Int Surg 2014;**99**:807–811
DOI: 10.9738/INTSURG-D-13-00184.1

Implantation of Nerve Stump Inside a Vein and a Muscle: Comparing Neuroma Formation in Rat

Theddeus O. H. Prasetyono[1], Erythrina Permatasari[2], Esti Soetrisno[3]

[1]*Division of Plastic Surgery, Department of Surgery, Faculty of Medicine University of Indonesia/Cipto Mangunkusumo Hospital, Jakarta, Indonesia*

[2]*Division of Plastic Surgery, Department of Surgery, Faculty of Medicine Diponegoro University/Kariadi Hospital, Semarang, Indonesia*

[3]*Department of Pathology of Anatomy, Faculty of Medicine University of Indonesia/Cipto Mangunkusumo Hospital, Jakarta, Indonesia*

Among many techniques independently reported to manage neuroma formation, manipulation of the nerve stump inside muscle and vein is the most advantageous technique. This study aimed to enrich the basic data of macroscopic appearance and histopathology regarding which technique generates less neuroma: nerve stump implantation inside vein or inside muscle. An experimental study with posttest-only control-group design was conducted in 24 rats that were randomly arranged into 3 groups. One centimeter of the lateral branch of the right ischiadic nerve was cut. Group A served as the control group, where the proximal nerve stumps were left as they were after the excision; whereas the stumps of groups B and C were implanted inside muscles and veins, respectively. The samples were assessed with histologic examination after 4 weeks to measure the morphometric changes in the nerve endings. The data were statistically analyzed with *t* test. All rats healed uneventfully. No thrombosis was found within group C, and the stumps were free of neuroma formation. The muscle group formed smaller neuroma than the control group. Statistical analysis showed significant differences between the groups ($P < 0.05$). The outcome of nerve stump implantation inside the lumen of a vein is superior to the implantation inside a muscle in preventing neuroma formation.

Key words: Amputation – Axons – Neuroma – Wound healing

Corresponding author: Theddeus O. H. Prasetyono, MD, Division of Plastic Surgery, Department of Surgery, Faculty of Medicine University of Indonesia/Cipto Mangunkusumo Hospital, Jl. Diponegoro 71, Jakarta 13410, Indonesia.
Tel.: +62 817858899; Fax: +62 21 31931424; E-mail: teddyohprasetyono@yahoo.com

Among many factors with a role in neuroma formation, local factors are the key. They include superficial location of the nerves, recurrent trauma, and infection.[1,2] There are many published surgical methods of nerve stump manipulation aimed at managing neuroma formation; they vary from excision, shortening the stump, sealing the stump with silicone, and implantation of the stump inside a vein, muscle, or bone, to suturing nerve to nerve in an end-to-side fashion.[1–10] Unfortunately, with regard to nerve stump management at the first surgery, the authors observed empirically that most surgeons only tie up the nerve stumps with threads without great concern on the future of potential painful neuroma formation.

Among these many techniques, manipulation of the nerve stump inside muscle or vein is the most advantageous technique as it is easy to find adjacent muscle and vein; also, the techniques need no exogenous implant and are considered less-extensive procedures. However, to the best of the authors' knowledge, there is only one clinical study comparing the treatment of painful neuroma between nerve stump translocation into muscle and into vein.[11] That study was in favor of the vein group, and it was conducted to measure clinical outcome without histopathologic study on the result of neuroma formation. This study, by comparison, is intended to enrich the basic data of macroscopic appearance and histopathology regarding which technique generates less neuroma: the nerve stump implantation inside a vein or inside a muscle. This study also aims to encourage surgeons to apply a simple and advantageous technique of nerve stump management to prevent the formation of painful neuroma.

Methods

A randomized experimental study with posttest-only control-group design was conducted on 24 pure Sprague-Dawley male rats aged 3 to 4 months, weighing 300 to 400 g. Ethical clearance certificate was issued by the Ethical Research Committee of the Ministry of Health of Indonesia. Each animal underwent a 1-cm excision of the right lateral branch of ischiadic nerve and was randomly arranged into 3 groups. In group A, the proximal end of the nerve stump was left as it was and only marked with one 10-0 nylon suture to make identification easier at the time of tissue harvesting during the second surgery. In group B, the proximal stump of the nerve was implanted inside an adjacent muscle and fixed by a no-tension simple interrupted epineurium suture on the site of the muscle entrance (Fig. 1). The nerve stump in group C was implanted inside the lumen of the femoral vein. The epineurium was stitched to the tunica adventitia with two 10-0 nylon simple interrupted sutures. In anticipation of the small tissue of the nerve stump taken from a small animal, the tissue specimens from proximal nerve stumps were harvested 4 weeks after the surgery for histologic assessment using hematoxylin and eosin (H&E) staining. The animals were killed by standardized laboratory euthanasia. The measurement of neuroma formation area used a magnification microscope (×400) defined in micrometers. Assessment was also directed to evaluate mini fascicles with irregular axons, Schwann cells, and fibroblasts. The result was statistically analyzed with t test using SPSS program Version 10.0 to identify the difference in neuroma formation area between groups. Level of significance was set at $P < 0.05$.

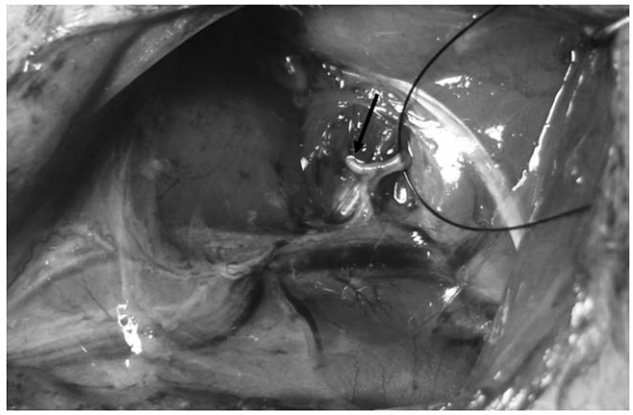

Fig. 1 Nerve stump of the muscle group. Nerve stump in the muscle group right after implantation. The arrow marks the lateral branch of the ischiadic nerve stump, which is buried in muscle.

Results

As baseline data, the diameter of the nerves did not differ between groups as shown in Table 1. In the control group, neuroma appeared as a white mass with a smooth surface, bigger than the proximal part, with adhesion to the surrounding tissue. Eight proximal nerve stumps that were buried inside the muscle were in place without any dehiscence. Minimal scar tissue with some neovascularization was seen on the muscle at the site where the nerve was inserted. Figure 2 illustrates the normal vein

Table 1 Diameter of lateral branch ischiadic nerve after transection

	Diameter, mm		
No.	Group A	Group B	Group C
1	0.62	0.67	0.62
2	0.62	0.58	0.56
3	0.58	0.56	0.75
4	0.73	0.55	0.70
5	0.59	0.64	0.69
6	0.72	0.70	0.76
7	0.63	0.72	0.63
8	0.73	0.62	0.67
Mean	0.66 ± 0.06	0.65 ± 0.07	0.66 ± 0.07

Table 2 Neuroma formation area

	Size of neuroma area, μm^2		
No.	Group A	Group B	Group C
1	19,551	2595	0
2	43,298	0	0
3	41,546	5892	0
4	12,168	12,265	0
5	35,840	14,527	0
6	36,866	6358	0
7	29,869	15,945	0
8	16,681	18,751	0
Mean	29,477.38 ± 11,915.08	9541.63 ± 6770.10	

outlook of group C, demonstrating good circulation without any thrombus formation. The femoral veins were of the same size with good flow performance as shown by patency test.

Table 2 shows details about the size of the neuroma area, which was measured under magnification microscope (×400). Neuroma formation in the muscle group was smaller (mean 9541.63 ± 6770.10 μm^2) than the control group (mean 29,477.38 ± 11,915.08 μm^2), whereas no neuroma was found in the vein group. Statistical analysis showed a significant difference between groups ($P < 0.05$).

Neuroma in the control group developed as a mass with fibroblast, Schwann cells, and irregular axons along with fibrotic tissue, while normal nerve with regular axons appeared in the vein group without any inflammation reaction (Fig. 3). Of interest, the muscle group showed nerve fibers infiltrating the muscle tissue.

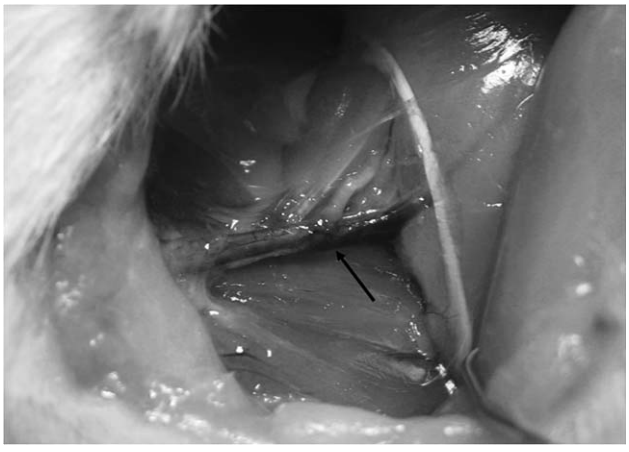

Fig. 2 Nerve stump of the vein group. Nerve stump of the vein group at the second look before sample harvesting. The arrow marks the nerve, which was implanted inside the vein. The picture was taken 4 weeks after the first surgery.

Discussion

This study showed that the muscle group demonstrated smaller neuroma formation, similar to the results reported by Sinis *et al*[10] and Baxun.[12] It seems that nerve stump implantation into the muscle is protective to the axon, where the muscle functions as a barricade to external stimulation. Muscle may prevent nerve regeneration because of its ability in superinnervation. Clinically, it is easy to find a selected host muscle that is sufficient in size to properly implant the stump inside it. Brooks *et al* performed this technique in 10 patients and showed that nerve implantation into a muscle is superior in preventing painful neuroma.[13]

Low *et al*,[4] Koch *et al*,[6,9] and Mobbs *et al*[8] found the neuroma when they implanted the nerve stumps inside veins. They found that the neuroma was formed with a more regular appearance and more myelinated axons compared with the stumps, which were not implanted. It turned out that blood flow did not act as a stimulant for the axons and thus prevented aggressive axon regeneration. Axons regenerate through vein walls that provide a non-immunogenic environment rich in collagen and laminin, which increases vascularization.[14] Low *et al*[4] highlighted that axons do not extend into the vein lumen but regenerate alongside its wall.

Surprisingly, our study failed to show neuroma formation in the vein group. This result may be interpreted as a positive outcome of the vein group in preventing neuroma formation. However, it may be too premature to draw such a conclusion, as longer observation time might have a different outcome. Low *et al*[4] and Koch *et al*[6] also reported that neuroma formation was smaller in the group where the nerve stumps were implanted into veins than in the control group, in which no manipulation was done to the stumps. Presumably, 3 to 8 months

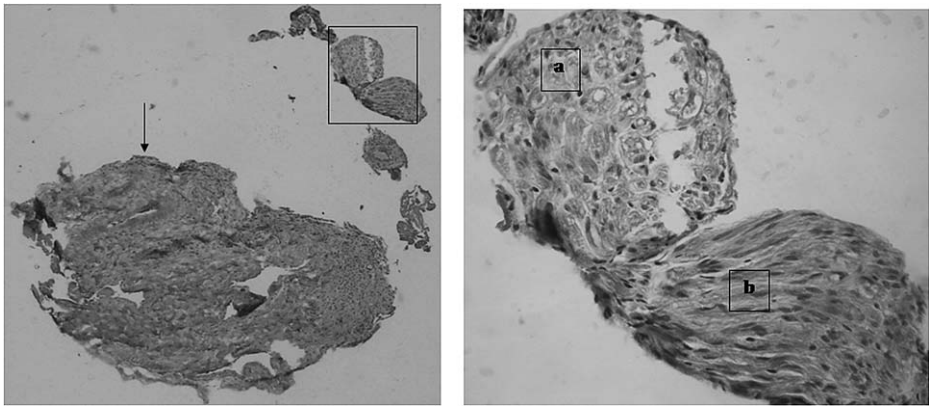

Fig. 3 Histologic examination of the vein group stump. Histology of the nerve stump in the vein group shows regular axon. Regular axons appear with transversal (a) as well as sagittal slice (b). The arrow marks the vein wall.

of observation would reveal neuroma formation. However, our study showed that implantation into veins posed better outcome within the same 4-week period than the group in which the nerve stumps were implanted into muscle. The results of our study are in accordance with the others[4,6,8,9,14] who suggested that implanting the nerve stump inside a vein would prevent painful neuroma formation in the clinical setting. This finding is in accordance with what Balcin *et al*[11] have observed in their clinical study, where nerve stump management is better in the vein group than in the muscle group.

What was also interesting to witness was that we did not observe thrombus in the vein group. We predict that the nerve stump is not a potential thrombogenic substance, although thrombolytic process may have occurred during the 4-week time. Low *et al*[4] reported a similar outcome in which they did not observe thrombus developed inside the veins. They also describe a histologic finding of reendothelization of the vessels covering the neuroma. It is unfortunate that we could not confirm the finding in our study to support the hypothesis that the reendothelization might act as a protective layer for neuroma formation.

In summary, implantation of nerve stump inside vein is potentially superior compared with muscle implantation in preventing neuroma formation. This study may support the clinical practice to manage nerve stumps by implanting them inside a vein in order to get the best outcome in patients.

Acknowledgments

Honored with Purple Lotus Award for Best Research Proposal Presentation in the 14th Association of Southeast Asian Nations (ASEAN) Congress of Plastic Surgery (ACPS) in Conjunction with 12th Annual Scientific Meeting of Indonesian Association of Plastic Surgeons, Jakarta, May 8–11, 2008.

References

1. Mackinnon SE. Evaluation and treatment of the painful neuroma. *Tech Hand Up Extrem Surg* 1997;**1**(3):195–212
2. Dellon AL. Painful neuromas. In: Mathes SJ, ed. *Plastic Surgery.* 2nd ed. Philadelphia, PA: Elsevier, 2006:929–947
3. Herndon JH. Neuromas. In: Green DP, ed. *Operative Hand Surgery.* New York, NY: Churchill Livingstone, 1982:939–955
4. Low CK, Chew SH, Song IC, Ng TH. Implantation of a nerve ending into a vein. *Clin Orthop Relat Res* 2000;**379**(10):242–246
5. Dellon AL, Mackinnon SE. Treatment of the painful neuroma by neuroma resection and muscle implantation. *Plast Reconstr Surg* 1986;**77**(3):427–436
6. Koch H, Herbert TJ, Kleinert R, Hubmer M, Scharnagl E, Pierer G. Influence of nerve stump transplantation into a vein on neuroma formation. *Ann Plast Surg* 2003;**50**(4):354–360
7. Lewin-Kowalik J, Marcol W, Kotulska K, Mandera M, Klimczak A. Prevention and management of painful neuroma. *Neurol Med Chir* 2006;**46**(2):62–68
8. Mobbs RJ, Vonau M, Blum P. Treatment of painful peripheral neuroma by vein implantation. *J Clin Neurosci* 2003;**10**(3):338–339
9. Koch H, Haas F, Hubmer M, Rappl T, Scharnagl E. Treatment of painful neuroma by resection and nerve stump transplantation into a vein. *Ann Plast Surg* 2003;**51**(1):45–50
10. Sinis N, Haerle M, Becker ST, Schulte-Eversum C, Vonthein R, Rosner H *et al*. Neuroma formation in a rat median nerve model: influence of distal stump and muscular coating. *Plast Reconstr Surg* 2007;**119**(3):960–966

11. Balcin H, Erba P, Wettstein R, Schaefer DJ, Pierer G, Kalbermatten DF. A comparative study of two methods of surgical treatment for painful neuroma. *J Bone Joint Surg* 2009; **91**(6):803–808
12. Baxun Z. Experimental study on prevention and treatment of neuroma by implanting nerve stump into muscle. *Chin J Reparat Reconstr Surg* 1998;**102**(6):2282–2283
13. Brooks D, Panthaki Z, Buncke HJ, Buncke GM. Neuroma transposition and intramuscular implantation using the Mitek "soft-tissue anchor": a new technique. *Ann Plast Surg*. 2001; **47**(3):257–262
14. Kelleher MO, Al-Abri RK, Eleuterio 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):53–57