

The Role of Muscle Cells and Fat Cells in Nerve Regeneration Evaluated by *in vivo* Electric Conductivity

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*Peripheral nerve reconstruction is a much debated subject nowadays that is why new surgical techniques are being performed. It has been demonstrated that after a complete section of a peripheral nerve, the healing process takes time, and most frequently the recovery is not complete. Muscle cells and fat cells due to their chemical components, can contribute in the healing process of the peripheral nerves. The purpose of this study is to evaluate the effect of wrapping a muscle flap or an adipose flap around the primary suture of a cut nerve in order to improve the motor nerve regeneration in a rat model by using *in vivo* electric conductivity measurements.*

Keywords: fat cells, muscle cells, nerve regeneration, *in vivo* electric conductivity

In plastic surgery one of the most challenging pathology is peripheral nerve injury. New surgical techniques are being developed and the old ones are being improved, because the functional recovery after nerve reconstruction is often incomplete [1, 2]. Peripheral nerve injuries may occur due to stretching, compressions (carpal tunnel syndrome), cut or damaged in accidents (sport injury or car crash). Several studies showed that if a complete nerve transection occurs, even if the surgery is perfectly adequate, full nerve recovery should not be expected [1, 3, 4]. There are several techniques that can improve the nerve functional and morphological recovery. One of these procedures is to wrap the site of the suture with a variety of substances, such as fat, muscle, vein [5-8]. Another technique is to use nerve conduits (autogenous biological conduits, nonautogenous, and nonabsorbable conduits), to protect the new scar formation, heaving the role of a nerve wrap [9-12]. Nerve conduits are useful when there is a nerve defect bigger than 5 cm. Muscle cells have in their component stem cells which are round, minimally adherent cells and have the property to contract spontaneously [13-16]. This muscle derived stem cells have a promising role in regenerative medicine. Since peripheral nerve injuries are most of the times associated with severe traumas, this cells can provide a unique advantage for the restoration of damaged tissues [13]. On the other hand, autogenous fat grafts consisting in whole adipose tissue have also a number of benefits. One of them is that they are rich in adipose stem cells even if they are not isolated. These adipose stem cells have a great therapeutic potential because of their trophic factor secretion and differentiation potential [17-24].

Several studies, using different methods, such as MRI (magnetic resonance imaging), and histologic analysis have demonstrated from the structural point of view that using nerve wraps (muscle cells and adipose cells) after primary repair can decrease scar tissue formation [9 - 11, 25]. The purpose of our study is to investigate, from the

functional point of view, using *in vivo* electric conductivity measurements how the fat cells and muscle cells help the nerve regeneration process. We assume that the use of these two types of cells would enhance motor nerve recovery in this study.

Experimental part

The Ethics Committee of The University of Medicine and Pharmacy of Targu Mures approved the experiment and study protocols, according to document 323/2017. In this study we have used twenty-four adult Wistar Albino rats that were divided in three equal groups. Nerve transection and repair procedure was performed under general anesthesia with ketamine/xylazine (75 mg/5 mg/ kg i.m). For all animals the left sciatic nerve was transected using a No. 11 blade and reconstructed under magnification loupes (2.5X Keeler). In all groups direct suture was performed using 8.0 Prolene sutures. For the first group the primary suture was performed without wrapping the nerve (fig. 1a), for group number two the reconstructed site was wrapped in a local muscle flap (fig. 1b), and the third group was wrapped in adipose flap harvested from the interscapular region of the same rat (fig. 1c). The right

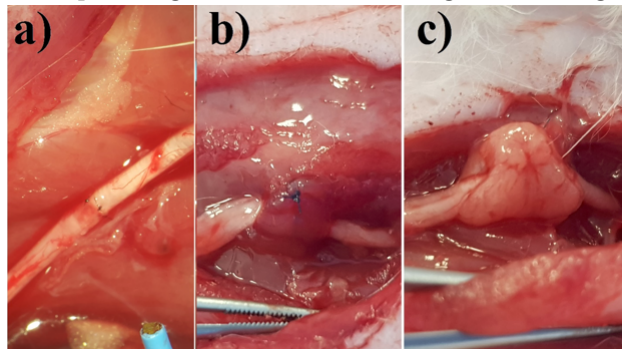


Fig 1. Picture of exposed Albino Wistar rats' sciatic nerve reconstruction after injury for a) the first group with direct suture; b) 2nd group with muscle flap wrapping the suture and c) 3rd group with adipose flap wrapping the suture

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sciatic nerves were used as control group. At 4, 6, 8 and 10 weeks *in vivo* electric measurements were performed for all rats.

For the electrical stimulation of the sciatic nerve, we developed an external programmable pulse generator based on the PIC16F877 microcontroller (Microchip, Inc. Arizona USA) which is capable to generate stimuli in a range of 0.2 to 6 ms (milliseconds) width. The stimulation frequency can be set from 60 ppm (pulses per minute) to 8000 ppm and the amplitude can be varied continuously from 0 to 24 V. The firmware was written in Pic Basic Pro a compiled with Proton Basic software (Crownhill Associates Limited, UK). The schematic of the logic unit can be seen in figure 2. To prevent any unwanted electric discharge, the device is operated from a battery pack of 6 Li-Ion accumulators, 4.2 V each, when fully charged. The device is permanently monitoring the charge status of the battery pack and warns user if the capacity drops below 1/3 of the full charge value. Ten stimulation protocols can be stored in the device's EEPROM area and recalled using the rotary switch when the stimulator is operating in memory recall mode.

The stimulus was delivered to the nerve in bipolar mode, using two parallel silver wires, 0.7 mm diameter spaced at 5 mm. The tip of the stimulating electrodes was bent, in order to avoid any damage of the stimulated nerve. The negative stimulating electrode was placed in distal position and the positive electrode was in proximal position. Any electrical contact between stimulation electrodes and surrounding tissue was avoided.

The acquisition of the compound muscle action potential (CMAP) was performed using 3 electrodes, two

signal electrodes and one electrode for active ground. The acquisition electrodes were 2 silver needles spaced at 5mm and inserted into the gastrocnemian muscle. A third electrode, served as active ground, was placed distally on the hind paw (fig. 3). CMAP potentials were amplified using an AD620 instrumentation amplifier (Analog Devices, Norwood, Massachusetts, USA). To avoid any significant input impedance, we used an LMC6484 quadruple CMOS operational amplifier with unity gain as buffer in the front of the AD620 amplifier. This configuration has input impedance in the range of 10 T Ω , making any tissue impedance imbalance insignificant. The third operational amplifier (IC1C) of the LMC6484 chip is used as active ground driver, as shown in figure 4.

For the signal acquisition we used a PCI-4452 acquisition card (National Instruments, Austin, TX, USA) connected to a standard P4 pc. The signals were measured, filtered and stored using LabVIEW software. Filters were configured as following: high pass filter cut-of frequency 10 Hz, low-pass filter cut-of frequency 10 kHz, and a 50 Hz notch filter ON. The sampling rate was set at 22 kHz. An image of the recorded signals is represented in figure 5. As it can be seen in figure 5, the conduction time was measured from the beginning (raising front) of the stimulus to the first spike of the muscle potential. In our protocol, stimulation width was 0.5 ms and stimulation frequency was 1 ppm.

Results and discussions

The average values of response voltage, response time and response velocity are presented in figure 6, figure 7 and figure 8 for all groups (direct suture, muscle flap

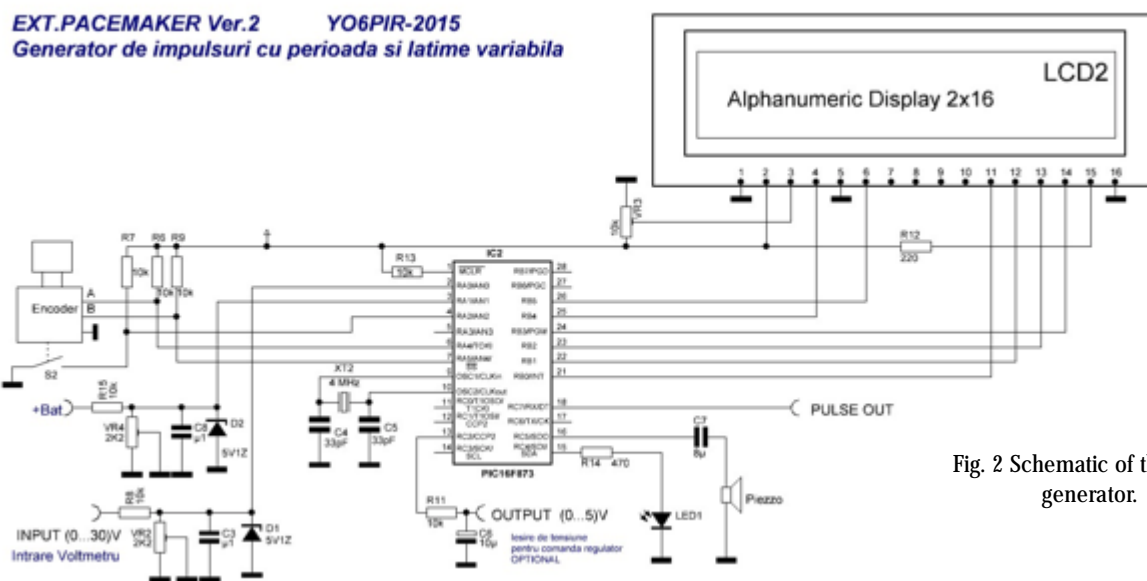


Fig. 2 Schematic of the pulse generator.

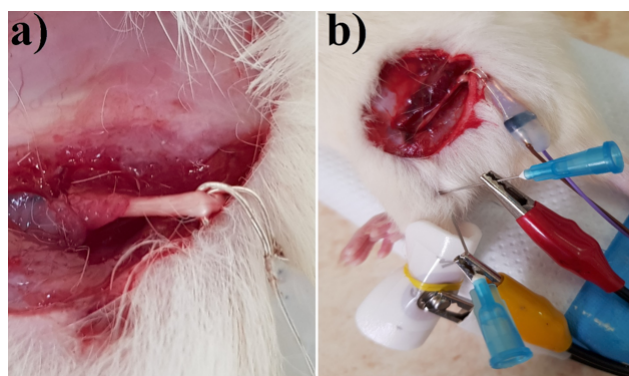


Fig. 3 Picture of an exposed Albino Wistar rats' sciatic nerve *in vivo* electric conductivity measurements using a) stimulating electrode positioned on the proximal part, above the repair site of the sciatic nerve and b) two acquisition electrodes inserted in the gastrocnemian muscle, and the third electrode served as active ground placed on the hind paw.

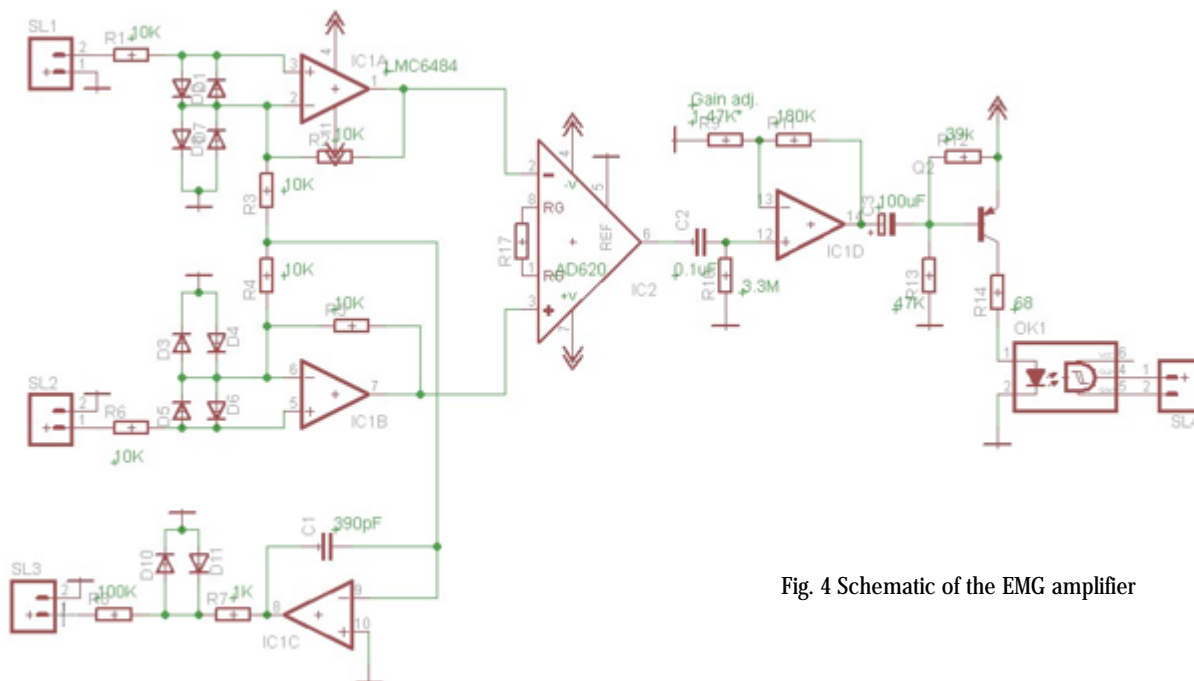


Fig. 4 Schematic of the EMG amplifier

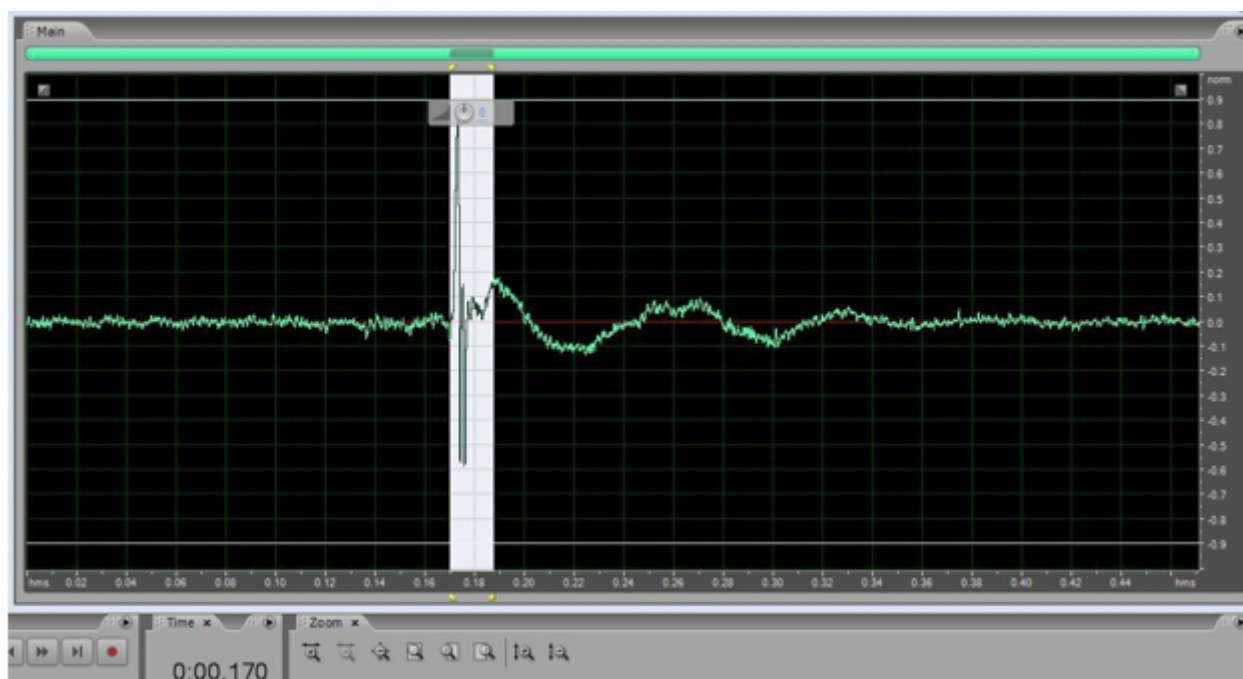


Fig. 5 Recorded CMAP (compound muscle action potential) and measurement

wrapping and adipose flap wrapping). In each case, the parameters measured for the healthy nerve (blue square) are compared with those measured for injured sciatic nerve (magenta circle). We can observe that in all groups there are significant differentiations between the healthy

nerve's average voltage (2.8-4 V group one figure 6a; 3-5 V group two figure 7a and 4-4.7 V group three fig. 8a) and under recovery sciatic nerve (4-6 V group one figure 6a; 4.7-7.8 V group two figure 7a and 5.7-7.7 V group three fig. 8a). The higher average voltage response is seen in the

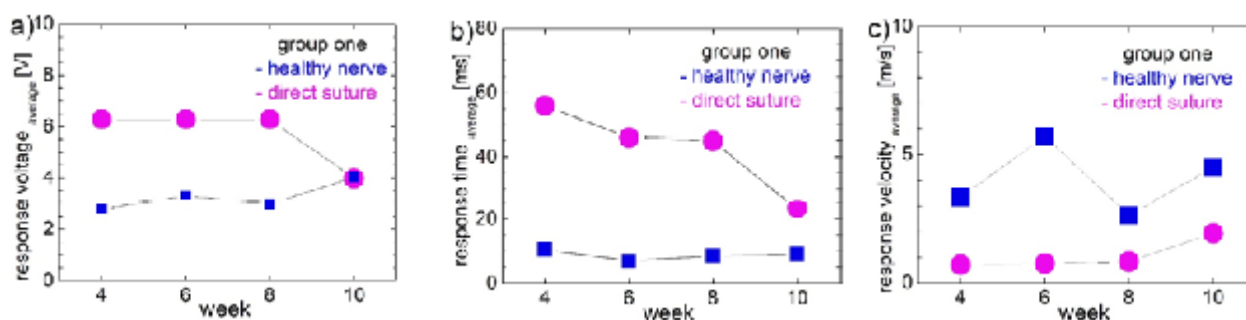


Fig. 6 *In vivo* electric conductivity parameters compared for healthy nerve and direct suture. The measured parameters were the average voltage response a), the time response b), and the velocity response c).

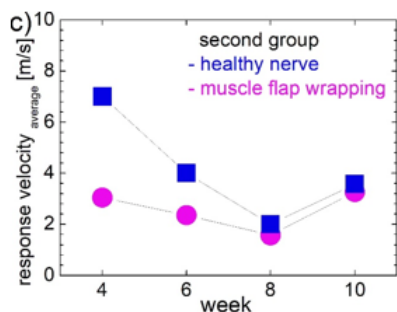
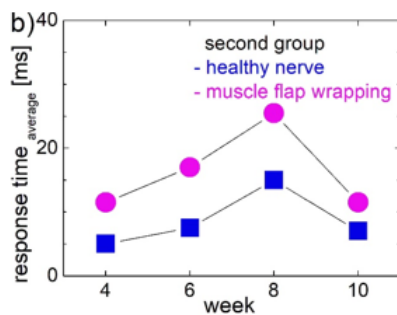
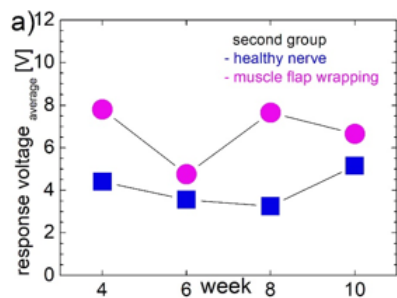


Fig. 7 *In vivo* electric conductivity parameters compared for the Albinos Wistar rats' healthy sciatic nerve and direct suture with muscle flap wrapping. The measured parameters were the average voltage response a), the time response b), and the velocity response c).

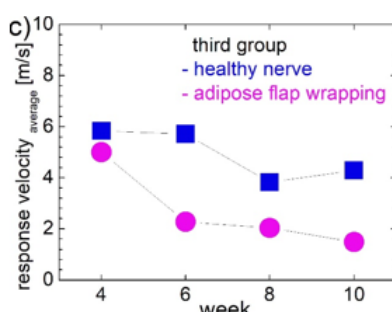
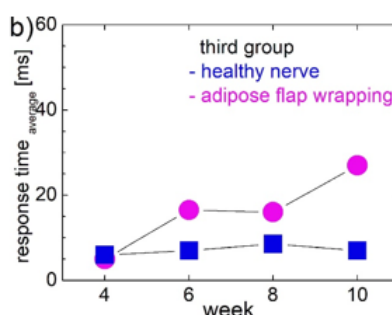
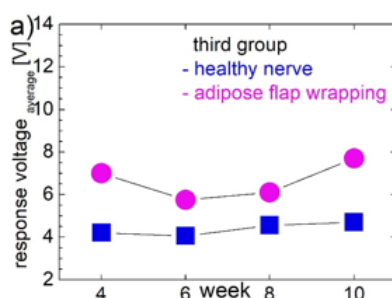


Fig. 8 *In vivo* electric conductivity parameters compared for the group of Albinos Wistar rats with healthy sciatic nerve and direct suture with adipose flap wrapping. The measured parameters were the average voltage response a), the time response b), and the velocity response c).

adipose flap wrapping group, and the smallest response is seen in the direct suture group.

The average time response is presented in figure 6b, 7b and 8b. The healthy nerve's behavior is similar in all three groups having measurements between 5-15 ms, while the injured and repaired nerve has big differences between all reconstruction procedures. The average time response for the direct suture group is much higher (23-56 ms, fig. 6b) than in group number two where the time response is between 11 and 17 ms (fig. 7b). Also the adipose flap wrapped group has higher response time (5-27 ms, fig 8b) than the muscle flap wrapped group.

The average response velocity is represented in figures 6c, 7c, and 8c. In the healthy nerves we observe a fluctuation between the values, but the interval between week 8 and 10 is characterized by increasing average response velocity values measured in all groups. The average velocity response is higher in the case of muscle flap wrapped group 1.00-3.26 m/s (fig. 7c), than in the direct suture group 0.71-1.92 m/s (fig. 6c), and larger than the average velocity response values measured for the third group 1-5 m/s (fig. 8c). The conduction velocity increased progressively from 4 to 10 weeks in the first (direct suture) and second (muscle flap wrapped) group, compared to the third group (adipose flap wrapped) where it decreased. We can observe that the response time in the injured nerve in the second and third group is increasing excepting the interval between the 8 and 10 week. In this period of time in the muscle flap wrapped group the values decrease (having the same behavior like the healthy nerve), while in the adipose flap wrapped group it increases. The average response time for the second group is longer than the other two groups.

The response voltage is an important parameter and we can see for the first group that the values between the healthy and injured nerve become almost the same, demonstrating that from the average voltage response point of view the nerve is healed after 10 weeks [25]. From the response velocity values point of view, the rats' sciatic nerve is not healed, but in the first and second group the values become closer to the healthy nerve values, in comparison with the third group where the values are decreasing.

We can consider that until the 8 week the recovery of the peripheral nerve is not restored, but after this period of time the healing process passes over the lesion and the functionality of the nerve is faster restored [25].

Conclusions

In vivo electric conductivity is a technique that can be used to evaluate the peripheral nerve healing process from the functional point of view. Significant functional differences were observed between the direct sutured group and the other two groups. From the average voltage response point of view, the rat's sciatic nerve from the first group is healed at 10 weeks after injury and repaired using direct suture. From the average response velocity one can say that the muscle cells wrapped around injury have a bigger role in regeneration than the fat cells. Overall, the electric conductivity measurements showed that the functionality of an injured sciatic nerve is restored faster using muscle cells and direct suture, compared to adipose cells wrapped around injured site.

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