Injury, Int. J. Care Injured xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Injury

journal homepage: www.elsevier.com/locate/injury

Full length article

The effect of in vivo created vascularized neurotube on peripheric nerve regeneration

Abdul Kerim Yapici[a,*], Yalcin Bayram[a], Hakan Akgun[b], Recep Gumus[c], Fatih Zor[a]

[a] Gulhane Military Medical Academy, Dept. of Plastic Surgery, Ankara, Turkey
[b] Gulhane Military Medical Academy, Dept. of Neurology, Ankara, Turkey
[c] Gulhane Military Medical Academy, Dept. of Histology, Ankara, Turkey

ARTICLE INFO

Article history:
Received 17 December 2016
Received in revised form 5 May 2017
Accepted 9 May 2017

Keywords:
Nerve regeneration
Vascularized conduit
Non-vascularized conduit
Biologic conduit

ABSTRACT

Introduction: Creating vascularized nerve conduits for treatment of nerve gaps have been researched, however, these methods need microsurgical anastomosis thereby complicating the nerve repair process. Thus, the concept of vascularized nerve conduits has not popularized up till now. The aim of this study is to evaluate the effects of vascularized and non-vascularized biological conduits on peripheral nerve regeneration.

Material and methods: Following ethical board approval, 15 Sprague-Dawley rats were used in the study. The rats were equally divided into three groups. In group I, a silicon rod was inserted next to the sciatic nerve of the rat and connective tissue generated around this rod was used as a vascularized biological conduit. In group II, a silicon rod was inserted into the dorsum of the rat and connective tissue generated around this rod was used as a non-vascularized biological conduit. In group III, autogenic nerve graft was used to repair the nerve gap. The contralateral sciatic nerve is used as a control in all rats. Macroscopic, electrophysiological and histomorphometric evaluations were performed to determine the nerve regeneration.

Results: There was no statistically significant difference between groups, in terms of latency. However, the mean amplitude of group I was found to be higher than other groups. The difference between group I and II was statistically significant. Myelinated axonal counts in group I was significantly higher than groups II and III.

Conclusion: Our results showed that vascularized biological conduits provided better nerve regeneration when compared to autografts and non-vascularized biological conduits. Creation and application of vascularized conduits by using the technique described here is easy. Although this method is not an alternative to autogenic nerve grafts, our results are promising and encouraging for further studies.

Introduction

Autologous nerve grafts are the gold standard for repair of nerve gaps as they have critical elements for nerve regeneration such as basal lamina and Schwan cells. [1–3] However, the results following autologous nerve grafts are still suboptimal and cause donor site morbidity. Stemming from these disadvantages, there are many studies searching for alternative nerve conduits to autologous nerve grafts. [4] Vein grafts, collagen, basal lamina scaffolds, de-cellularized allografts and synthetic conduits are all considered as an alternative to nerve autografts. [3–11] Tissue engineered nerve conduits were also studied. These grafts included various growth factors, mediators and cellularization of the nerve conduit by Schwann or stem cells. [4] The effect of vascularization of the nerve graft was first mentioned by Kanaya et al., however, vascularized nerve grafts have not gained popularization due to surgical complexity and donor side morbidity. [12] Despite all these studies, autologous nerve grafts still remain the best option.

Hunter et al. presented the histological features of pseudo-synovial sheath which is generated around a silicone rod. [13] They demonstrated that this pseudo-synovial sheath had three distinctive layers, namely intima, media and adventitia. The intimal cells contain a glycosaminoglycan substance and have a secretory capacity, the media cells have large amounts of collagen and provide structural and vascular support and the adventitia is a

* Corresponding author at: General Dr. Tevfik Sağlam Cad. GATA Plastik, Rekonstrüktif ve Estetik Cerrahi Anabilim Dalı, Keçiören/Ankara, Turkey.
E-mail address: drakyapici@gmail.com (A.K. Yapici).

http://dx.doi.org/10.1016/j.injury.2017.05.014
0020-1383/

highly vascular structure. The idea of using this pseudo-synovial sheath for nerve repair was first mentioned by Lundborg. [14] Following Lundborg, several studies reported successful usage of biological conduits as nerve conduits, [14–21] however, there is no study comparing the peripheral nerve regeneration with vascularized and non-vascularized biological conduits.

In this study, we aimed to evaluate the effect of vascularization of biological nerve conduits on peripheral nerve regeneration on a rat sciatic nerve model.

Material and methods

Following Local Ethical Board approval, a total of 15 Spraque-Dawley rats, weighing between 250-300 g were included in the study. All operations were performed under ketamine (Ketalar®, Eczacibasi, Istanbul, Turkey, 35 mg/kg i.m.) anesthesia. All surgeries were performed on the right sciatic nerves of the rats and left sciatic nerves were used as controls. In order to create biological conduits, 1.5 cm silicone rods 1 mm in diameter were placed either in the rat dorsum or next to the sciatic nerve. The fibrovascular sheath generated around the rod after 8 weeks was used as a biological conduit. Nerve coaptation was performed under the microscope magnification (Leica M525 F40, Germany), using 9/0 Ethilon (Ethicon®, US, LLC) sutures.

The rats were randomly divided into three groups. In groups I and II, the operations were performed in two stages and in group III a single stage was performed. The experimental protocol is seen in Table 1. In group I (the vascularized biological conduit group), the right sciatic nerve was exposed with the gluteal split technique and a 1.5 cm silicone rod (1 mm in diameter) was placed parallel and next to the sciatic nerve (Fig. 1a). Following 8 weeks, the second operation was performed. The right sciatic nerve and its three main trunks were exposed from the sciatic notch at the proximal end to the tarsal tunnel at the distal end by a gluteal split technique. A 1 cm nerve defect was created just 0.5 cm distal to the sciatic notch. The silicone rod, which was placed next to the sciatic nerve, was removed without harming the fibrovascular sheath created around the rod. Briefly, in order to preserve the vascularization of the nerve conduit no dissection was performed of the conduit. the process performed only involved opening both ends of the conduit, removing the silicone rod and using the tube as a nerve conduit. The proximal and distal ends of the fibrovascular sheath were trimmed in order to leave a 1 cm fibrovascular sheath. The proximal and distal ends of the sciatic nerve were re-routed to the proximal and distal openings of the fibrovascular sheath. In this way, the nerve gap was bridged by a vascularized fibrovascular sheath, referred to as a vascularized biological conduit (Fig. 1b–d). In group II (the non-vascularized biological conduit group), a 1.5 cm silicone rod (1 mm in diameter) was placed to the dorsum of the rat in a subcutaneous pocket and following 8 weeks, the second operation was performed. In the second operation, the right sciatic nerve was exposed and a 1 cm nerve gap was created 0.5 cm distal to the sciatic notch. The fibrovascular sheath, created at the dorsum of the rat was harvested as a graft and trimmed in order to leave 1 cm of fibrovascular sheath (Fig. 2a). This sheath was used to bridge the nerve gap (Fig. 2b), which is referred to as a non-vascularized biological conduit. In group III (autograft group), the right sciatic nerve was exposed and a 1 cm nerve defect was created 0.5 cm distal to the sciatic notch and the nerve gap was bridged by autologous nerve graft.

Gross examination

Following 8 weeks from the nerve repair surgery, both sciatic nerves of the rats were explored. All animals received general anesthesia (with ketamine), and sciatic nerve explorations were performed by experienced microsurgeons under magnification and the sciatic nerve was protected from iatrogenic injury. Nerve repair sites were evaluated for scar formation, adhesion, and neuroma formation.

Electrophysiological evaluation

Following macroscopic evaluation of the nerves, electrophysiological evaluation was performed with electromyography (EMG). EMG is performed with a (2 + 8) channel EMG device (Medelec Synergy, USA). The entire electrophysiological procedure was carried out at room temperature. The stimulating electrode was a Teflon-coated bipolar hook stimulator and applied to the nerve just distally to the sciatic notch. Supramaximal stimulus was given to the sciatic nerve. The recording electrodes were Dantec 13L0512 concentric EMG needles and applied to the soleus muscle 10 mm distally to the tibial tubercle. The distance between the stimulating electrode (as cathode) and the recording electrode was 40 mm. The frequency limits of the amplifier were 500 Hz to 10 kHz. Each potential was recorded five times. The measurement of latency was performed according to the point where the first peak was seen at the isoelectric line. The amplitude measurement was taken from peak to peak. The same measurements were made at the left sciatic nerve and used as a control.

Histomorphometric evaluation

Histomorphometric evaluation included myelinated axonal counts. The rats were euthanized following the electrophysiological procedure. For assessment of myelinated axon counts, 2 mm of sciatic nerve samples were obtained from 5 mm distally to the distal nerve repair site. Control samples were obtained from the same site at the left sciatic nerve. All samples were fixed in 2.5% glutaraldehyde and post-fixed in 2% osmium tetroxide and embedded in Araldite

Table 1
Experimental protocol.

	First operation		Second operation		Evaluation
Group I (vascularized conduit)	Silicone tube placed next to sciatic nerve	8 weeks	Creation of nerve defect + Repair with vascularized conduit	8 weeks	• Gross examination • Electrophysiologic evaluation (EMG) • Histomorphometric evaluation
Group II (non-vascularized conduit)	Silicone tube placed to dorsum of the rat		Creation of nerve defect + Repair with non-vascularized conduit		
Group III (autograft)	–		Creation of nerve defect + Repair with autograft		

Please cite this article in press as: A.K. Yapici, et al., The effect of in vivo created vascularized neurotube on peripheric nerve regeneration, Injury (2017), http://dx.doi.org/10.1016/j.injury.2017.05.014