8:110.
Gordon T (2014) Neurotrophic factor expression in denervated motor and sensory Schwann cells: relevance to specificity of peripheral nerve regeneration. Exp Neurol 254:99.
Gu X, Ding F, Yang Y, Liu J (2011) Construction of tissue engineered nerve grafts and their application in peripheral nerve regeneration. Prog Neurobiol 93:204-230.
Guaiquil VH, Pan Z, Karagianni N, Fukuoka S, Alegre G, Rosenblatt MI (2014) VEGF-B selectively regenerates injured peripheral neurons and restores sensory and trophic functions. Proc Natl Acad Sci U S A 111:17272-17277.
Hoke A, Redett R, Hameed H (2006) Schwann cells express motor and sensory phenotypes that regulate axon regeneration. J Neurosci 26:9646-9655.
Jesuraj NJ, Nguyen PK, Wood MD, Moore AM, Borschel GH, Mackinnon SE, Sakiyama-Elbert SE (2012) Differential gene expression in motor and sensory Schwann cells in the rat femoral nerve. J Neurosci Res 90:96-104.
Kim JR, Oh SH, Kwon GB (2013) Acceleration of peripheral nerve regeneration through asymmetrically porous nerve guide conduit applied with biological/physical stimulation. Tissue Eng Part A 19:2674-2685.
Ladak A, Olson J, Tredget E, Gordon, T (2011) Differentiation of mesenchymal stem cells to support peripheral nerve regeneration in a rat model. Exp Neurol 228:242-252.
Lee BK, Ju YM, Cho JG, Jackson JD, Lee SJ, Atala A, Yoo JJ (2012) End-to-side neurorrhaphy using an electrospun PCL/collagen nerve conduit for complex peripheral motor nerve regeneration. Biomaterials 33:9027-9036.
Lieberman AR (1971) The axon reaction: a review of the principal features of perikaryal responses to axon injury. Int Rev Neurobiol 14:49-124.
Lundborg G (2000) A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance. J Hand Surg Am 25:391-414.
Lundborg G, Dablin L, Danielsen N, Nachemson AK (1986) Tissue specificity in nerve regeneration. J Plast Reconstr Surg 20:279-283.
Mackinnon S, Dellon L, Lundborg G, Hudson AR, Hunter D (1986) A study of neurotrophism in a primate model. J Hand Surg 11A:888-896.
Madison RD, Robinson GA, Chadaram SR (2007) The specificity of motor neurone regeneration (preferential reinnervation). Acta Physiol 189:201-206.
Marquardt LM, Sakiyama-Elbert SE (2013) Engineering peripheral nerve repair. Cur Opin Biotechnol 24:887-892.
Muheremu A, Wang Y, Peng J (2013) Advances in experimental and clinical studies of chemotaxis. Can J Neurol Sci 40:292-298.
Napoli I, Noon LA, Ribeiro S, Kerai AP, Parrinello S, Rosenberg LH (2012) A central role for the ERK-signaling pathway in controlling Schwann cell plasticity and peripheral nerve regeneration in vivo. Neuron 73:729-742.
Nectow AR, Marra KG, Kaplan DL (2011) Biomaterials for the development of peripheral nerve guidance conduits. Tissue Eng Part B Rev 18:40-50.
Politis MJ (1985) Specificity in mammalian peripheral nerve regeneration at the level of the nerve trunk. Brain Res 328:271-276.
Politis MJ, Spencer PS (1983) An in vivo assay of neurotropic activity. Brain Res 278:229-231.
Politis MJ, Ederle K, Spencer PS (1982) Tropism in nerve regeneration in vivo. Attraction of regenerating axons by diffusible factors derived from cells in distal nerve stumps of transected peripheral nerves. Brain Res 253:1-12.
Pujic Z, Goodhill GJ (2013) A dual compartment diffusion chamber for studying axonal chemotaxis in 3D collagen. J Neurosci Methods 215:53-59.
Ruiter G, Malessy M, Alaid A (2011) Misdirection of regenerating motor axons after nerve injury and repair in the rat sciatic nerve model. Exp Neurol 2008:339-350.
Scheib J, Höke A (2013) Advances in peripheral nerve regeneration. Nat Rev Neurol 9:668-676.
Seckel BR, Chiu TH, Nyilas E, Sidman RL (1984) Nerve regeneration through synthetic biodegradable nerve guides: regulation by the target organ. Plast Reconstr Surg 74:173.
Seidel MF, Wise BL, Lane NE (2013) Nerve growth factor: an update on the science and therapy. Osteoarthritis Cartilage 21:1223-1228.
Suganuma S, Tada K, Hayashi K, Takeuchi A, Sugimoto N, Ikeda K, Tsuchiya H (2013) Uncultured adipose-derived regenerative cells promote peripheral nerve regeneration. J Orthop Sci 18:145-151.
Summa PG, Kingham PJ, Raffoul W, Wiberg M, Terenghi G, Kalbermatten DF (2010). Adipose-derived stem cells enhance peripheral nerve regeneration. J Plast Reconstr Aesthet Surg 63:1544-1552.
Taylor CA, Braza D, Rice JB, Dillingham T (2008) The incidence of peripheral nerve injury in extremity trauma. Am J Phys Med Rehabil 87:381-385.
Tsubokawa N, Maki Y, Yoshizu T, et al (1999) Comparison of the neurotropic effects of motor and sensory Schwann cells during regeneration of peripheral nerves. Scand J Plast Reconstr Hand Surg 33:379-385.
Vetter I, Pujic Z, Goodhill GJ (2010) The response of dorsal root ganglion axons to nerve growth factor gradients depends on spinal level. J Neurotrauma 27:1379-1386.
Vyas A, Li Z, Aspalter M, Feiner J, Höke A, Zhou C, O'Daly A, Abdullah M, Rohde C, Brushart TM (2010) An in vitro model of adult mammalian nerve repair. Exp Neurol 223:112-118.
Wang R, Rossomando A, Sah DW, Ossipov MH, King T, Porreca F (2012) Artemin induced functional recovery and reinnervation after partial nerve injury. Pain 155:476-484.
Weiss P, Taylor AC (1943) Histiomechanical analysis of nerve reunion in the rat after tubular splicing. Arch Surg 47:419-447.
Weiss P, Taylor AC (1944) Further experimental evidence against "neurotrophism" in nerve regeneration. J Exp Zool 95:233-257.
Whitlock EL, Tuffaha SH, Luciano JP (2009) Processed allografts and type I collagen conduits for repair of peripheral nerve gaps. Muscle Nerve 39:787-799.
Wright MC, Mi R, Connor E, Reed N, Vyas A, Alspalter M, Coppola G, Geschwind DH, Brushart TM, Höke A (2014) Novel roles for osteopontin and clusterin in peripheral motor and sensory axon regeneration. J Neurosci 34:1689-1700.
Xin H, Wang G, He X (2011) Acellular nerve allograft promotes selective Regeneration. Neural Regen Res 6:257.
Xu H, Holzwarth JM, Yan Y, Xu P, Zheng H, Yin Y, Ma PX (2014) Conductive PPY/PDLLA conduit for peripheral nerve regeneration. Biomaterials 35:225-235.
Yuan J, Chan S, Mortimer D, Nguyen H, Goodhill GJ (2013) Optimality and saturation in axonal chemotaxis. Neural Comput 25:833-853.
Zhou X, Liu Y, Ma J, Sui T, Ge Y, Cao X (2014) Extradural nerve anastomosis technique for bladder reinnervation in spinal cord injury: anatomical feasibility study in human cadavers. Spine 39:635-641.

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