Tips and Tricks in Experimental Nerve Defect Surgery
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Abstract
Microsurgery in itself might be considered a challenge for some plastic surgeons, as it requires patience and perseverance. Furthermore, when it comes to nerve surgery, the challenge is even greater as it implies a longer time necessary to observe results. In clinical practice, a good technique is associated with a good outcome. However, the problem of the recovery time remains an issue to be addressed by future generations.

Nerve defects represent an even greater challenge in traumatology. Crush injuries, lacerations and avulsions may all lead to irreversible damage of the nerves. In such cases, special surgery techniques are required to restore function¹-⁴. In order to prepare for these special techniques, a surgeon must begin with experimental in vivo nerve surgery and one that has the highest similarity with humans is the sciatic nerve of the Wistar rat⁵.

Keywords: sciatic nerve defect, rat model, experimental surgery, nerve tubes.

INTRODUCTION
Although quite resistant to shear stress, nerves can be injured in different ways at different sites⁶,⁷. Nerve defects up to 0,5cm can be sutured sometimes primarily, with proximal and distal dissection. However this results in higher tension in the suture, increasing the risk of nerve rupture and influencing the regeneration process. For this situation, coaptation of the nerve endings is better performed indirectly by using a nerve conduit⁸,⁹.

The theoretical knowledge of different types of nerve injuries helps to properly evaluate each case and decide upon specific actions¹⁰,¹¹. A rigorous training in experimental microsurgery is to be performed before conducting sutures on humans, as there is a learning curve to be achieved¹²,¹³.
One of the most suitable laboratory animals for in vivo experimentation is the Wistar rat, due to its high similitude in nerve regeneration; furthermore its availability ease for manipulation make the rat the ideal candidate for experimental microsurgery. What is more, the sciatic nerve in the case of the Wistar rat is easily accessible and has suitable size and length for different microsurgical procedures\(^\text{14}\). The sciatic nerve runs along the inferior border of the femur for 3cm before dividing into 3 terminal branches – common peroneal, tibial and sural nerve\(^\text{15}\).

Apart from the surgical technique, good outcomes in the \textit{in vivo} experiments depend on proper anesthesia\(^\text{16}\).

**MATERIAL AND METHOD**

Wistar rats in different microsurgery training laboratories were used. The weight of the rats varied between 250-350g being of different ages (7 to 9 weeks). The anesthesia protocol consisted of a mixture of ketamine (75mg/kg) and xylazine (10mg/kg) administered intraperitoneally. For a correct dose administration, a scale was used to weigh every animal. In all laboratories, different microscopes with magnification varying between 3x-20x were used, as well as suitable microsurgical instruments appropriate sutures (8-0.9-0.10-0).

After administrating the anesthesia, the rats were shaved in the gluteal area and the thigh region and pla-
placed in prone position, with the limb to be operated on in extension. A syringe was placed under the hip joint in order to achieve maximal limb extension and to push the sciatic nerve more superficial. An oblique-transverse skin incision was made 1cm inferior to the border of the femur and dissection through the biceps muscle fibers was performed in order to expose the underlying sciatic nerve.

After creating a 0.5cm nerve defect, a surgical repair using different procedures was performed. Some involved using a silicone tube, others a biologic vessel harvested from a rat. Both procedures require placing the nerve endings inside the conduit for 0.3cm and 2-3 sutures at both ends.

**RESULTS**

In all cases when a defect is involved, the nerve must grow through 2 different sites – proximal and distal. The outcome after nerve repair depends on the technique used, on the type of nerve conduit as well as on the type of the initial nerve injury. The regeneration speed of the sciatic nerve in rats is estimated at 1.5mm/day and final results may be observed several weeks after the intervention. This growth rate may be increased by using different growth factors inside the nerve conduits17.

**DISCUSSIONS**

Choosing adequate size rats is important in order to have large enough nerves to practice the microsurgical technique. Therefore, it is recommended not to operate rats which are lighter than 250g or younger than 7 weeks of age.

The exact dosage of anesthesia is vital to be administered in order for the animals to survive and to have a fast recovery (if a longer study is in question). When the correct dosage doesn’t have the desired effect, it is better to postpone the operation and choose another rat if possible (multiple doses may cause respiratory distress which can finally kill the lab animal).

Synthetic nerve tubes represent a good therapeutic option for nerve defects. They do not require a prior intervention for harvesting; their lumen is generally open (no external growth resistance) and can be filled with different growth factors. They can be manufactured to
sizes. The major problem with veins is that they tend to collapse, making it difficult for the nerve to grow to reach the distal ending. A solution for this issue is to fill their lumen with either muscle fibers or different growth factors, in order to keep the lumen open. Manoli et al showed no difference between nerve autografting and muscle in vein conduits for defects of the digital nerves.

Arteries can also be used as nerve conduits. They are scarcer to find and when harvested may result in some degree of functio laesa but they have the advantage of a relative rigid wall, keeping the lumen open. Therefore they can be more easily filled with semiliquid substances (nerve factors) that remain inside. The difference in caliber in comparison to the nerve may determine notching of the vessel. To prevent this, equally distanced sutures on the circumference of the vessel should be performed.

In case of a small caliber artery to be used as a nerve conduit, this can be adjusted by making fishmouth incisions in order to fit the nerve endings inside the lumen.

Although nerve conduits may be used to bridge a nerve gap and facilitate grow-through process, autografts/allografts have similar result and are frequently used in nerve defects; there are however pitfalls in using either methods, which need to be kept in mind when dealing with nerve defects.

**CONCLUSIONS**

Nerve surgery requires special microsurgical training. Experimental microsurgery performed on living rats offer a real experience when it comes to handling live tissue. Nerve defects can be solve only by using different types of nerve conduits, each having advantages and disadvantages.
Knowing the shortcomings of each conduit (biological or synthetic) and what can be done in each situation is the prerogative to performing a nerve repair with good expected outcomes.

References

1. Hu M, Xiao H, Niu Y, Liu H, Zhang L. Long-Term Follow-Up of the Repair of the Multiple-Branch Facial Nerve Defect Using Acellular Nerve Allograft. J Oral Maxillofac Surg. (2016)

2. Xu Z, Chen Z, Feng W, Huang M, Yang X, Qi Z. Grafted muscle-derived stem cells promote the therapeutic efficiency of epimysium conduits in mice with peripheral nerve gap injury. Artif Organs. 2019 Dec 2. doi: 10.1111/aor.13614

3. Guo Q, Wang G, Lin W, Huang Z, Tan Z, Liu H, Huang F. RESEARCH PROGRESS OF AUTOLOGOUS VEIN NERVE CONDUIT FOR REPAIR OF PERIPHERAL NERVE DEFECT. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi. 2015 Nov;29(11):1446–50. Review

4. Wu, Fei & Xing, Danmou & Peng, Zhengren & Rao, Ting. (2008). Enhanced Rat Sciatic Nerve Regeneration through Silicon Tubes Implanted with Valproic Acid. Journal of reconstructive microsurgery. 24. 267-76. 10.1055/s-0028-1078696.

5. Andrei Marin, Georgiana Gabriela Marin, Anca Patea, Dan Mircea Enescu. Timing, Logistics and Bureaucratic Process in Planning an Experimental In Vivo Nerve Regeneration Project from A to Z. Modern Medicine | 2019, Vol. 26, No. 2

6. Battitson B, Titolo P, Ciclamini D, Panero B. Peripheral Nerve Defects: Overviews of Practice in Europe. Hand Clin. 2017 Aug;33(3):545-550. doi: 10.1016/j.hcl.2017.04.005. Review

7. Welch JA. Peripheral nerve injury. Semin Vet Med Surg (Small Anim). 1996 Nov;11(4):273-84. Review

8. Ravinder Bamba, D. Colton Riley, Nathaniel D. Kelm, Nancy Cardwell, Alonda C. Pollins, Ashkan Afshari, Lyly Nguyen, Richard D. Dortch & Wesley P. Thayer (2018) A novel conduit-based coaptation device for primary nerve repair, International Journal of Neuroscience, 128,6, 563-569, DOI: 10.1080/00207454.2017.1398157

9. Babovic N, Klaus D, Schessler MJ, Schimoler PJ, Kharlamov A, Miller MC, Tang P. Assessment of Conduit-Assisted Primary Nerve Repair Strength With Varying Suture Size, Number, and Location. Hand (N Y). 2019 Nov;14(6):735-740. doi: 10.1177/10813818198769382. Epub 2018 Apr 5.

10. Andrei, M., Ioana, M., & Mirccea, E. (2019). Underlying histopathology of peripheral nerve injury and the classical nerve repair techniques. Romanian Neurosurgery, 33(1), 17-22. https://doi.org/10.33962/roneuro-2019-003

11. Lastofka, D., Manolescu, N., Balint, E., Costea, R., & Ionescu, M. M. (2017). Histopathological modifications in sciatic nerve allograft in Wistar rats. Journal of Biotechnology, 256, S66.

12. Christensen TJ, Anding W, Shin AY, Bishop AT, Moran SL. The Influence of Microsurgical Training on the Practice of Hand Surgeons. J Reconstr Microsurg. 2015 Jul,31(6):442-9. doi: 10.1055/s-0035-1549443. Epub 2015 Apr 20.

13. Andrei, M., Gabriela, M., Amalia, D., & Mirccea, E. (2019). Learning curve in rat dissection for experimental sciatic nerve repair. Romanian Neurosurgery, 33(3), 243-248. https://doi.org/10.33962/roneuro-2019-041

14. Ionescu. M. M., Costea, R., Balint, E., & Lastofka, D. (2017). Surgery technique models of sciatic nerve allograft in Wistar rats. Abstracts/Journal of Biotechnology 256S, 44, S116

15. Mihai Meheditu-Ionescu, Ovidiu Stefanescu, Radu Cristian Jecan. Nervous Regeneration Allograft Type of Periferic Nerv. Modern Medicine | 2019, Vol. 26, No. 2

16. Costea Ruxandra, Daniel Lastofka, and Mihai Meheditu. “Comparison of Ketamine–Medetomidine-butorphanol and Ketamine–dexmedetomidine-butorphanol Anesthesia in Rats.” Agriculture and Agricultural Science Procedia 6 (2016): 305-308.

17. Fujimaki H, Uchida K, Inoue G, Miyagi M, Nemoto N, Saku T, Isobe Y, Inage K, Matsuhashi O, Yagishita S, Sato J, Takano S, Sakurnya Y, Ohtori S, Takahashi K, Takaso M. Oriented collagen tubes combined with basic fibroblast growth factor promote peripheral nerve regeneration in a 15 mm sciatic nerve defect rat model. J Biomed Mater Res A. 2017 Jan;105(1):8-14. doi: 10.1002/jbm.a.35866. Epub 2016 Aug 21

18. Mohammad J, Delaviz H, Mohammadi B, Delaviz H, Rad P. Comparison of repair of peripheral nerve transaction in pregenerated muscle with and without a vein graft. BMC Neurol. 2016 Nov 22;16(1):237.

19. Manoli T, Schulz L, Stahl S, Jaminet P, Schaller HE. Evaluation of sensory recovery after reconstruction of digital nerves of the hand using muscle-in-vein conduits in comparison to nerve surgery or nerve autografting. Microsurgery. 2014 Nov;34(8):608-15. doi: 10.1002/micr.22302. Epub 2014 Aug 2.

20. Marin Andrei, Mihai Ruxandra Ioana, Marin Georgiana Gabriela. Pitfalls and problems encountered in rat model sciatic nerve surgery. Romanian Neurosurgery (2019) XXXIII (4): pp. 396-399DOI: 10.33962/roneuro-2019-064

21. Moosavizadeh SM, Alizadeh Otaghvar H, Baghae M, Zavari A, Mohyeddin H, Fatahian H, Farazmand B, Moosavizadeh SMA. Comparison of conduit and autograft efficiency in repairing femoral nerve injury in New Zealand rabbits. Med J Islam Repub Iran. 2018 Oct 13;32:99. doi: 10.14196/mjiri.32.99. eCollection 2018.
