GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

ABSTRACT

PhD Thesis

Possibilities of increasing the length of the *muscle in vein* type grafts

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Content

Key words: peripheral nerve, regeneration, speed, grafts, muscles, vein, stimulation, electromagnetic waves, microsurgery, innovation, functionality, cost.

The motivation and research objectives

The increasing incidence of home and road accidents has led us to identify a solution to facilitate the functional recovery and reintegration of all the patients affected in the field of work. At the same time, the need to save the budget allocated for the surgical interventions and the need to have a fast recovery led us to develop this experimental study.

1. Development of an algorithm to increase the MVNG length by the technique: vein windows from 1.5 cm long to 3 cm long.
2. Acceleration of axonal growth rate by MVNG using stimulation with EMPF and Umm UHF.
3. Faster recovery after grafting with MVNG (sensitivity and motor function) stimulated with EMPF compared to conventional grafts.
4. Reduce the cost price by using long MVNG compared to neural tubes.

Introduction

Nerve grafts

The history of nerve grafts started from the time when direct co-operation was observed that it may not always be possible, especially in complex traumas with large losses of substance when the resurgence of a limb is very important.

At a defect of more than 3 cm the direct suture is impossible because the tension created would destroy the axonal migration and cause only another trauma.

The nerve graft was not always the salvage solution but rather a compromise, but the evolution of microsurgery has made this method a common one in peripheral nerve reconstructions.

Material and methods

The mass of surgical instruments was composed of the basic instruments in microsurgery (forceps, needle-holder, scissors, vascular clamps, separators, suture threads of different thicknesses: 3/0 Nylon, 6/0 Prolene, 7/0 Premilene, syringes of 2,5 and 10 mL, kidney tray, classic scalpel with disposable blades size 23, sterile fields, compresses, physiological serum, Sinerdol capsules dissolved in physiological serum, Betadine.

The operator (the author) wore disposable robes, sterile gloves after surgical washing, mask and cap.

For magnification we used a lamp with a classic 100W bulb and 8x magnification surgical magnets adjusted to each operating stage.

Postoperatively, all subjects were carefully transported to separate cages and monitored until awakening. After waking up each was placed in the same cage depending on the lot of which it is part.

At the slaughter, at 12 weeks postoperatively, the protocol in force of the UMF Iași was respected.

The procedure of harvesting the segments of the operated nerve was performed in the same way as the one described at the surgery.

The segments were kept in formaldehyde for preservation and further processing.

The subjects are represented by: Wistar rats weighing 300-350 g on a standard diet. The rats benefited from water and food at their discretion. The average temperature was 21 degrees Celsius, humidity 46-65%, light cycle was 12/12.

All the ethical norms were observed throughout the experiment in accordance with the request submitted and approved by the ethics committee of UMF Iași.

Groups:

50 experience animals divided into separate lots (10 rats in 5 lots each)

A. for defects of length x.

1) classic, autologous grafts

2) GNMV

B. for defects of length 2x.

1) classic, autologous grafts

2) 2 centrally sutured muscle-in-vein grafts

3) **Unique GNMV with *vein windows*.**

All 2x length batches were stimulated with postoperative EMPF.

The length X was 1.5-1.7 cm.

General anesthesia performed with sedation:

-xylazine 2% used for sedation 0.1 µg / 100 g animal

-ketamine 1mg / ml-0.1µg / 100g per animal at 10-15 minutes after injection of sedative

Group 1-x long autologous nerve graft

- after the proper hygiene of the area to be operated (epilated, washed with water and surgical soap, betadinate) and the installation of anesthesia, the experienced animal is positioned in the ventral decubitus with the lower left limb elevated using a syringe positioned below it.

- the incision was made with the blades with blade 23, parallel to the iliac crest on a length of 6 cm

- the dissection started from the distal to the proximal carefully so as not to damage the vessels

-The sciatic nerve was discovered by an incision in the form of s italic at the level of the femoral biceps muscle, posterior of the femur and of the knee joint, thus dividing the muscle into two flaps, anterior and posterior, by removing them highlighting the sciatic nerve. At about 0.5 cm cranial of the knee joint, the sciatic nerve is divided into 3 branches:

in a ventrally oriented branch (tibial nerve)

#a dorsal localized branch (peroneal nerve)

a branch with the smallest dimensions (sural nerve).

The posterior member of the Wistar rat has primary innervation provided by the sciatic nerve and its branches.

- a portion of 1.5 cm starting from below the branch of the sciatic nerve was taken
- the suture of the graft segment was performed by inverting its ends
- the suture was made with 7/0 Premilene thread with separate, epiperineural threads.
- I washed the wound intensely with physiological serum and Sinerdol
- 3/0 Nylon skin suture with separate threads
- clean the wound with Betadine
- sending on awakening

Group2- x long classical MVNG

- The principle of MVNG is the replacement of the missing portion of the peripheral nerve by an autologous graft but constructed from a biological tube filled with skeletal muscle. The biological tube is a vein segment.

-experienced animals were prepared in the same way mentioned in group 1

Group 3-2x long autologous classical nerve grafts

-the animal preparation, dissection and sampling of the sciatic nerve segment were performed in the same way as described in group 1, with the indication that the segmented sciatic nerve segment is 3.4 cm (2x) long.

- the segment thus sectioned is sutured in the opposite direction at the ends of the sectioned nerve acting as an autologous nerve graft - the steps are subsequently those described in group 1

Group 4-2 x long MVNG centrally sutured

- animal preparation, incision and dissection are affected as in group 1, but the length of the segment of sciatic nerve taken is 3.4 cm

- the preparation of the composite nerve graft involves the preparation of two grafts of length x, exactly like those of group 2

Groups 5-Single MVNG with *windows-vein*

-application of an innovative principle for the elongation of nerve grafts

- creation of fenestrations in the surface of the vein to slice a whole muscle of length 2x through the venous path

- in this way no axons are lost and the regeneration is more uniform

- the speed of regeneration can be accelerated by applying EMPF and Umm UHF

***Windows-vein* method**

-a segment of muscles of length 2x + 0.5 cm (3.5 cm) adjacent to the sciatic nerve is harvested

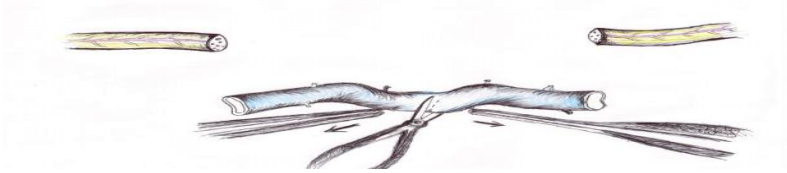

Figure 7.12. Harvesting of the 2x length venous segment and central sectioning. Sketches made by Prof Univ Stamate Teodor. Used with the author's consent.

-in the same way, a vein segment of the same length is harvested

-both are processed according to the protocol explained in group 1

-from 1.5 cm to 1.5 cm it is made with the scalpel of the incision perpendicular to the length of the vein (in the case of a graft of 3 cm length it is incised centrally)

-a microsurgical forceps slides the vein to the first fenestration

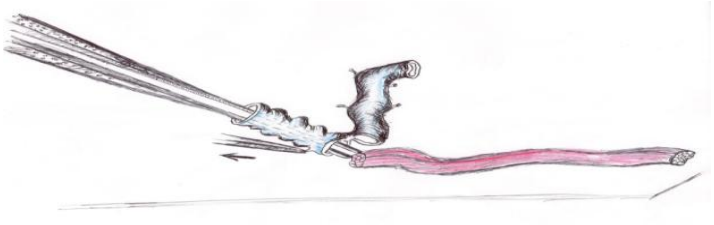

Figure 7.13. Sliding the muscle into the vein until the first fenestration. Sketches made by Prof Univ Stamate Teodor. Used with the author's consent.

-with another forceps, the muscle slips along the length of the muscle fibers inside the vein.

-the muscle slips to the end leaving only the portion until the first window inside the vein

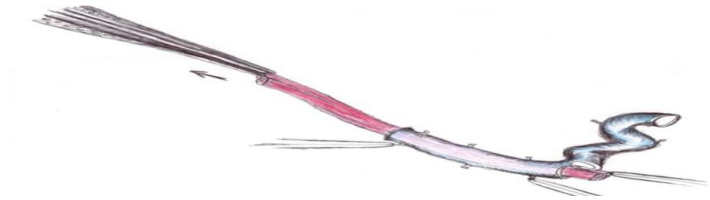

Figure 7.14 Complete sliding of the muscle segment inside the vein. Sketches made by Prof Univ Stamate Teodor. Used with the author's consent.

-with another forceps sliding in the opposite direction through the window obtained the vein segment

-between the ends of the forceps, the muscle is grasped and the segment of slipped vein is filled

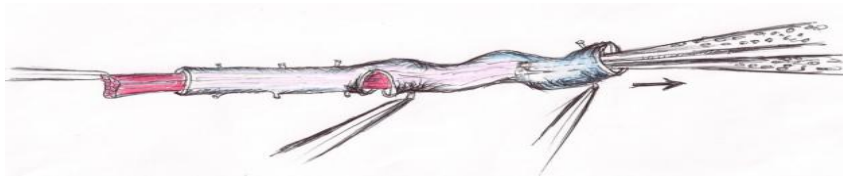

Figure 7.15. Final appearance before suturing. Sketches made by Prof Univ Stamate Teodor. Used with the author's consent.

-similarly, it is performed with the other windows

-NB-method is time consuming and anesthesia is limited-so exercise is required beforehand

-after sliding the entire muscle through the vein the sutures are threaded with 7/0 Premilene

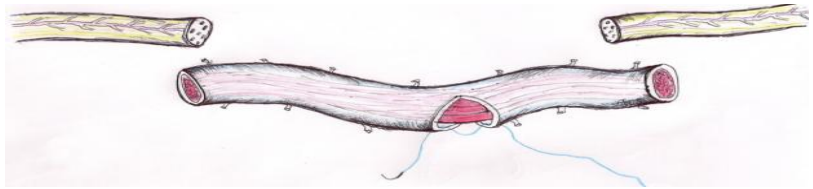

Figure 7.16. Suturing of the venous fenestra. Sketches made by Prof Univ Stamate Teodor. Used with the author's consent.

- the “windows-vein” type suture is sutured at the ends of the sciatic nerve in the same way as the other lots

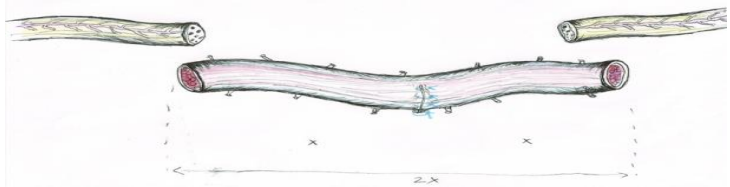

Figure 7.17. Obtaining the graft segment of length 2x, final appearance. The surgical technique is such that the nerve end is inside the nerve graft. Sketches made by Prof Univ Stamate Teodor. Used with the author's consent.

Figure 7.18. The final result of the muscle-in-vein nerve graft, "windows-vein". Sketches made by Prof Univ Stamate Teodor. Used with the author's consent.

Sciatic functional index (SFI)

Gutmann's (1942) research has shown that the inability to remove the fingers from the hind limb to rats is a significant parameter for assessing the degree of injury and subsequent recovery. This method has been quite elementary (1).

De Medinaceli and collaborators developed a quantitative, reproducible and reliable method of evaluating the functional status of the sciatic nerve in the rat, by analyzing the characteristic data of the plantar imprint of the hind limb.

Tactile sensitivity testing-Von-Frey test (mechanical nociception testing)

After emptying the bladder with the help of the Credè maneuver, the experienced animal is separated from the group and placed in a separate room for 30 minutes for acclimatization. On a Plexiglas platform are attached 8 filaments that can apply a strength from 1.1 g to 50 g. The force is applied in series: 5 repeated stimulations for each filament 3 minutes apart.

The behavior of the experimental animals is observed and noted separately in the tables (seconds until the withdrawal of the leg versus the applied force).

The method is noninvasive, does not traumatize the experimental animals, which is why it was chosen to test the tactile sensitivity (2).

Stimulation with EMPF and UmmUHF

Postoperatively, groups 3, 4 and 5 were stimulated with EMPF and Umm UHF (7.1 / 42.19 GHz) through the equipment provided by the Faculty of Electronics, Telecommunications and Information Technology (ETTI), Polytechnic University of Bucharest, courtesy of Mr. Lecturer Rusu Ion.

Basically, around groups 3, 4 and 5 we mounted 2 devices that generated a distance of 1 m EMPF and UmmUHF. The devices worked intermittently.

Daily, subjects were stimulated for 2 hours in the 10-12 hour range and later at 16-18. The stimulation was performed daily for 28 days.

The chosen time interval has significance from the point of view of the metabolism of the nervous system. Studies have concluded that during these time intervals the activity of the central and peripheral nervous system is at maximum levels (3, 4).

Gastrocnemian index

For calculating this, the muscles of both lower limbs were excised. Gastrocnemius muscles were weighed 12 weeks postoperatively. The gastrocnemius index measures the atrophy of the muscles after denervation.

Gastrocnemius muscle index = Weight of the denervated muscle / weight of the contralateral muscle.

A value as close to 100% represents good re-innervation. A distance of 100% means a pronounced denervation with muscular atrophy.

The gastrocnemius index tests the motor integrity of the sciatic nerve after re-innervation or denervation. It is a valuable indicator of the nervous regeneration process (5).

Statistical analysis-methods

Microsoft Excel and SPSS 13.0 were used to describe and analyze the data. Statistical data processing was done by applying several specific tests (such as Kruskal-Wallis). The t-Student test was used to compare the quantitative data between the sample pairs studied. In order to make comparisons between the types of lesions, an analysis of the ANOVA variant was performed.

Results

Table 8.4. Descriptive data on rank averages, according to experimental groups.

Studied parameters	Group	N (subjects no)	Mean range
SFI (T1)	1.00	10	37.75
	2.00	10	20.85
	3.00	10	20.25
	4.00	10	5.50
	5.00	10	43.15
	Total	50	
SFI (T2)	1.00	10	41.15
	2.00	10	21.35
	3.00	10	19.70
	4.00	10	5.50
	5.00	10	39.80
	Total	50	
SFI (T3)	1.00	10	43.35
	2.00	10	26.75
	3.00	10	17.25
	4.00	10	5.50
	5.00	10	34.65
	Total	50	
Axons diameter	1.00	10	45.40
	2.00	10	28.00
	3.00	10	17.15
	4.00	10	5.50
	5.00	10	31.45
	Total	50	
Axons number	1.00	10	45.45
	2.00	10	27.60
	3.00	10	15.95
	4.00	10	5.50
	5.00	10	33.00
	Total	50	
Myelin sheath thickness (mμ)	1.00	10	41.35
	2.00	10	24.60
	3.00	10	17.30
	4.00	10	5.50
	5.00	10	38.75
	Total	50	
Epineurium thickness (μm)	1.00	10	18.70
	2.00	10	31.95
	3.00	10	5.95
	4.00	10	45.50
	5.00	10	25.40
	Total	50	
Perineurium thickness (μm)	1.00	10	44.05
	2.00	10	15.50

	Groups	N (subjects no)	Mean range
	4.00	10	5.50
	5.00	10	36.95
	Total	50	
Studied parameters	3.00	10	25.50
IG	1.00	10	40.70
	2.00	10	30.15
	3.00	10	20.70
	4.00	10	5.55
	5.00	10	30.40
	Total	50	
Fibroblasts	1.00	10	19.90
	2.00	10	9.45
	3.00	10	22.80
	4.00	10	45.50
	5.00	10	29.85
	Total	50	
Mastoid cells	1.00	10	8.20
	2.00	10	26.05
	3.00	10	28.80
	4.00	10	45.50
	5.00	10	18.95
	Total	50	
Endoneural vessels thickness	1.00	10	42.15
	2.00	10	26.35
	3.00	10	15.50
	4.00	10	5.50
	5.00	10	38.00
	Total	50	

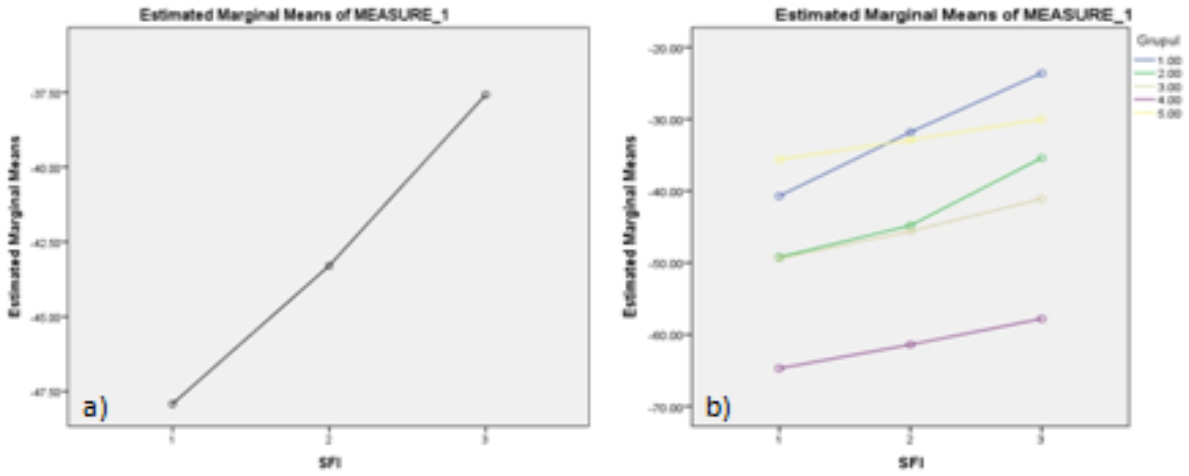

Figure 8.1.a). Improvement of the level of indicators over time. Figure 8.2.b). Improvement of the index level in each of the experimental groups included in the study.

Anatomopathological analysis

Group 1

(simple graft (x=1,5 cm))

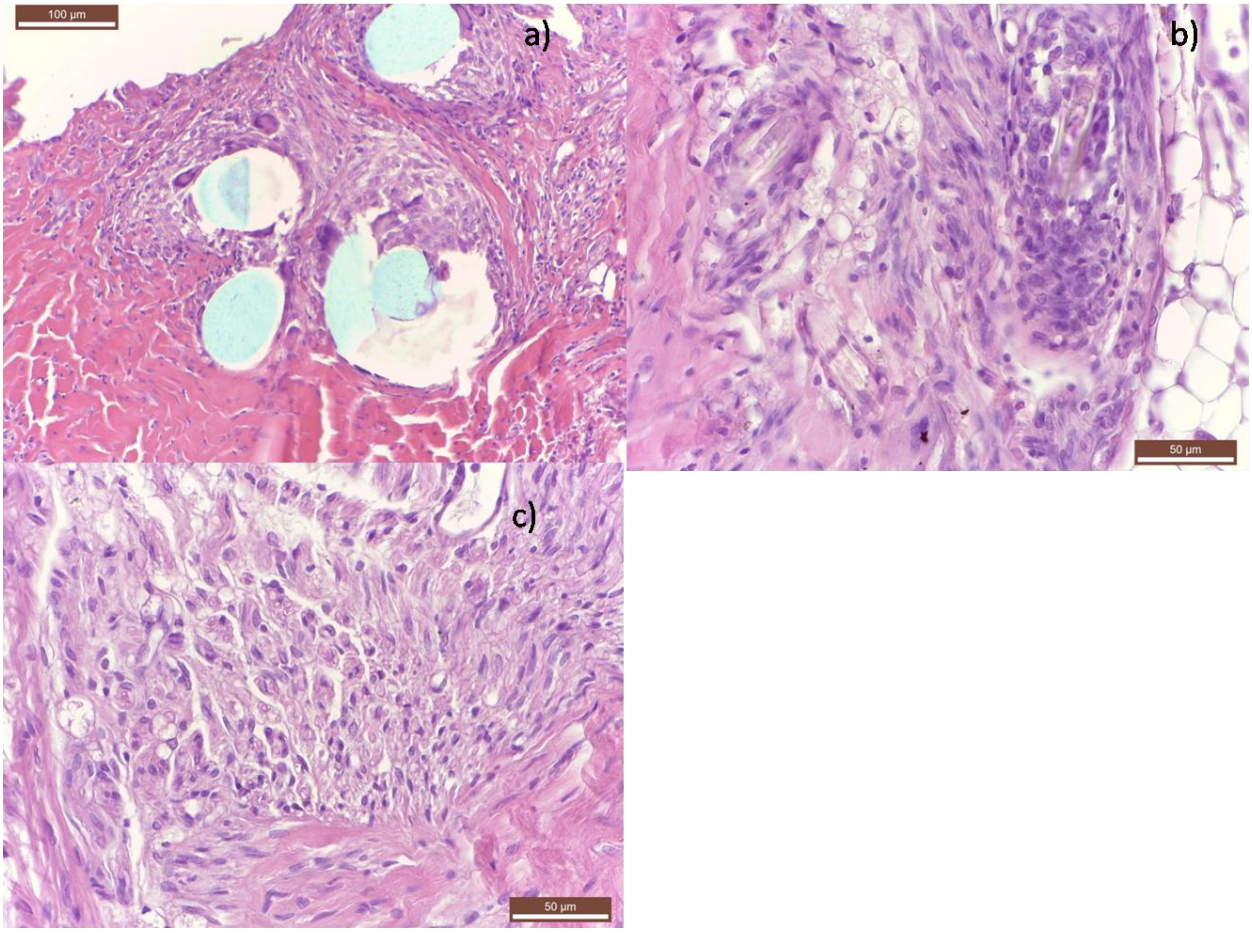

Figure 8.3. Analysis of the sciatic nerve in group 1; May-Grunewald-Giemsa coloring, 40x.
1a) At the level of the epinervum (epineurium) we notice an accentuated inflammatory infiltrate consisting of giant cells of foreign body, lymphocytes, histiocytes and fibroblasts (especially around the suture threads).

1b) Outside the epinerval, we find the regeneration of axons surrounded by Schwann cells (axonal regeneration outside the connective tissue - of the epinerv).

1c) In the perimeter of the epinervum, the regeneration of axons with heterogeneous appearance and disordered orientation is observed. Noteworthy vessels are noted (neoangiogenesis present).

Macroscopically, we observe the areas of the suture threads surrounded by numerous nerve cells located in concentric layers. The appearance is rich in regenerating cells, which gives the preparation the environment necessary for peripheral axonal regeneration.

A uniformity in distribution is observed at all cell levels, constant density of fibroblasts, lymphocytes, Schwann cells, and neoangiogenesis cells can be observed.

Group 5

(MVNG 2x length with „window-vein”)

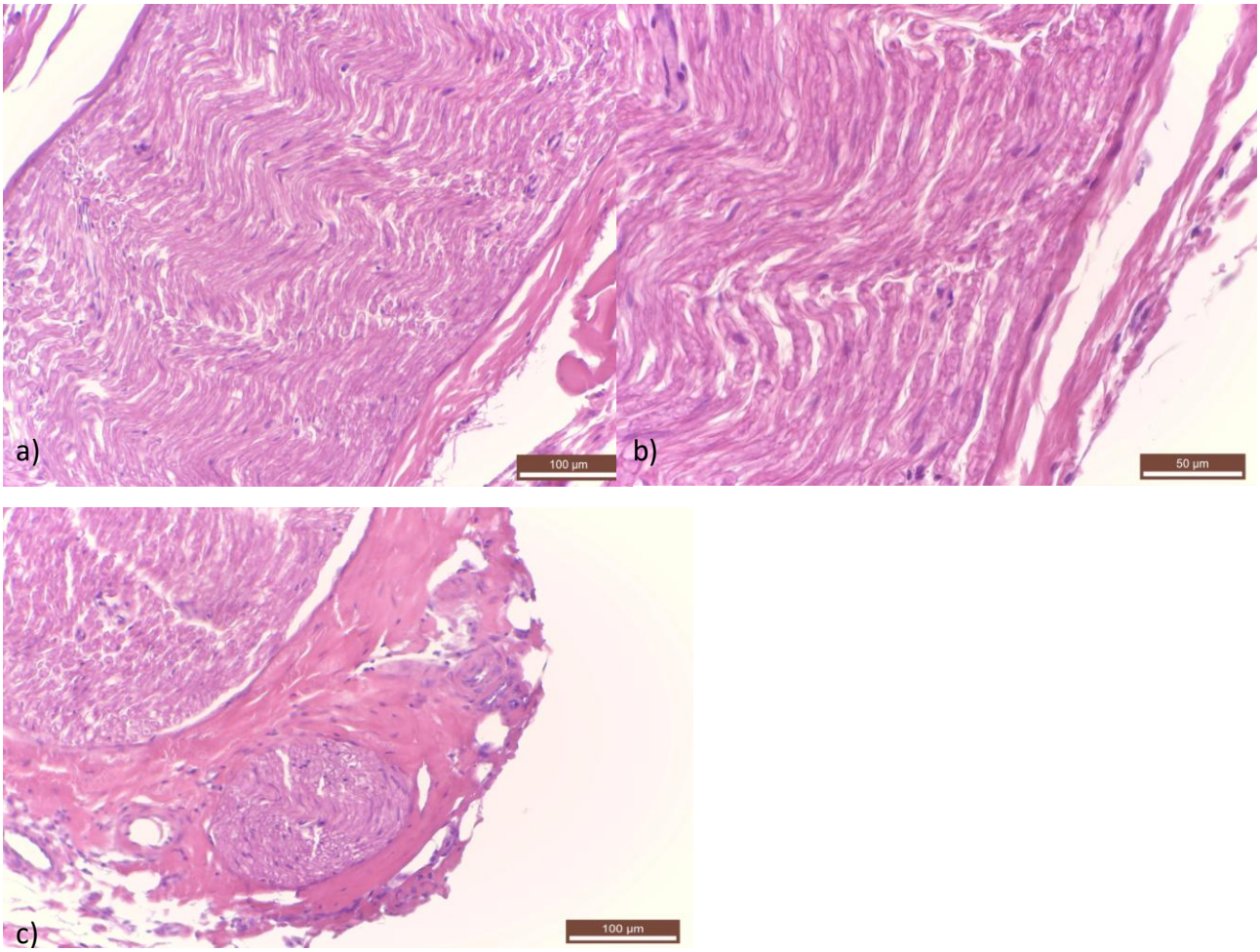

Figure 8.7. Sciatic nerve analysis in group 5; May-Grunewald-Giemsa coloring, 40x.
a) Appropriate axonal regeneration, with normal / ordered natural axons orientation, with a delicate neural stroma. Neoangiogenesis present. Structural homogenization of the venous wall with the formation of a homogeneous epinerv, with well oriented connective fibers, without inflammatory infiltrate.
b) Ordered arrangement of myelin and myelin fibers, differentiation of structural cells of the vein wall into fibroblasts producing conjunctive fibers, thus participating in the formation of the epinerv.
c) Axons regenerated outside the epinerv, organized in a bundle surrounded by a sheath / sheath consisting of connective tissue with the role of epinerv.

Discussion

Although peripheral nerve surgery has always been an integral part of neurosurgery, its status within the global landscape has long been in the shadow of more imposing areas, such as skull base neurosurgery or vascular neurosurgery. There are many reasons, one of them being plausible by the word peripheral itself, which evokes the notions of marginality and less importance. Some of the common misconceptions associated are that the neurological deficit in peripheral nerve injury is permanent and irreversible, that peripheral nerves do not have the capacity to regenerate, that the results of surgical treatment are

insignificant and that peripheral nerve surgery is not attractive to neurosurgeons, but is more rather reserved for plastic surgeons and orthopedics. Due to the dedicated work of a relatively small but enormously dedicated group of neurosurgeons around the world in recent decades, these preconceptions have been eliminated and it is becoming increasingly clear that the operation of the peripheral nerves is not peripheral in the above sense and that it is not it deserves less attention than the aforementioned summers (6, 7, 8).

Namely, although peripheral nerve surgery is not a life-saving surgery, it has proven to be life-changing surgery, with a major impact on the patient's quality of life, as it improves the patient's ability to perform daily and professional activities and thus affecting his physical and psychological well-being. Moreover, since most patients with peripheral nerve injury and brachial plexus injury belong to the active population, peripheral nerve surgery also has substantial socioeconomic implications. In contrast to the aforementioned opinion, the peripheral nervous system has been shown to have immense potential for regeneration, with significant results improved by various stimulation modalities where recent brain plasticity research indicates that experience-dependent reorganization of networks plays an important role in recovery. functional. For all these reasons, systematic research, education and practice in peripheral nerve surgery are certainly worth the effort (9, 10).

In the surgery of the peripheral nerves an important role is played by both the aspects mentioned and discussed above but above all the mastery of the surgeon to successfully perform the surgery. Both the biological, financial, organizational elements and the timing of the surgical intervention build a unitary whole that results in the proper regeneration or on the contrary its failure.

In our study, we proposed a new, new method, the "windows-vein" method for repairing defective lengths exceeding 3 cm in humans to double the defect length and 3.5 cm in the Wistar rat. The results obtained lead us to the conclusion that the method is efficient and can be implemented in the current treatment of extensive peripheral nerve injuries.

Innovation should be one of the objects of study in medical universities as new methods are always needed in niche surgery. In a society where trauma is becoming more common, occupational diseases that affect peripheral nerves become important in plastic surgery, and repair methods are still limited to classic neurorphs, autologous nerve grafts and behaviors. carpet with various substances that stimulate axonal growth or budding. New methods bring an added reinforcement of these annoying pathologies that decrease the quality of life constantly and continuously. At the same time, the application of the new methods becomes difficult due to the bureaucracy, the limitation of the case law and the necessary logistics. Thinking beyond barriers can bring constant and visible benefit to the patient's benefit if applied as quickly as possible to cases of trauma without functional recovery and reintegration into daily activities. The long-term benefits of introducing new surgical methods into current practice lead to lower costs for recovery therapies and post-traumatic psychotherapy. But the cornerstone in applying these easy methods is the surgeon's skill, stepping outside the classical protocols and opening the patient to new ones.

Conclusions

1. The grafting of the peripheral nerves is a useful solution for solving polytrauma cases. However, graft donor areas are limited.

2. Alternative grafting with composite grafts in specific cases gives good functional results.

3. Reducing comorbidities by taking biological material from the same site as trauma is an undeniable advantage.

4. The method of grafting the peripheral nerves by constructing a muscle-in-vein graft has favorable results in both humans and experienced animals, but is limited by the graft length (3-4 cm).

5. The method of increasing the length of the moss-in-vein graft from 3 cm to 6 cm, by adding venous fenestrations formed the "windows-vein" type graft. The functional results in the experimental animal proved its integration and the early re-establishment of the usual activity.

6. The cost of producing these types of nerve grafts is minimal, practically depending entirely on the biological material of the animal / patient experience and the skill of the surgeon.

7. The repair of the peripheral nerves is not only mechanical as of other organs, the axons needing to resume the intercellular connections with the peripheral target organs and at the same time they must mature. During these processes, a multitude of cellular processes intervene that can contribute to or diminish the success rate of a nerve repair.

8. Biological mechanisms of regulation of nerve repair represent the future of research in peripheral nerve trauma. In addition to the difficulty of reconstructing the physical nerve continuity, a more accurate reconstruction of the local biological conditions will be required for the integration of the injured peripheral nerve into the biological circuit.

9. Stimulation with EMPF and UmmUHF is an efficient method of accelerating functional recovery after nerve graft, as speed of regeneration has always been a controversial issue. Therefore, the use of electromagnetic energy brings an advantage in the recovery of peripheral functions with faster integration compared to unstimulated groups.

Meeting the objectives

The objectives of this study were successfully achieved. Namely, we have developed an efficient method of increasing MVNG length by adding "windows-vein". The group of experienced animals that was treated by this method had functional results similar to the group treated by neuroraphobia and the group treated by autologous grafting. We managed to double the length of the MVNG by the "windows-vein" technique from 1.5 to 3 cm in the experience animal.

At the same time, the use of EMPF and Umm UHF stimulated axonal growth and faster integration of the grafts used, so it is a simple, non-invasive and inexpensive method to shorten the duration of convalescence and recovery after complex traumas involving peripheral nerves.

The price of a nerve graft increased by the "windows-vein" method is reduced. Basically, the biological material used in the vicinity of the affected area and the surgeon's skill is used. In this way the additional costs of a possible neural tube can be cut that can cost 5000 euros for a single cm.

Originality and innovative contributions of the thesis

The originality of the thesis is the implementation of a new method of increasing the GNMV length without using synthetic grafts or having other donor sites and thus adding comorbidities. The fenestrations made along the length of the vein help to manipulate the skeletal muscle used to fill the vein easily, and the regenerating axons are not lost through the small breaches in size. A loss of axons regenerated by large gaps, for example the union of two complete GNMVs, is a failure of nerve repair as the axons will not reach the terminal end and

will not reach their initial purpose. On the contrary, they will produce dysesthesia, neuralgia and neuroma.

Stimulation with EMPF increased the rate of regeneration compared to the groups without stimulation. Therefore this simple and noninvasive method is useful for the harmony and success of the surgical method.

One resolution discovered during the course of the experiment was the type of anesthesia used. The addition of bupivacaine to the edges of the operative wound resulted in the reduction of postoperative pain and autophagy phenomena of the experimental animals. At the same time, the integration in the current activities was faster compared to the experimental animals where Bupivacaine was not used.

Total numbers of tables: 52

Total numbers of figures: 28

Total number of references: 224

All the figures and the tables from the abstract respected the numbering and name from the PhD thesis.

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