A FREE VEIN GRAFT CAP INFLUENCES NEUROMA FORMATION AFTER NERVE TRANSECTION

MARIAROSARIA GALEANO, M.D.,1 BENEDETTO MANASSERI, M.D.,1 GIOVANNI RISITANO, M.D.,1∗
STEFANO GEUNA, M.D.,2 FEDERICA DI SCIPIO, B.Sc.,2 PAOLA LA ROSA, M.D.,2 GABRIELE DELIA, M.D.,1
FRANCESCO STAGNO D’ALCONTRES, M.D.,1 and MICHELE R. COLONNA, M.D.1

Introduction: Neuroma formation is a major problem in nerve surgery and consensus about its prevention has not been reached. It has been suggested that vein covering can reduce neuroma formation in transected nerves. In this article, the Authors propose an easy and novel method of covering by nerve stump capping with a free vein graft. Methods: Neuroma-like lesions were created on the rat thigh sectioning the femoral nerve above its division in 16 animals. The proximal nerve stump was invaginated into the lumen of a 1.5 cm long femoral free vein graft on the right side, and the vein was closed on itself by microsurgical sutures to form a cap for the nerve stump. On the left side acting as the control neuroma, the nerve was cut and left uncovered. Histological and immunohistochemical assessment was used to quantify the degree of neuroma formation. Results: Our results confirm that vein-covering of a transected nerve stump can be effective in reducing neuroma formation. Moreover, unlike previous works that buried the nerve into an adjacent vein left in place, our experiments showed that also the use of a free vein graft cap can hinder neuroma formation. Although translation of rat experiments to the clinics should be dealt with caution, our data suggest a careful clinical use of the technique. © 2009 Wiley-Liss, Inc. Microsurgery 29:568–572, 2009.

Peripheral nerve lesions represent a common problem for the reconstructive surgeon.1 After transection of a peripheral nerve, Wallerian degeneration occurs distally to the lesion site, whereas proximally regenerating axons sprout into the surrounding tissue and may grow aberrantly forming dense nerve tangles called neuromas, which are often painful and become a source of functional and psychological disability for the patient.2–4 Therefore, one of the main challenges in treating damaged nerves is to prevent the patho-physiological response of transected nerves which leads to neuroma formation.

Despite the variety of surgical treatments that have been explored in an effort to prevent neuroma formation and treat painful neuromas, there is not yet a universally accepted method.3,5 Neuroma prevention methods can be broadly divided in two approaches, namely shortening, or covering the nerve stump. Although nerve stump shortening6 can provide very good clinical results in terms of pain reduction, its positive effects are very often transitory because of neuroma’s natural tendency to recurrence at the new nerve transection site. Searching for alternative prevention strategies, surgeons have attempted to cover the nerve stump with the goal of isolating it from the inflammatory cascade and neurotrophic factors production triggered by nerve trauma in surrounding tissues. Nerve covering can be obtained either by burying the nerve stump into a nearby anatomic structure or by capping it with either biological or synthetic material.7–19

Among the various attempts, burying of the nerve stump into an adjacent vein have been tested both experimentally12,15,20 and with patients10,11,13,14,21 with promising results. Vein tissue are used in nerve surgery also in case of nerve defects where free vein grafts can be used as conduits for bridging the gap.22–24 From this experience, we took the idea to further explore vein tissue covering for neuroma prevention in a rat experimental model using a technique which differs from previous published,12,15,20 because it is based on capping the transected nerve ending with a free vein graft closed with microsurgical sutures, instead of burying the nerve stump into an adjacent vein left in place.

MATERIALS AND METHODS

Experimental Design

All animal procedures were undertaken according to the declaration of Helsinki and to the Guide for the Care and Use of Laboratory Animals. Sixteen male Sprague-Dawley rats weighing 200–300 g were used. During the experiments, the animals were housed one per cage, maintained under controlled environmental conditions (12 hours light/dark cycle, temperature approximately 23°C) and provided with standard laboratory food and water ad libitum.
Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital 40 mg/kg. Both lower limbs were shaved, cleaned with povidone iodine, and draped. A longitudinal skin incision was made along the left lower limb from the midgroin to the knee. The femoral vein, artery, and nerve were identified from medial to lateral. The femoral vein was isolated. The femoral nerve was exposed and transected proximal to its bifurcation. After transection distal nerve elements were resected at least 2 cm distally to avoid spontaneous nerve regeneration. In the same animal, the proximal nerve stump was left free on the left side, while on the right side the whole surgical procedure was repeated, and the proximal nerve stump was implanted into the lumen of a 1.5 cm long vein graft, harvested from the femoral vein and sutured to the vein wall with 10-0 nylon sutures. The distal end of the vein graft was closed with 4-0 nylon. All surgical procedures were performed under an operative microscope (Zeiss OPMI 7). The animals were sacrificed 8 weeks after the operation and the specimens, proximal nerve stump together with its vein cap on the treated side (Fig. 1B), and “nude” proximal nerve stumps on the control side, were excised and processed for morphological analysis.

**Histology**

After fixation in 10% formalin overnight and washing in phosphate buffer saline (PBS), the samples were dehydrated and embedded in paraffin and cut at 8-μm perpendicular to the main nerve axis. For light microscope analysis, sections were stained with Masson’s trichrome stain (Bio-Optica, Milan, Italy) and observed with a Leica DM400 microscope equipped with a Leica DFC320 digital camera and a IM50 image manager system (Leica Microsystems, Wetzlar, Germany).

**Immunohistochemistry and Confocal Laser Microscopy**

For immunohistochemistry and confocal laser microscopy, sections were incubated overnight in a solution containing the anti-neurofilament-200kD (a-NF) (monoclonal, mouse, which recognizes the pig 200 kD subunit of neurofilaments, dilution 1:200, Sigma, St. Louis, MO), and anti-S100 (polyclonal, rabbit, which recognizes the bovine 21 kD monomeric units of S-100 calcium-binding protein, dilution 1:600, Sigma, St. Louis, MO). After washing in PBS, double immunolabeling was carried out by incubating the cuts for 1 hour in a solution containing two different secondary antibodies: CY3-conjugated anti-rabbit IgG (dilution 1:400, Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania) and a goat anti-mouse IgG Alexa-Fluor-488-conjugated (dilution 1:400, Molecular Probes, Eugene, Oregon). The sections were finally mounted with a Dako fluorescent mounting medium and analyzed by a LSM 510 confocal laser microscopy system (Zeiss, Jena, Germany), which incorporates two lasers (Argon and HeNe) and is equipped with an inverted Axiosvert 100M microscope. For specificity assessment, the two antibodies were checked by Western blotting, which showed a single band of staining, and secondary antibody labeling with omission of the primary antibody which led to no immunopositivity. In addition, we have also carried out a “morphology-based specificity test”, consisting of the same immunolabeling protocol to stain sections from normal peripheral nerve where the two proteins are known to be present with a clear localization, easily detectable from a morphological point of view. This test supported the specificity of each reagent by demonstrating that it specifically labels only the tissue elements where the immunogen is known to be present.

---

**Figure 1.** Operative microscope view of the vein-capped proximal femoral nerve stump at time of surgery ([A] original magnification = ×10) and at time of withdrawal (month-2 postoperative) ([B] original magnification = ×6). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
Quantitative Morphological Analysis

In each sample, neuroma formation was quantitatively assessed at both light and confocal microscopy. For light microscopy assessment, neuroma-like cross-sectional area was measured on Masson’s stained sections using point counting technique similar to the technique proposed by Sinis et al.\textsuperscript{18} For confocal laser microscopy assessment, a semi-quantitative evaluation of the axo-glial cytoarchitecture was given to all nerve specimens by the same observer. Each specimen was given a score on a scale ranging from 0 to 4, where 0 is completely disorganized and 4 is normal organization of the axo-glial complex. All assessments were made in blind by the same observer and, in order to standardize the site of quantitative analysis, the sections for both light and confocal quantitative analysis were randomly selected at a distance ranging from 400 to 600 µm from the nerve stump distal ending.

Statistical comparison of quantitative data was subjected to one-way ANOVA test for neuroma size data and to chi\textsuperscript{2} test for axo-glial cytoarchitecture semi-quantitative data. Statistical significance was established as $P < 0.05$. All statistical procedures were performed by using the software “Statistica per discipline bio-mediche” (McGraw-Hill, Milan, Italy).

RESULTS

During the postoperative period, none of the animals showed signs of autotomy or contractions. Histological and immunofluorescence analysis (see Fig. 2) revealed the presence of a disorganized tissue structure in the control neuroma group with haphazardly arranged axons mingled with S100-positive spindle cells (Figs. 2A and 2C). By contrast, the parallel organization of nerve fibers was more preserved in the proximal stump vein-encapsulated nerves displaying an overall normally arranged neural architecture (Fig. 2B). Also myelination was better in vein-encapsulated nerves resembling almost normal (Fig. 2D), while in control neuromas myelination was poor (Fig. 2C). Quantitative evaluation of the size of neuroma-like formation showed that neuromas were significantly ($P < 0.05$) smaller in the vein-capped group ($0.14 \pm 0.11$ mm) in comparison to the control group ($0.32 \pm 0.14$ mm). Yet, semi-quantitative evaluation at laser con-
focal microscopy after a-neurofilament and a-S100 double immunostaining showed that axo-glial cytoarchitecture was more preserved in the vein-capped group in comparison to the control group (2.4 ± 0.5 vs 1.4 ± 0.5, P < 0.05, on a scale where 0 is completely disorganized and 4 is normal nerve cytoarchitecture).

**DISCUSSION**

Transection of a peripheral nerve may result in the formation of a painful neuroma and numerous surgical strategies have been proposed to prevent this occurrence.3

In particular, previous evidences12,14,15,20 have been published suggesting that veins could be used to prevent neuroma formation, and it has been demonstrated that burying transected nerve stumps into an adjacent vein left in place was an effective method in preventing neuroma formation.

Starting from these experiences, we conceived an original approach, consisting of capping the transected nerve stump with a free vein graft. To be validated, the results of our novel technique had to be compared first with a control group of simply transected (“nude”) stumps, as we made in this study. To obtain the most homogeneous results, avoiding individual differences and reactivity, control and treated stumps were obtained in the same animal, respectively, on the different lower limbs. Moreover, the surgical procedures were easy and reproducible.

The results of the present experimental study confirmed the previous evidence that covering the transected nerve stump with vein tissue can reduce neuroma formation.

We showed neuroma reduction both by a reduced area of disorganized nerve tissue at light microscopy observation and by a better axo-glial cytoarchitecture at confocal laser microscopy observation.

Neuroma formation is the result of different factors that lead to a regenerative overstimulation of the proximal nerve fibers.4

In injured nerves inflammation occurs and macrophages, lymphocytes, mast cells and Schwann cells up-regulate the expression of neurotrophins, neural cell adhesion molecules, cytokines, and other soluble factors and yet infiltration of inflammatory cells is closely associated with neuropathic pain.28,29

We propose two mechanisms to explain the effectiveness of vein capping of a transected nerve stump in preventing neuroma formation.

The first, by invaginating the nerve stump into the vein graft closed on itself, the nerve is mechanically isolated from the surrounding inflammation and scar tissue.

The latter, on the inside of the venous conduit the effects of distal and surrounding growth factors and of the dynamic environment are reduced and the regenerative response can be attenuated.5,15,20 In this view, it is interesting to note that, in the rat, it has been shown that neuroma formation and neuropathic pain can be reduced by inactivation of both NGF30 and BDNF.31 It can thus be hypothesized that isolation of the nerve stump from the environment exerts a preventive effect on neuroma formation also by isolating transected axons from the neurotrophins that are produced by the tissues surrounding the injury sites.

Moreover, from a technical point of view, there are several advantages of our technique if compared to other experiences with veins in neuroma formation: first, neither major venous trunks nor important segmental veins, even if adjacent to the nerve stump (as in case of severe digital trauma with few dorsal vein still draining) should be sacrificed, such as in the method proposed by other Authors using vein lumen as a recipient bed for nerve stumps.

Moreover, veins potentially available for nerve capping (e.g. subcutaneous veins) are easily accessible and available throughout the body; however, to preserve segmental vein drainage, we actually recommend to find donor veins close to the severed parts, but in safe segments and compartments.

Yet, technically speaking, veins can be easily dilated with fine surgical forceps to adapt the lumen to the nerve size32 and can thus be used to treat large nerves, such as the sciatic or the common peroneal nerves.

Finally, although successful capping of nerve stumps with numerous synthetic materials (such as silicon17 and collagen19 conduits) has been reported, veins have the advantage of being obtainable at no cost, and, unlike nerve stump burying into an intact and perfused adjacent vein,14 this technique does not cause any risk of venous thrombosis.

However, in this study we can compare our vein capping method with the other methods of vein burying only from a technical point of view, and we can not demonstrate whether vein caps rather than vein burying are more effective in neuroma prevention.

We understand that this comparison can be important from the speculative point of view and propose that it could be the aim of further studies.

**CONCLUSIONS**

The experimental study reported in this article confirms that implantation into a vein cap can represent an important alternative in surgeon’s hands for neuroma prevention. The use of a free vein graft for nerve stump capping that we proposed is original and makes the surgical technique easy and fast without requiring extensive
dissection. Therefore, on the basis of our experimental findings, its clinical use could also be encouraged.

REFERENCES