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Modern Diagnostics of Nerve Regeneration after Surgery

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Annotation: A fundamentally new approach in experimental neurohistology can be called tubulation using conductors - tubes, which serve as a substrate for the growth of nerve fibers through the site of injury of the nerve trunk [25, 26]. In conditions of diastasis, such a substrate can also be a reservoir for a filler that stimulates regeneration processes. In addition, the conductor prevents the penetration of surrounding connective tissue into the site of injury, which limits the formation of a connective tissue scar that prevents the passage of growing nerve processes to the distal tail [21, 27].

Keywords: peripheral nerve; nerve regeneration; nerve graft channel; Reperen; regeneration.

The aim of the study was to investigate the effect of non-absorbable conductors from Reperen and non-absorbable fibrin from Tissukol on the regeneration of the rat sciatic nerve in conditions of diastasis of its tails.

Materials and methods. The experiments were conducted on white non-striped male rats of reproductive age (n = 14). The animals were divided into three groups: 1 - intact (n = 5) - the morphology of the sciatic nerve in the area of the planned plastic surgery was studied; 2nd - (n = 4) nerve grafting was performed using a conductor made of non-resorbable Reperen material; 3rd (n = 5) - plastic surgery was performed using a conductor made of absorbable Tissucol material. The animals were anesthetized with isoflurane. After completely transecting the sciatic nerve at the level of the middle third of the thigh, its tails were inserted into a conductor filled with saline with an internal diameter of 2 mm and a length of 10 mm. A 5 mm diastasis was created by spreading the ends of the nerve and fixing the epineurium to the edges of the tube with interrupted

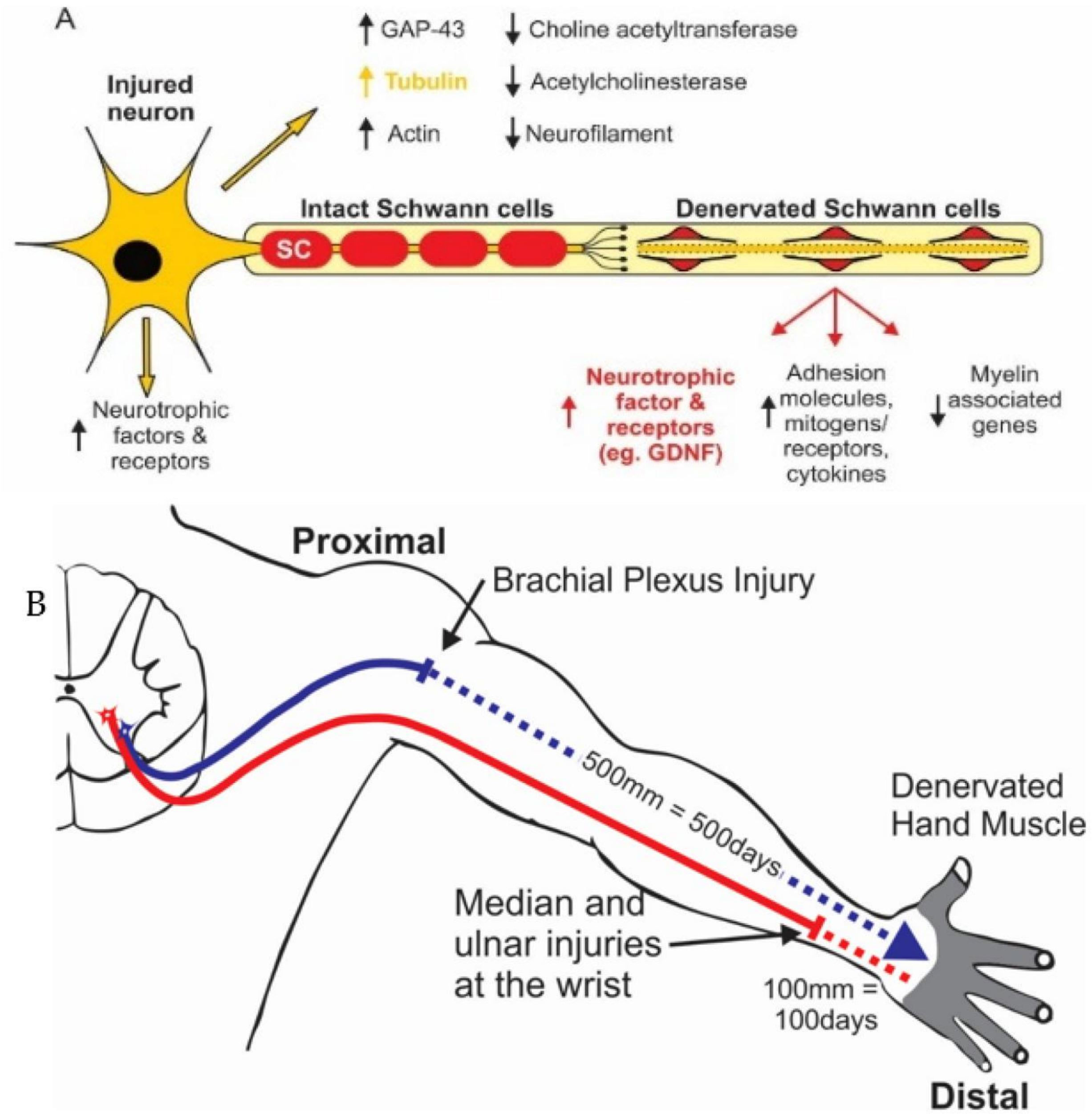
sutures using 8/0 polypropylene monofilament. The total number of myelinated nerve fibers in the area of plasticity (tubulation) in the distal part of the nerve was measured, and the characteristics of the formation of the connective tissue sheath were determined 14 weeks after injury.

Results. The structural features of the sciatic nerve in the tubular area were studied using resorbable and non-resorbable conductors for its reconstruction. When using both types of conductors, stereotypy of the number of nerve fibers in the distal part of the reconstructed nerve and close to normal clinical parameters were revealed.

Conclusion: The results obtained allow us to consider a non-absorbable Reperen material conductor, along with a resorbable Tissucol conductor, as promising for neuroplasticity.

Introduction

Peripheral nerve damage leads to a decrease or loss of function of the affected limb and disability of the patient, therefore, the restoration of nerve structures is one of the main directions of neurosurgery and neurohistological research [1].



Suturing of nerve trunks is inevitably accompanied by the formation of connective tissue scarring, which leads to only partial restoration of limb function [2-4]. The main method of microsurgical treatment of diastasis of the damaged nerve trunk is its autoplasty with sections of other peripheral nerves [5-13]. During this operation, the blood supply to the repaired nerve is disrupted, which reduces the rate of its regeneration and contributes to the formation of scars in the connective tissue [14-20]. In addition, autoplasty is accompanied by pain due to additional incisions in the donor areas, impaired sensitivity, and the formation of neuromas [21-24].

Currently, there are studies with empty non-biodegradable and biodegradable tubes made of various materials, which are used to study the effect of fillings on nerve regeneration [5, 23, 24, 27-32]. Good results have been obtained with biodegradable conductors, including those made of fibrin glue - Tissucol [27, 31, 32]. In neurosurgery, Reperen is successfully used - a non-biodegradable biocompatible material used in dura mater plastic surgery, which keeps brain structures intact and prevents the formation of adhesions with surrounding tissues [33]. Reperen plate has been used to isolate the trigeminal nerve root by wrapping it to eliminate severe pain attacks in the innervation zones during arterial pulsation [34]. However, in the clinic, tubes made of non-biodegradable materials are rarely used for peripheral nerve plastic surgery, and preference is given to biodegradable ones.

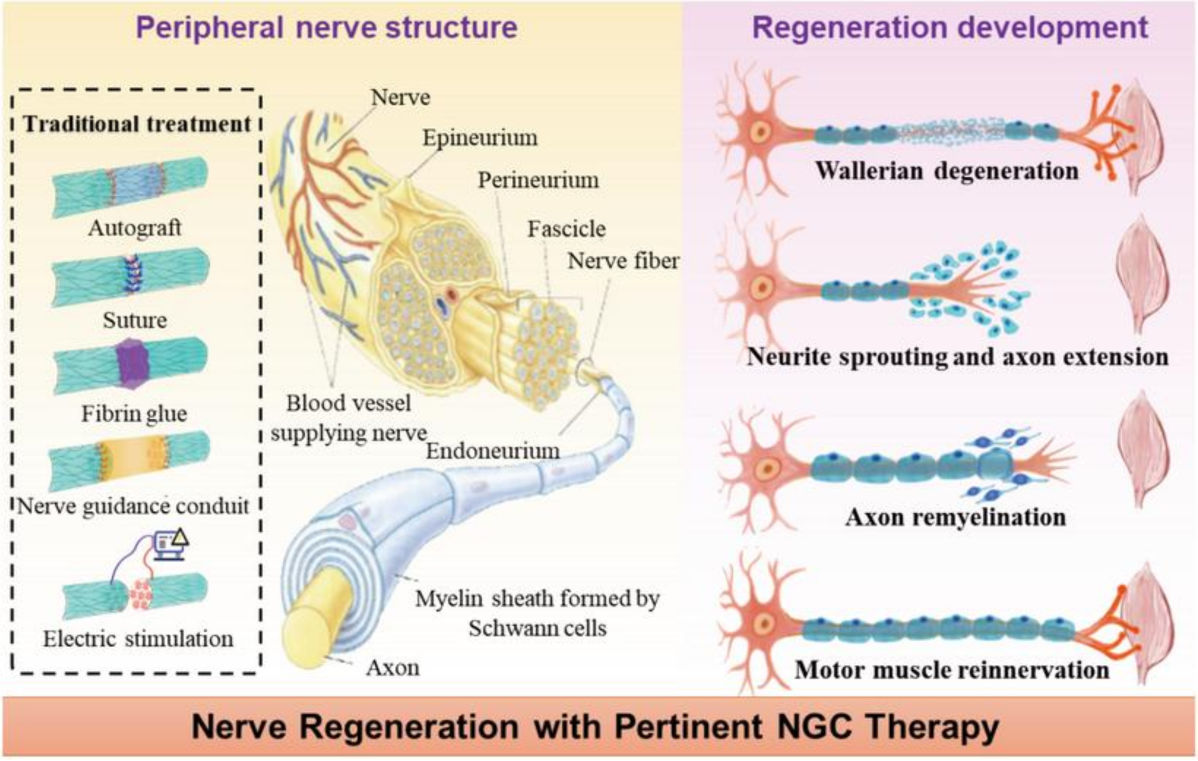
In this regard, the aim of the study was to compare the properties of using rigid tubular conductors made of the non-absorbable Reperen material and the resorbable fibrin material Tissucol and their effect on the regeneration of the sciatic nerve in rats.

Materials and methods

The experiments were conducted in accordance with Good Laboratory Practice (GLP) standards on male white non-striped rats of reproductive age (n=14) weighing 350–400 g. Animal care and experiments were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 2020), as well as the ethical principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 2016). The work was approved by the Ethics Committee of the Volga Regional Research Medical University.

The animals were divided into 3 groups: 1st (n=5) - intact, the morphology of the sciatic nerve in the area of the planned plastic surgery was examined; 2nd (n=4) - neuroplastic surgery was performed using a guide made of non-absorbable Reperen material; 3rd (n=5) - neuroplastic surgery with a guide made of absorbable Tissucol material.

Animals were anesthetized with isoflurane using a Zoomed minor veterinary anesthesia machine (Beijing Read Eagle Technology Co., Ltd., China) and a 7F-3L oxygen concentrator (Jiangsu Yuyue Medical Equipment and Supply Co., Ltd., China). Access to the sciatic nerve of the right hind limb was achieved by cutting the skin along the projection of the femur and then bluntly dissecating the muscle fascia. The isolated nerve was transected at the level of the middle third of the thigh, and its tails were inserted into a saline-filled conduit with an internal diameter of 2 mm and a length of 10 mm. A 5-mm diastasis was created by spreading the ends of the cut nerve, immersing these ends in the conduit to a depth of 2.5 mm on both sides, and securing the epineurium to the edges of the conduit with interrupted sutures using 8/0 polypropylene monofilament.

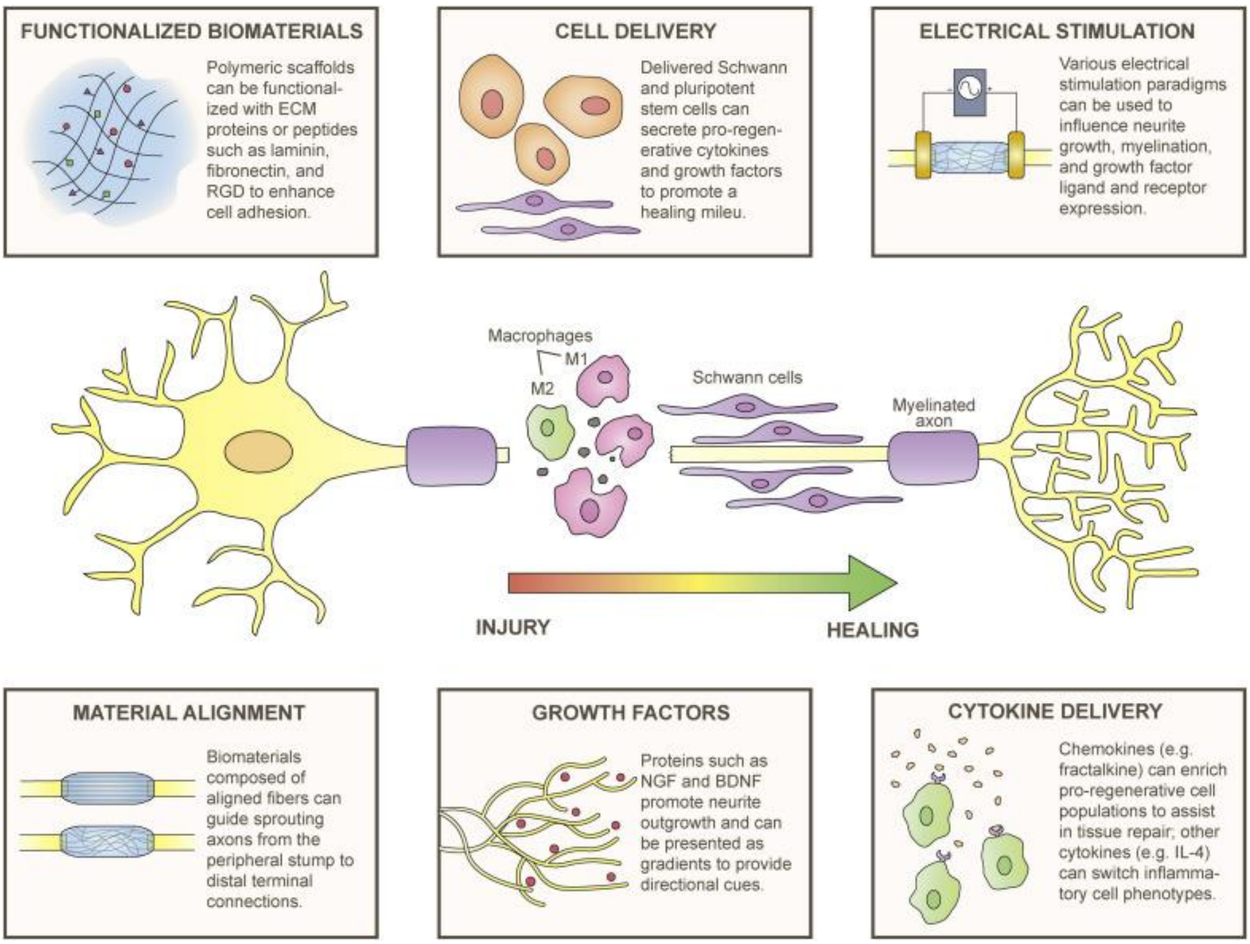


Two types of conductors were used in the experiments. The conductors from Reperend (Icon Lab, Russia) were supplied in the form of ready-made tubes in sterile packaging;

Morphological analysis was performed on semi-thin (0.5 μm) transverse sections of the sciatic nerve prepared on a Leica EM UC7 ultramicrotome (Leica, Germany) and stained with methylene blue and fuchsin. The developing nerve was examined in its diastasis area and in the distal tail 1 mm from the conductor. The material was fixed in a 2.5% solution of glutaraldehyde in phosphate buffer (pH = 7.4) and post-fixed in a 1% solution of osmium tetroxide, then embedded in an Epon-Araldite resin mixture. Photographs of the sections were taken using a Nikon Eclipse 80i light microscope (Nikon DS-Fi1 camera) (Nikon, Japan) at ×10 and with ×10 and ×20 objectives.

Morphometric analysis of the regenerated nerve sections was performed using the NIS-Elements BR 4.0 software. The following was performed: 1) the total number of regenerated myelinated nerve fibers and their assessment in size groups based on the diameter of the nerve fiber according to the classification [35] that distinguishes small (less than 4 μm in diameter), medium (4–7 μm), and large (more than 7 μm) fibers; 2) analysis of the total cross-sectional area of the nerve, the area of the nerve fiber bundles, and the area of the epineurium.

Statistical processing of the obtained data was carried out using the Statistica 10.0 program using a set of basic and non-parametric statistics. The statistical significance of the differences in the values between the compared quantities was assessed using the Mann-Whitney test at $p<0.05$.



Results

Morphology of the intact sciatic nerve. Typically, the studied section of the sciatic nerve consists of an average of 4 nerve bundles, each of which is surrounded by a perineurium. The epineurial membrane is thin, with a moderate development of adipose tissue (Fig. 1, a). The total area of the sciatic nerve of a mouse is normally 1,365,632.0 ± 180,727.5 m². In our case, the area of the nerve without the epineurium is 782,832.3 ± 160,703.0 μm², and the area of the epineurium is 582,799.5 ± 110,457.5 μm².

a - intact animal; b - resuspended in the Reperen conduit at the end of the 14th week of the experiment; in - resuspended in the conduit from Tissukol at the end of the 14th week of the experiment; A - nerve fibers, B - perineurium, B - epineurium; staining with methylene blue and fuchsin

Analysis of the size groups of myelinated nerve fibers showed that the largest number of large fibers (d>7 μm), their number is 57.7%, the share of medium fibers (4<d<7 μm) is 37.3%, and small fibers (d<4 μm) - 5.7%.

Morphology of the developing sciatic nerve in the area of tubulation. At week 14 of the experiment, the area of injury showed significant morphological differences when using saline-filled Reperen and Tissucol conduits.

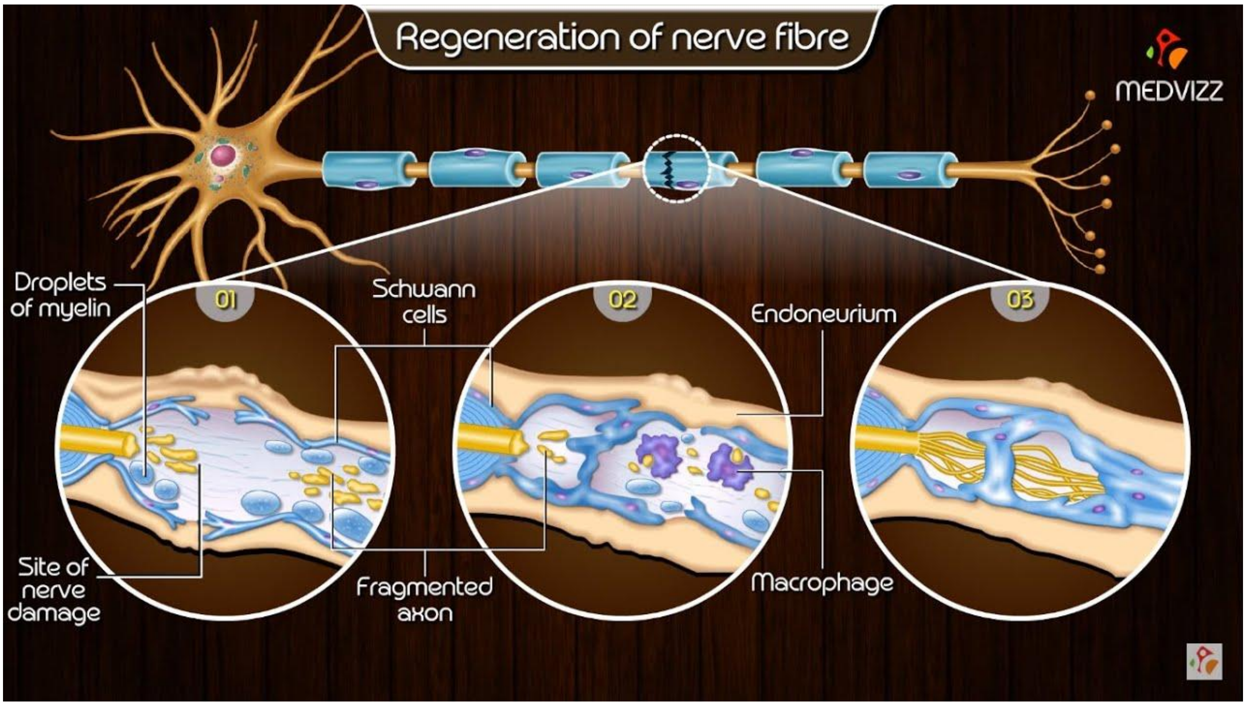
In the regenerative state, the developing nerve was thinner than its distal part and the intact nerve. The regenerating nerve trunks were located inside the intact resorption tube and did not adjoin its inner surface (Fig. 2, a). It contained a single bundle of nerve fibers (Fig. 1, b). The average cross-sectional area of the nerve with the epineurium was 1.7 times smaller than normal, and without the epineurium 2.1 times smaller than normal. The cross-sectional area of the epineurium was 1.37 times smaller than intact values. Among the size groups, medium-sized myelinated nerve fibers predominate, accounting for 53.7% of the total number (see table). The proportion of small fibers is 35.1%, large fibers are 11.2%. Moreover, the number of myelinated nerve fibers in all size groups is statistically significantly different from normal values.

a - From Reperendan; b - From Tissukol (indicated in brackets)

velichanskaya-tablet.jpg The number of myelinated nerve fibers in the sciatic nerve using non-biodegradable and biodegradable conductors

14 weeks after surgery, the Tissucol canal was completely resorbed. The regenerated area was significantly thicker than the nerve in the Reperen canal. The perineurium was not defined and the nerve fibers were presented in the form of a single bundle. The epineurium was very thickened and had a loose structure. In this regard, the total area of the nerve increased significantly and exceeded the intact values by 2.68 times. In addition, the average area of the epineurium was almost 4.0 times higher than normal. The average area of the nerve without epineurium was 1.76 times larger than the intact nerve.

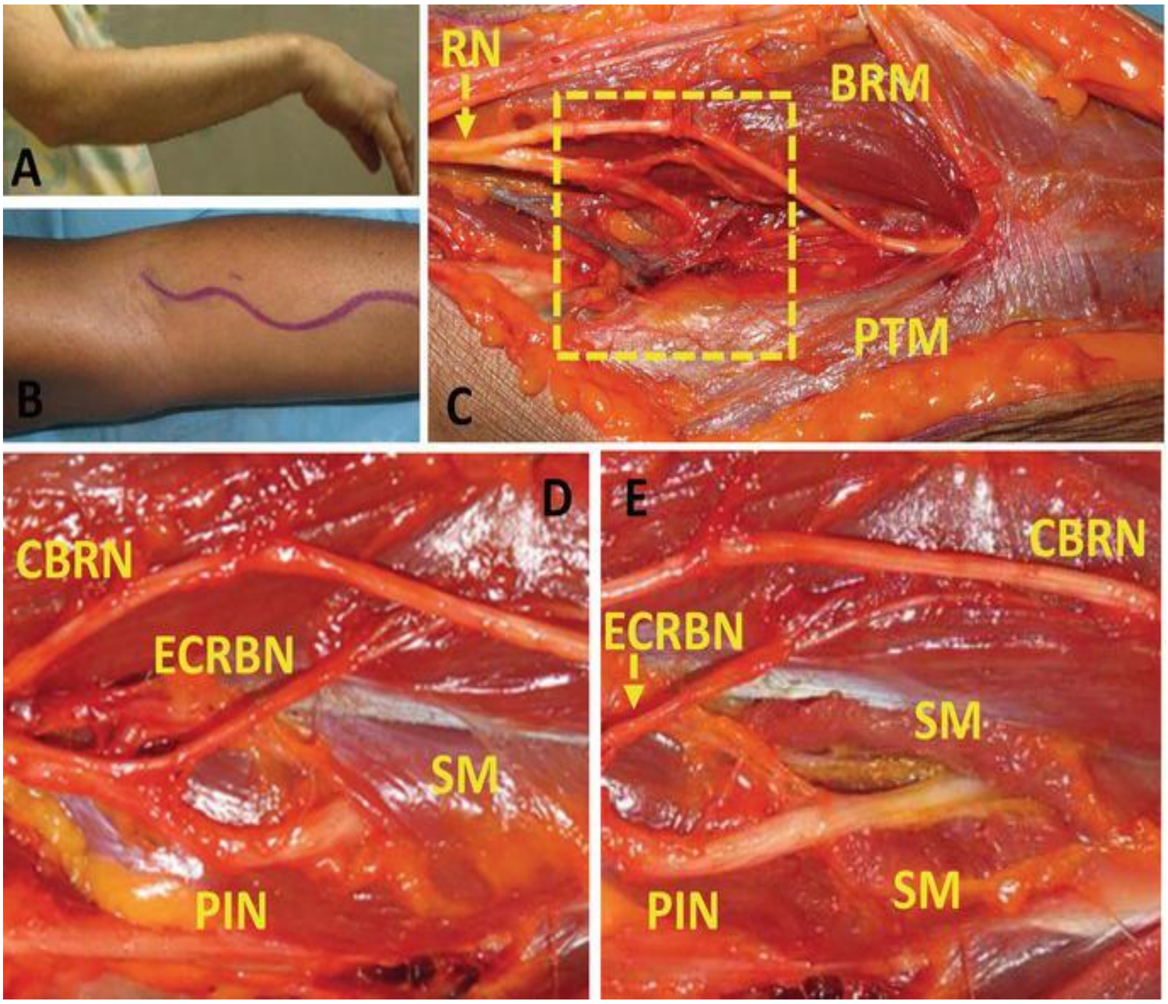
The total number of myelinated fibers in the lesion area increased by 3.6 times compared to the norm (see table). Among the size groups, small myelinated fibers predominate, accounting for 65.3% of the total number. The amount of medium fibers was 29.6%, which was not statistically significantly different from the norm, the proportion of large fibers was only 5.1%.



In addition, significant differences were found when using the named conductors. At the end of the experiment, no adhesion was observed around the Reperen. The conductor was covered with a thin layer of connective tissue and was easily separated from the surrounding tissues. When using Tissucol, in all animals, after the conductor resorption, the newly formed nerve area was separated from the surrounding tissues with difficulty due to a pronounced adhesion process.

Morphology of the distal sciatic nerve. The morphology of the distal nerve trunk had significant differences when using both types of conductors.

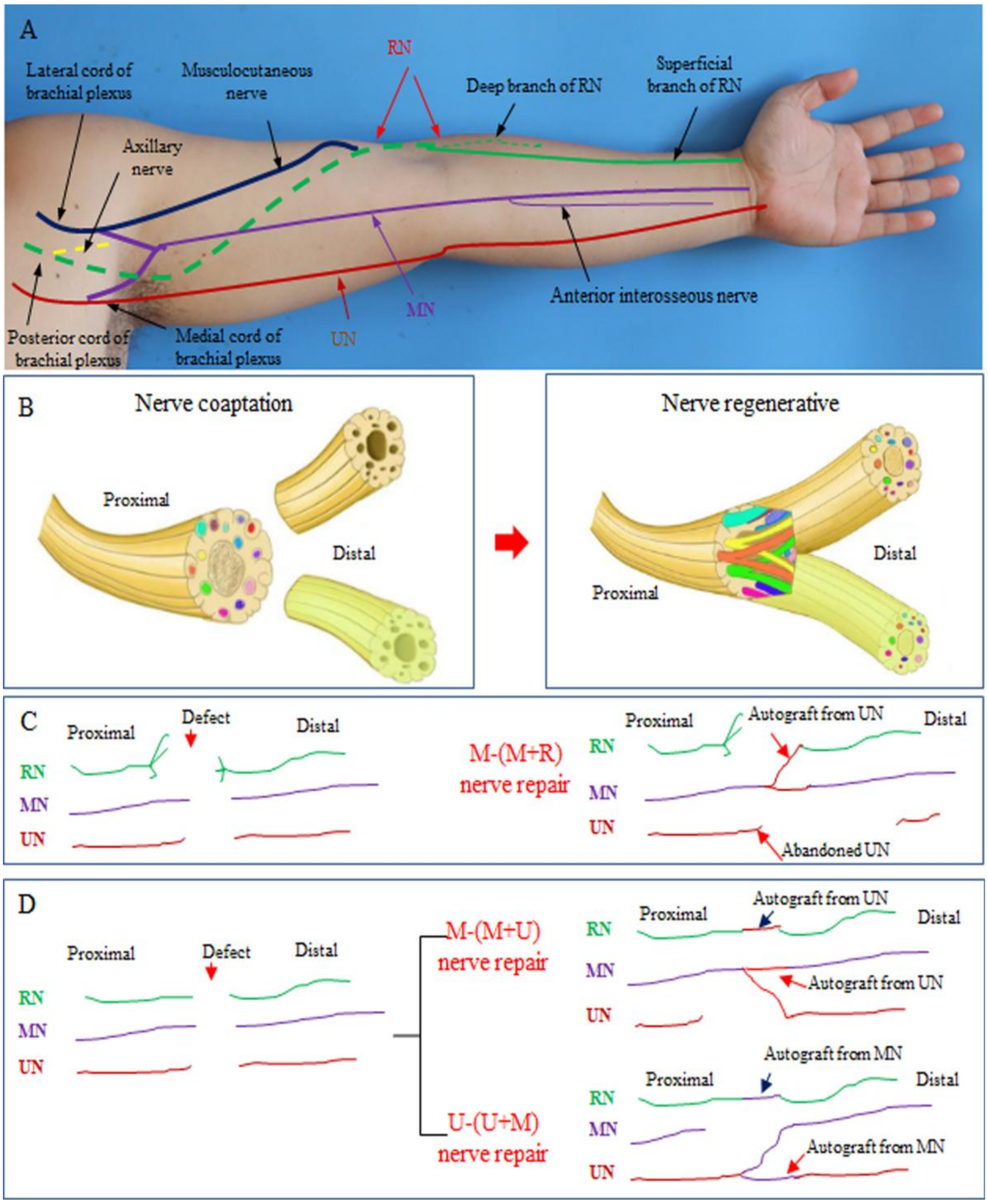
By the end of the experiment (week 14), after nerve tubulation damaged by Reperen, morphological analysis of its distal section 1 mm from the edge of the tube showed the preservation of multifascicularity characteristic of an intact nerve (Fig. 3, a). However, the total cross-sectional area of the nerve trunk increased by 2-3 times compared to intact values due to the increase in epineurium. The average area of nerve bundles was reduced compared to normal. The number of small myelinated fibers was 71.0% of the total number, medium - 26.5%, large - 1.8%.



a - When using Reperen; b - When using Tissukol; A - nerve fibers, B - epineurium; staining with methylene blue and fuchsin

After tubulation with tissul, at the end of the experiment, a bundle of nerve fibers surrounded by a wide, loose epineurium was observed in the distal space. The perineural membrane was not expressed. The total area of the nerve in the distal region exceeded the area of the intact nerve due to the expansion of the epineurium. The total number of myelinated nerve fibers was almost 2 times higher than in the intact nerve. The number of large myelinated fibers was 4.1%, medium - 30.8%, small - 65.0%.

Clinical indicators of recovery of the injured limb. Normally, in animals, the free angle of the leg in the ankle joint area is 180 ° [36]. In the case of sciatic nerve injury, the clearance of the ankle joint can quantitatively characterize its contracture.



The control clinical indicators of the condition of the injured right hind limb were: 1) the angle of the foot; 2) the position of the limb; 3) inflammation of the ankle joint with the appearance of trophic ulcers; 4) the number of fingers. The use of both types of conductors in nerve regeneration showed similar results at the end of the experiment. The angle of extension of the ankle joint was on average 170 °, and no inflammation or thickening of the joint was observed. When walking, the animals leaned on their feet and straightened the fingers of the injured limb (Fig. 4).

Discussion:

The experiment investigated the morphological characteristics of the sciatic nerve, including counting the total number of myelinated nerve fibers and dividing them into three size groups, as well as estimating the total cross-sectional area of the sciatic nerve, the cross-sectional area without the epineurium, and the epineurium itself. The percentages obtained for the size groups of myelinated nerve fibers differ from the results of the authors [35] who counted nerve fibers by area.

In both experimental groups, a significant increase in the total number of nerve fibers in the tubulation area was observed 14 weeks after surgery compared to the norm. According to the authors [37, 38], this fact may be due to the increase in the number of regenerating nerve fibers during the transition from the proximal tail to the injured area. A tendency for myelinated nerve fibers to decrease in size when passing through the proximal border zone of the tubulation area was also detected, which was also shown in experiments conducted by the authors using other types of conductors and when conducting the total number of nerve fibers 8 weeks after surgery; However, a greater number of small myelinated nerve fibers was found in the fibrin conductor (Tissukol), which ultimately affected the total number of conductors.

Conclusion: The differences observed in the formation of the regenerated nerve with different types of tubes are due to the fundamental difference in the structure of the conductors. Fibrin glue contains fibrinogen and fibronectin, which are components of the extracellular matrix and are able to stimulate the proliferation of connective tissue components [40, 41]. Apparently, the pronounced growth of the epineurium in the experiment with Tissukol is associated with the fibrin conductor, which acts as a cellular matrix that forms the epineurium, which subsequently occupies the thickness of the fibrin tube. In addition, the process of adhesion between the conductor and the surrounding tissues was noted, which prevented the natural movement of the nerve in its bed (tunnel syndrome) and may have a negative effect on the formation of the nerve.

Reperen is a spatially crosslinked polymer made of methacrylic oligomers that does not contain micropores. It has a high degree of biocompatibility, biostability and inertness for the body, due to which it does not age and does not cause adhesion. It is known that fibroblasts multiply in a single layer on its surface, which prevents the growth of connective tissue in a 3D shape and serves as a basis for preventing the formation of adhesions [33]. Reperen also prevents the penetration of actively dividing connective tissue into the site of injury through its structure.

After 14 weeks of tissue conduit tube, a bundle of nerve fibers is observed in the distal tail, surrounded by a wide, loose epineurium, without clear boundaries. The perineural membrane is not expressed. A similar structure of the distal part of the reconstructed rat nerve was also observed by the authors [27] when the tube was tubed with a fibrin conduit, but with a diastasis of 1 cm.

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