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Inside-out Vein Graft vs Autogenous Nerve Graft in Promoting Axonal Regeneration: An Experimental Study in a Rat Model

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Background: An experimental study was done to compare the efficacy of inside-out vein graft versus autogenous nerve graft as nerve conduit in promoting axonal regeneration in a rat model.
Methods: The study used 16 Spraque-Dawley rats randomly divided into two groups: the inside-out vein graft group and control group (autognous nerve graft). The outcomes measured were histomorphology (axon number and diameter), muscle twitch response (amplitude) and the walking track analysis at 2, 4 6 and 8 weeks.
Results: The inverted vein graft and control groups showed similar axon diameter (P=0.76), and axon number (P=0.85), weeks and similar muscle twitch responses (P=0.87) after eight weeks. The walking track analysis showed no significant difference between the two groups at eight weeks.
Conclusion: The study showed that the inside-out vein graft group had similar motor recovery as compared to control group based on the muscle twitch analysis and walking track analysis in a rat model. In terms of histomorphometric analysis, the two groups were similar in terms of axon diameter and axon count.

Key words: nerve regeneration, nerve repair, muscle contraction

In managing peripheral nerve injuries, primary nerve repair is the treatment of choice. However, in cases where a nerve gap is present, autogenous nerve grafting becomes the standard of treatment.[1-3] Morbidities arising from nerve graft donor sites are often debilitating and may account to as much as 30% of complaints, years after the index surgery.[4]

In order to circumvent such morbidities, substitute nerve conduits have been investigated. Among the most commonly used nerve conduits are of the synthetic type, of which some have been approved by the Food and Drug Administration for clinical use.[5] These synthetic conduits offer no donor site morbidity and with minimal tissue reaction. Commercially available synthetic conduits, such as the polyglycolic acid (PGA) conduit have been studied and were reported to have favorable results both in animal[6,7] and human studies[8-10].

However, natural nerve conduits have been identified and among these, the use of vein grafts was promising in promoting axonal regeneration. There have been an increasing number of studies investigating the use of grafts because of its availability and lack of rejection. The use of vein grafts and its variations to bridge nerve gaps have been described in several studies. In one study, inside-out vein grafts (IOVG) and autogenous nerve grafting were compared in terms of promoting nerve regeneration in Sprague Dawley rats.[11] The results showed comparable or even superior results of the IOVG as compared to nerve grafts in terms of nerve conduction velocities and histologic results (higher axonal counts).

Clinical application of vein grafts for nerve regeneration has been done on neglected digital nerves for the recovery of digital sensation.[12] It was found that vein conduits yielded better sensory recovery in terms of static and moving two point discrimination. These studies showed that vein grafts for nerve regeneration has been limited to sensory evaluation only. Studies evaluating motor recovery from vein conduits were lacking.

Some compared inside out vein graft and a standard vein graft in bridging nerve gaps in rats. Histological analysis of vein grafts showed that the mean axon diameter, fiber diameter and myelin sheath thickness was statistically greater than the standard vein graft.[13] In another study, it was also found that inside out vein

grafts have greater regenerated axon number than standard vein grafts.[14]

Because of the promising results obtained in sensory regeneration using the IOVG model[15], the present authors wanted to determine if motor recovery was also possible using inverted vein grafts as nerve conduits. This may provide a significant alternative to standard autogenous nerve grafting techniques in motor reanimation. The purpose of the study was to compare inverted vein grafts with conventional autogenous nerve grafts in terms of histomorphometric analysis and motor recovery in a rat model.

Methods

In an experimental study design, sixteen Sprague Dawley rats, each weighing 150 to 250 grams were randomly assigned to two groups: the inverted vein graft or inside-out vein graft (IOVG) group and the autogenous nerve graft group (control). Randomization was carried out through sealed, opaque envelopes prior to experimentation. The rats were kept individually in numbered cages with food and water ad libitum. Each rat was assigned an identification number. In cases of rat mortality prior to the end of the study period, each rat was replaced and was assigned to the original treatment group of the deceased rat. An overview of the methodology is seen on Figure 1. The authors adhered to the institutional guidelines for the care and use of animals in research.

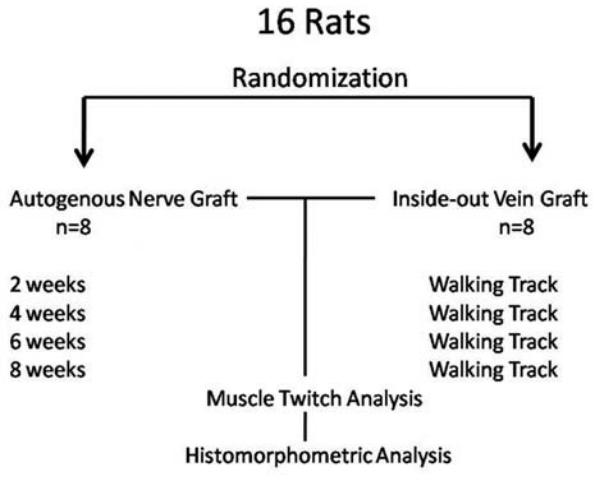

Figure 1. An overview of the methodology.

Nerve Grafting Procedure

The animals were anesthetized by using a combination of 5% Ketamine and 2% Xylazine at 1:4 ratios, applied intra-peritoneally at a dose of 0.10 ml/100 g body weight. The left lower limb was prepared for the surgical procedure using antiseptic solution and site protection with surgical drapes. The animals were positioned at ventral decubitus, with paws tied together in abduction and the sciatic nerve were addressed through a longitudinal straight lateral skin incision, about 3-cm long. The incision extended from femoral major tubercle to lateral femoral condyle, followed by blunt dissection between ischio-tibial and major gluteus muscles, exposing the nerve from its emergence to its distal trifurcation.

After identification of the sciatic nerve, creation of a 10mm nerve gap was done. Radomization was then carried out after exposure of the sciatic nerve. For the autogenous nerve graft group, the 10mm nerve was resected and was then sutured back into place using two or three interrupted 10-0 epineural nylon sutures using an operating microscope with standard microsurgical techniques. The sciatic nerve repair was performed by two surgeons trained in microsurgical procedures. The skin was closed with simple interrupted 3-0 nylon sutures.

For the IOVG group, a 10 mm vein was then harvested from the ipsilateral jugular vein. The vein was then inverted inside-out as described by Rodrigues and Silva.[15] The IOVG was then used to bridge the nerve gap in the sciatic nerve with similar microsurgical technique. No antibiotic prophylaxis was given to the operated rats. Total time for each surgery ranged from 45 minutes to 90 minutes.

Outcomes Assessment of Motor Nerve Recovery

Walking Tract Analysis

Each rat was subjected to a walking track analysis at 2, 4, 6 and 8 weeks after nerve surgery. The animals were made to walk on a 43-cm long x. 8.7 cm wide and 5.5-cm high wooden track, with a dark small shelter at

the end. The hind paws of the rats were dipped in methylene blue solution (5% w/w in water) and the rat permitted to ambulate down the walking track into which a strip of white paper had been placed. Measurements were made from the prints and the Sciatic Function Index (SFI) was computed based on the described formula.[18-19]

Sciatic Functional Index (SFI) Formula

SFI = -38.3 x ([EPL-NPL]/NPL) + 109.5 x ([ETS-NTS]/NTS) + 13.3 x ([EIT- NIT]/NIT) -8.8

Where:
N: Normal or non operated
E: Experimental or Operated
PL: Print Length
TS: Toe Spread or the distance between the first and fifth toe
ITS: Intermediate Toe spread or the distance between the second and fourth toes.

Muscle Twitch Response using Powerlab Instrument

After the rats were subjected to the walking track analysis at the 8th week, the muscle twitch response was measured using the Powerlab® recorder (ADInstruments, Colorado Springs, CO, USA). The rats were re-anesthetized and the sciatic nerve reopened. A nerve stimulator was applied to the sciatic nerve proximal to the repair site at 0.1 mA until reproducible twitches were recorded unto the machine. The recording needles were applied to the gastrocnemius muscle belly and the amplitude (Newtons) were recorded. Eight readings were taken per rat and the average was taken.

As a form of control, the contralateral sciatic nerve was opened in four rats and the twitch response and contraction times were taken. Eight readings were taken and the average was used as a comparator to the inverted vein graft and control groups.

Morphometric Analysis of Nerve Regeneration

After the muscle twitch analysis, the rats were sacrificed. The animals were sacrificed by cervical dislocation after anesthesia with ketamine. and were disposed according to the animal guidelines set by the committee on disposal animals used in research. The sciatic nerves of the operated side were harvested and evaluated for histologic examination. Specimens for histologic examination were taken from the middle of the graft of the two groups (approximately 0.5 cm from the proximal and distal suture sites). The specimens were fixed 0.1% osmium tetroxide in 0.1 M sodium cacodylate buffer. The nerves were sectioned at 1 μm thickness, mounted and stained with toludine blue; they were viewed using a compound microscope at a magnification of 400x. Morphometric analysis (measurement of axon diameter, fibre diameter and myelin sheath thickness) was carried out. The system had been calibrated using a standard 1 mm graticule. The axon and fibre diameters of 200 nerve fibres were measured from each section using a random sampling technique.

Statistical Analysis

All data were encoded in Microsoft Excel XP and analyzed using STATA 10.1 (College Station, Texas). The study used the t-test to evaluate for statistical significance for the differences in histologic (mean axonal count and diameter), the walking track analysis per week and lastly the muscle twitch. The Kruskal-Wallis test was used to compare the muscle twitch groups. If a difference was detected, a pair-wise comparison using Dunn's test was performed. All statistical significance was set at $P \leq 0.05$.

Results

At the end of the study, there were three drop outs because of mortality from the sixteen rats. This included two control group rats and one inverted vein group rat. The other animals showed good health with no signs of infection from the surgical wound. Replacement rats were used as stated in the methodology and completed the eight weeks of evaluation.

On re-exploration of the rats (8th week), the gross appearance of the sciatic nerves in the control group showed that the graft was intact with no signs of

neuroma formation. For the IOVG group, almost all rats had intact sciatic nerves except for one rat which showed partial rupture of the repair at the proximal end. Histomorphometric analysis of the grafted nerves was done, measuring axon diameter and number in each of the grafts. Axon diameter in the IOVG group was comparable to the control group (P=0.7624). Axon numbers in both groups were similar (P=0.855) (Table 1).

In terms of muscle twitch response, only six controls and seven IOVG were available for evaluation of the muscle twitch response. Table 2 shows the median responses of the control, the IOVG and the normal contralateral sciatic nerve groups. Both the control and the IOVG groups showed lower twitch response compared to the contralateral normal sciatic nerve. There was a significant difference among at least one of the medians of the three groups on the Kruskal-Wallis test (P=0.0113). Dunn's multiple comparison test detected a difference between the medians of the control vs contralateral groups (P=0.0280) and the IOVG vs contralateral groups (P=0.0054). No difference, however, was detected between the medians of the control and IOVG groups (P= 0.8726).

Table 1. Summary of histologic and morphometric analysis after 8 weeks.

	Control (n=8)	IOVG+ (n=8)	P-value*
Average Axon Diameter (μm, SD)	3.5, 1.1	3.3, 1.0	0.76
Average Axonal Count	2.2 , 1.2	2.4, 1.5	0.85

t-test, significant at $P \leq 0.05$. +IOVG - Inside-out vein graft

Table 2. Mean muscle twitch response between groups at 8 weeks.

	Control (n=6)	IOVG + (n=7)	Contralateral Thigh (n=4)	P-value*
Median	139.9	98.4	313.2	0.0113
25th Centile	113.5	84.2	303.6	
75th Centile	173.1	232.0	436.4	

Kruskal-Wallis test: significant at $P \leq 0.05$

For the walking track analysis, both control and IOVG groups showed a trend of increasing scores with each time frame (approaching normal). At the second and eighth weeks, the scores of the two groups were not statistically different but the IOVG group showed a strong trend toward better scores with a p value of 0.13 and 0.08, respectively (Table 3).

Table 3. Mean SFI (Sciatic Foot Index) in the Walking Track Analysis at 2, 4, 6 and 8 weeks.

	Treatment Group		
Time	Control (n=8)	IOVG (n=8)	P value*
2 weeks	-74.4, 9.0	-69.2, 6.6	0.13
4 weeks	-74.0, 5.2	-63.7, 6.2	0.005
6 weeks	-68.9, 7.3	-59.9, 7.5	0.02
8 weeks	-68.8, 6.3	-60.5, 8.7	0.08

*t-test, significant at $P \leq 0.05$; IOVG-inside-out vein graft

Discussion

The use of IOVG has been described as a viable alternative for recovery of sensory nerves in humans. In animal studies, IOVG has been shown to be comparable to conventional vein or arterial grafting in terms of axonal anatomy and morphology.[13-15,16] However, assessments of motor recovery after inverted vein graft conduits are lacking. In this study, the axon diameters and axon counts were similar for the IOVG group and control group. This finding was contrary to a study done by Wang and his colleagues where the axon count was found to be better in the IOVG graft as compared to the control group in terms of axon count and conduction velocities.[11] Sacrifice time for that study was at 8 and 12 weeks. The longer duration of their study may be a reason why axon counts in the IOVG was better than the control group.

In order to assess muscle recovery, the present study made use of two methods: the muscle twitch

analysis and the walking track analysis. A powerlab receiver was used to assess muscle twitch strength. Logistical concerns prevented the use of the more ideal examination which was the EMG-NCV test. Thus, muscle twitch was measured as a means to assess motor response to nerve stimulation. Results showed that both the control and IOVG graft groups have smaller twitch amplitude as compared to the contralateral, normal thigh. However, compared to each other, the IOVG graft and control group were similar. The lower amplitude of muscle response in both treatment groups compared to normal showed that eight weeks was not enough for complete recovery.

The walking tract analysis was an objective measure of sciatic nerve motor recovery as described by De Medinaceli et al.[17] It measured the degree of motor recovery by measuring the print length, toe spread and intermediate toe spread as compared to the contralateral, normal foot print. An increasing toe spread and intermediate toe spread with decreasing print length correlated with motor recovery. The sciatic functional index (SFI) described the recovery of the sciatic nerve. The closer the SFI approaches zero, the better the recovery. For the control and IOVG group, there was a trend towards improved SFI scores at the end of the study, which indicated nerve recovery, having no significant difference in SFI at the end of the study period (8th week). However, at four and six weeks, there was a significant difference between the two groups with the IOVG group having better scores. Higher scores for the IOVG graft group correlate with better motor recovery of the sciatic nerve.

At present, the standard of treatment for bridging nerve defects is the use of autogenous nerve grafting. However, because of the similarity in results between the standard method and the use of IOVG, it may be possible to use IOVG as an alternative for bridging nerve defects, for both sensory and motor reconstruction. Comparable results were achieved between autogenous nerve graft or arterial grafts and IOVG in terms of sensory recovery in both animal and human studies.[11-15,18] The good response of the IOVG was attributed by Wang, et al.[11] to the presence of the tunica adventitia that promoted nerve regeneration by providing an environment rich with collagen, laminin and Schwann cells. Levine, et al.[19] further clarified that the success of vein grafts was in part due to the nerve growth factor (NGF) that was present in veins which was responsible for its use in entubilization. In a rat model, immunohistochemical staining showed the presence of NGFs in sciatic nerves and vein graft models. The NGF immunoreactivity was present in the tunica intima and tunica adventitia of femoral veins and arteries but not in the tunica media. Since most arteries are indispensible, the use of veins as graft substitutes is a practical alternative to nerve grafts to serve as nerve conduits. The rationale of inverting the vein rests on the premise that the presence of valves in the veins may block axonal regeneration and thus the inside-out technique of vein grafting was carried out to avoid this.

The IOVG groups showed good promise in this experimental study such that it was comparable to the standard technique of nerve reconstruction using autogenous nerve grafting, both in the anatomy, morphology and motor recovery. At 10 mm defects, the IOVG proved to be satisfactory and comparable to standard nerve grafting. It is possible that IOVG may also be applicable in larger defects.

Conclusion

In summary, the use of IOVG was comparable to the standard autogenous nerve grafting in promoting axonal regeneration as shown in the size and number of the regenerated axons, and on motor recovery of the sciatic nerve as shown in the muscle twitch analysis and sciatic foot index analysis.

References

1. Terzis J, Faibisoff B, Williams B. The nerve gap: suture under tension vs. graft. Plast Reconstr Surg 1975; 56(2):166-170.
2. Lundborg G. 25-Year Perspective of Peripheral Nerve Surgery: Evolving Neuroscientific Concepts and Clinical Significance. J Hand Surg 2000; 25A (3):391-414.
3. Wang WZ, Crain GM, Baylis W, Tsai TM. Outcome of digital nerve injuries in adults. J Hand Surg 1996; 21A: 138-143.
4. IJpma FF, Nicolai JP, Meek MF. Sural nerve donor-site morbidity: thirty-four years of follow-up. Ann Plast Surg 2006; 57(4):391-395.

5. Meek MF, Coert JH. US Food and Drug Administration /Conformit Europe- approved absorbable nerve conduits for clinical repair of peripheral and cranial nerves. Ann Plast Surg 2008; 60(4):466-72.
6. Nakamura T, Inada Y, Fukuda S, Yoshitani M, Nakada A, Itoi S, Kanemaru S, Endo K, Shimizu Y. Experimental study on the regeneration of peripheral nerve gaps through a polyglycolic acid-collagen (PGA-collagen) tube. Brain Res 2004; 1027(1-2):18-29.
7. Matsumoto K, Ohnishi K, Kiyotani T, Sekine T, Ueda H, Nakamura T, Endo K, Shimizu Y. Peripheral nerve regeneration across an 80-mm gap bridged by a polyglycolic acid (PGA)-collagen tube filled with laminin-coated collagen fibers: a histological and electrophysiological evaluation of regenerated nerves. Brain Res 2000; 868(2):315-38.
8. Inada Y, Hosoi H, Yamashita A, Morimoto S, Tatsumi H, Notazawa S, Kanemaru S,Nakamura T. Regeneration of peripheral motor nerve gaps with a polyglycolic acid-collagen tube: technical case report. Neurosurgery 2007; 61(5):E1105-7.
9. Weber RA, Breidenbach WC, Brown RE, Jabaley ME, Mass DP. A randomized prospective study of polyglycolic acid conduits for digital nerve reconstruction in humans. Plast Reconstr Surg 2000; 106(5): 1036-45.
10. Rinker B, Liau JY. A Prospective Randomized Study Comparing Woven Polyglycolic Acid and Autogenous Vein Conduits for Reconstruction of Digital Neve Gaps. J Hand Surg 2011; 36A: 775-81.
11. Wang KK, Costas PD, Bryan DJ, Eby PL, Seckel BR. Inside-out vein graft repair compared with nerve grafting for nerve regeneration in rats. Microsurgery 1995; 16(2):65-70.
12. Lee YH, Shieh SJ. Secondary nerve reconstruction using vein conduit grafts for neglected digital nerve injuries. Microsurgery 2008; 28(6):436-40.
13. Kelleher MO, Al-Abri RK, Eleutério ML, Myles LM, Lenihan DV, Glasby MA. The use of conventional and invaginated autologous vein grafts for nerve repair by means of entubulation Br J Plast Surg 2001; 54(1):53-7.
14. Ferrari F, De Castro Rodrigues A, Malvezzi CK, Dal Pai Silva M, Padovani CR. Inside-out vs. standard vein graft to repair a sensory nerve in rats. Anat Rec 1999; 256(3):227-32.
15. Rodrigues Ade C, Silva MD. Inside-out versus standard artery graft to repair a sensory nerve in rats Microsurgery 2001; 21(3):102-7.
16. Mohammadi R, Azizi S, Delireh N, Hobbenaghi R, Amini K. Functional recovery of sciatic nerve through inside-out vein graft in rats. Chin J Traumatol 2011; 14: 46-52.
17. De Medinaceli L, Freed WJ, Wyat RJ. An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. Exp Neurol 1982; 77: 469-487.
18. Jeon WJ, Kang JW, Park JH, et al. Clinical application of inside-out vein grafts for the treatment of sensory nerve segmental defect. Microsurgery 2011; 31(4):268-73.
19. Levine MH, Yates KE, Kaban LB. Nerve growth factor is expressed in rat femoral vein. J Oral Maxillofac Surg 2002; 60(7):729-33.