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THE EFFECT OF USING OF PLATELET RICH FIBRIN ON SCIATIC NERVE REGENERATION AFTER END-TO-END NEURORRHAPHY IN RAT MODEL

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ABSTRACT

Surgical repair or neurorrhaphy of peripheral nerves is a major surgical and clinical challenge with disappointing results. End-to-end neurorrhaphy is a gold standard technique in nerve repair, many techniques have been developed to improve the healing of the nerve after surgical repair, one of these techniques is the use of Platelet Rich Fibrin (PRF) in conjunction with end-to-end neurorrhaphy. This study aims to evaluate the effect of platelet rich fibrin in enhancing nerve regeneration of the sciatic nerve after end-to-end neurorrhaphy in rat model.

Material and methods: A double-blind randomized study was conducted on 20 Albino Wistar rats at the animal house of Misr University for Science and Technology, rats were divided into two groups, group I in which end-to-end neurorrhaphy was performed. In group II, after end-to-end neurorrhaphy, PRF in form of a membrane was used to wrap the area of neurorrhaphy. Preoperative and postoperative evaluation of nerve regeneration was performed at one and three weeks postoperatively using electromyography for nerve conduction measurements. Three parameters were used, the latency in conduction, the amplitude of voltage, and the reaction of degeneration postoperatively.

Results: latency, amplitude, and the reaction of degeneration within each group showed improvement along follow-up periods with a significant difference. Comparison of results between the two groups showed a significant difference along the follow-up periods in favor of group II.

Conclusion: PRF with end-to-end neurorrhaphy is superior to the end-to-end neurorrhaphy and is associated with improvement in nerve regeneration of sciatic nerve in rat model.

INTRODUCTION

Peripheral nerve injury (PNI) either motor or sensory is a major surgical and clinical challenge with often disappointing results and impaired sensory and motor function.[1] Nerve repair is an essential procedure to achieve function restoration after nerve injury. The process of healing is a multifactorial process, among these factors are axonal regeneration rate, type of injury and the

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injured nerve, age and the physiological status of the patient, the technique of repair, and the skills of the surgeon.[2] Despite the advances and evolution of microsurgery, new technologies involved, and the non-tension suturing techniques, the obtained results are disappointing and with difficult complete functional recovery, in addition to development of neuropathic pain in about 45% of cases, especially when there is total nerve transection.[3-7]

Rat sciatic nerve model has been commonly used for nerve repair studies as regeneration of sciatic nerve is very efficient, fast and the animal model is of low cost. Evaluation of regeneration is performed through different methods as histological examination, behavior and electrophysiological assessment.[8]

One of the most used nerve repair techniques is the end-to-end neurorrhaphy which is considered the gold standard technique in nerve repair surgery and when circumstances allow, it yields the best results.[6, 9]

About two decades ago, an autogenous components, platelet rich fibrin (PRF) attracted the attention of researchers and has been used in improvement of regeneration of peripheral nerves after surgical repair or neurorrhaphy.[10] Platelet rich fibrin (PRF) is prepared from autogenous platelets concentrate that contains progenitor cells that are essential in healing process in addition to growth factors as platelet-derived growth factor (PDGF), transforming growth factor (TGF)-b, vascular endothelial growth factor (VEGF), and platelet derived endothelial cell growth factor.[10]

Despite that, platelet rich fibrin (PRF) and platelet rich plasma (PRP) are nearly matching in the components regarding growth factors, PRF is considered advantageous over PRP due to simpler preparation with shorter duration, beside that there is no need for addition of heterogenous material during the preparation.[11] Moreover, PRF can be prepared in form of a membrane that can be wrapped around the nerve in form of a tube or conduit, tubulation has many advantages as it enhances the regeneration capacity with growth guidance inside the conduit-like form with prevention of collagen fibers growth around the transected area.[12, 13]

Successful neurorrhaphy is not only a clinical judgement of proper alignment of the proximal and distal ends of the nerve or tension free suturing based on the surgeon point of view, it should be also based on results obtained after the correct surgical neurorrhaphy. One of the methods used in evaluation of nerve repair surgical procedure is the use of electromyography in nerve conduction measurements regarding latency in conduction in milliseconds, amplitude of voltage in millivoltages and reaction of degeneration postoperatively.[14] Nerve conduction study is a well-established reliable measure for peripheral nerve function, it supports the clinical observation by accurately detecting the locus of the lesion and specifying any abnormality of conduction.[15, 16]

The aim of the study was to evaluate the effect of platelet rich fibrin (PRF) in enhancing the regeneration of the sciatic nerve after end-to-end neurorrhaphy in rat model.

MATERIALS AND METHODS

A double-blind randomized study was conducted on 20 Albino Wistar rats in the animal house of Misr University for Science and Technology. Albino Wistar rats were males and females weighing from 250 to 300 grams, aged from 10 to 12 weeks. The twenty animals were divided into two groups, each animal was kept in a separate box that was numbered by the veterinary staff members so that neither the surgeon nor the physiotherapy teams knew which type of repair was applied on the animal, both were totally blind. Feeding and medication during the study period were managed by the veterinary staff members. "Group I" was managed by performing end-to-end neurorrhaphy while Group II was treated with

performing end-to-end neurorrhaphy in addition to PRF membrane wrapping at the repair area.

Preoperatively, each animal was anesthetized with xylazine hydrochloride (20mg/kg) and ketamine (50mg/kg) by intraperitoneal injection, to prepare the animal for electromyography measurements and then surgery. After shaving of the area of the lower back and left hind limb of the rat, insertion of a concentric needle in the hamstring muscle was performed and by using of the stimulator, stimulation of the sciatic nerve was done and response from hamstring muscle was recorded. Using Cadwell Sierra II Wedge EMG system (Kennewick, Washington, USA), stimulation and recording were performed by application of an electrical stimuli to the sciatic nerve trunk at its proximal portion at its origin from the spinal segments L4-L6. Three parameters were recorded preoperatively for both groups, the latency in conduction in milliseconds, the amplitude of voltage in millivoltages and the reaction of degeneration of the sciatic nerve which is the resultant of postoperative amplitude divided by the preoperative amplitude (amplitude $_{post}$/ amplitude $_{pre}$).

After application of betadine as an antiseptic, skin incision was performed to expose the vastus lateralis and the biceps femoris on the left leg, sciatic nerve was exposed and identified then transected with lancet. Fig. (1).

Group I:

In this group, the transected sciatic nerve was sutured under microscope and with microsurgical instruments, end-to-end neurorrhaphy was performed using 8-0 proline suture, two simple interrupted sutures at sides of the nerve at 0 and 180 degrees was performed to approximate and stabilize the proximal and distal nerve segments with no tension. Fig. (2).

Closure of the skin was performed using 3/0 silk sutures in an interrupted fashion. Postoperative antibiotic was prescribed by the veterinarian staff member.

Group II

End-to-end neurorrhaphy of the transected nerve was performed in the same surgical procedures used in Group I; preparation of platelet rich fibrin (PRF) was delayed until neurorrhaphy was completed to avoid dryness of the PRF. Blood was collected by the veterinarian staff member through orbital plexus, 1 ml was collected in a plain clot activator tube. After using an electric centrifuge (Model: 80-1B Electric Centrifuge 4000rpm/min, Jiangsu, China) at 4000 rpm for 5 minutes, blood is separated into tree layers: acellular plasma at the top, red blood cells at the bottom, and the PRF clot in the middle. PRF clot was removed from the tube using forceps and separated from the red blood cells with forceps and scissors. PRF was then squeezed between two glass slaps to form a membrane. Fig. (3,4).

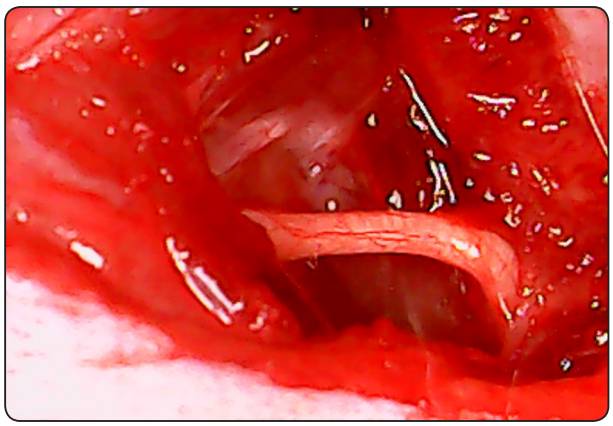

Fig. (1): Exposed and identified sciatic nerve.

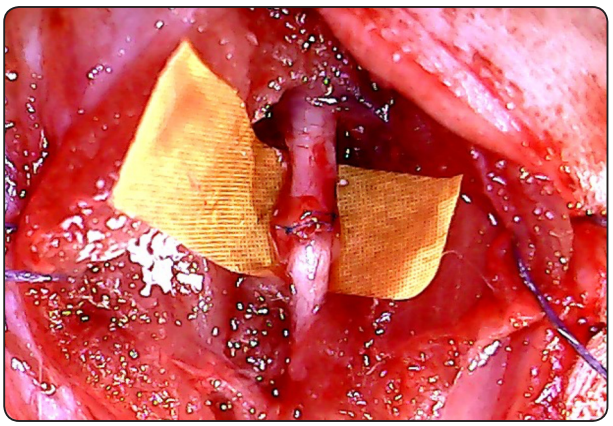

Fig. (2): End to end neurorrhaphy

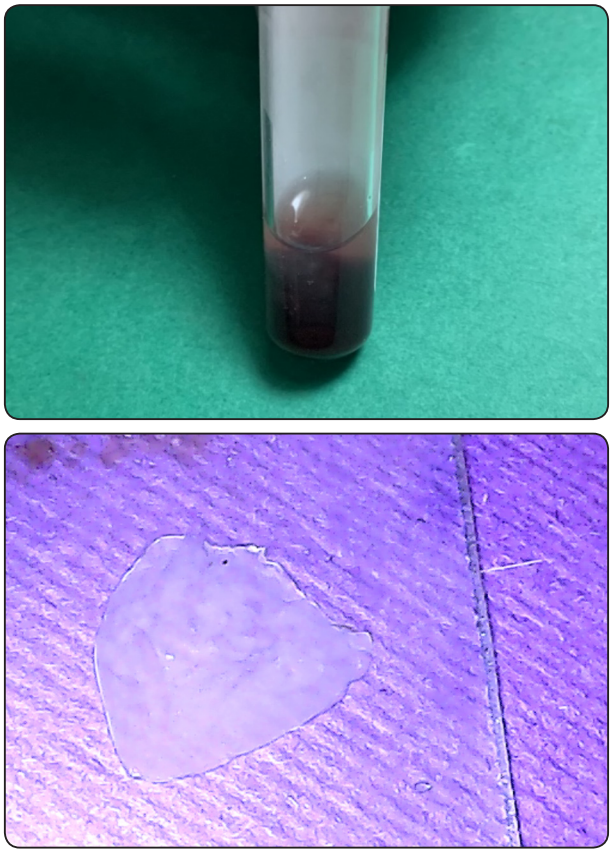

Fig. (3, 4): Obtained PRF clot in the tube, to be squeezed between two glass slaps to form a membrane.

Under the microscope the membrane was introduced underneath the sutured nerve, PRF membrane was wrapped around the nerve and tightened to provide intimate contact between the nerve and the membrane. Fig. (5).

Closure of the skin was performed using 3-0 silk sutures in an interrupted fashion. Postoperative antibiotic was prescribed by the veterinarian staff member as performed in Group I.

Follow-up was performed at two follow-up periods, one week and three weeks postoperatively using a Cadwell Sierra II Wedge EMG system, records were obtained from the left operated leg of the rat after the animal was anesthetized with intraperitoneal Ketamine/Xylazine in the same doses and technique used for the preoperative measurements and surgical procedures. Three

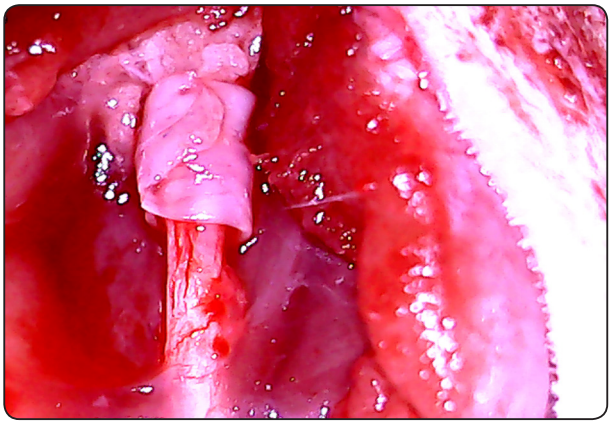

Fig. (5): PRF in a membrane form that is wrapped around the area of neurorrhaphy

parameters were recorded at the follow-up periods for both groups as performed preoperatively, the latency in conduction in milliseconds, the amplitude of voltage in millivoltages and the reaction of degeneration of the sciatic nerve.

Statistical analysis

Data collected throughout examination and obtained measurements were coded, entered, and analyzed using Microsoft excel software a statistical package for the social sciences (SPSS version 20.0). Descriptive statistical analysis was performed for all rates in the two groups for the preoperative and the postoperative records at one week and three weeks follow-up periods. Variables and all data were expressed as the means and ± standard deviation. A non-parametric statistical analysis was used because of the small sample size. Analysis of variance (Kruskal–Wallis test) was used to compare between preoperative and postoperative data at one week and three weeks follow-up periods. Comparison between reaction of degeneration data within the same group was performed using paired t-test (Wilcoxon matched pairs test). Comparison of data between the two groups was performed by using unpaired t-test (Mann–Whitney's test). The data were significant if the P values were equal to or less than 0.05.

RESULTS

On comparing results obtained from preoperative and postoperative readings of the latency in conduction in group I, it was found that, there is a significant improvement between the first and second postoperative follow-up periods (7.1±2.1 and 4.35±0.92 with P value of 0.0001). In addition to the significant improvement in amplitude (0.40±0.11 and 1.5±0.62 with P value of 0.0001) along the periods of follow-up. Reaction of degeneration showed a significant improvement at the postoperative three weeks follow-up on comparing with the first week postoperative follow-up (3.93±0.94 and 14.55±4.73 with P value of 0.002) as shown in Table (1) and Fig. (6).

Within group II we found that, on comparing results obtained from preoperative and postoperative readings, there was a significant improvement in the latency in conduction (2.79±1.32 and 1.63 ± 0.57 with P value of 0.0001) along the follow-up periods, at the same time, amplitude records showed improvement with significant difference (1.69± 0.51 and 3.38± 1.37 with P value of 0.0001) between the one week follow-up and three weeks follow-up periods. Reaction of degeneration records showed improvement along the follow-up periods at one week and three weeks postoperatively with significant difference (14.93± 3.12 and 29.24± 9.0 with P value of 0.002) as illustrated in table (2) and fig. (7).

Regarding the comparison of the three parameters between group I and group II, it was found that, there was no statistically significant difference between the preoperative readings of both latency in conduction and amplitude of voltage between the two groups. On the other hand, between the two groups, statistical difference was very significant in latency in conduction, amplitude of voltage, and the reaction of degeneration along the follow-up periods, one and three weeks postoperatively as shown in table (3) and fig. (8).

TABLE (1): The mean values and P scores of results of end-to-end neurorrhaphy (Group I) preoperatively and after one and three weeks postoperative follow-up periods respectively.

No.	Parameter	Preoperative		One week		Three weeks		P value	Significance
		Mean	SD	Mean	SD	Mean	SD		
1	Latency *(msec)*	1.0	0.11	7.1	2.1	4.35	0.92	0.0001	extremely significant
2	Amplitude *(mV)*	10.15	1.24	0.40	0.11	1.5	0.62	0.0001	extremely significant
3	RD* *(mV)*	--	--	3.93	0.94	14.55	4.73	0.002	Verry Significant

Kruskal–Wallis test, Statistical significance if P value ≤ 0.05. ***RD*: Reaction of degeneration.***

TABLE (2): The mean values and the P scores of results of end-to-end neurorrhaphy with PRF (Group II), preoperatively and after one and three weeks postoperative follow-up periods respectively.

No.	Parameter	Preoperative		One week		Three weeks		P value	Significance
		Mean	SD	Mean	SD	Mean	SD		
1	Latency *(msec)*	0.95	0.21	2.79	1.32	1.63	0.57	0.0001	extremely significant
2	Amplitude *(mV)*	11.47	2.5	1.69	0.51	3.38	1.37	0.0001	extremely significant
3	RD* *(mV)*	--	--	14.93	3.12	29.24	9.0	0.002	Very Significant

Kruskal–Wallis test, Statistical significance if P value ≤ 0.05. ***RD*: Reaction of degeneration.***

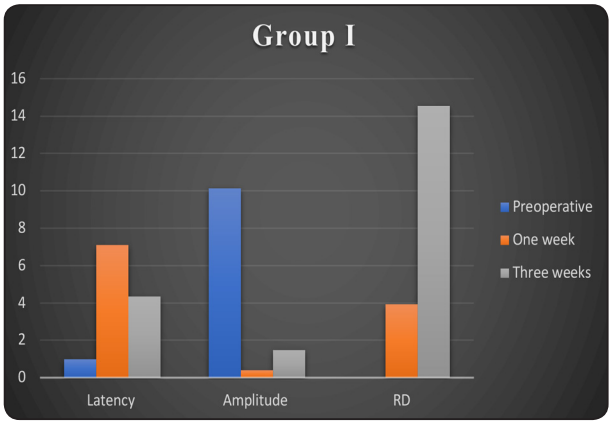

Fig. (6) Chart shows results of the three parameters within group I.

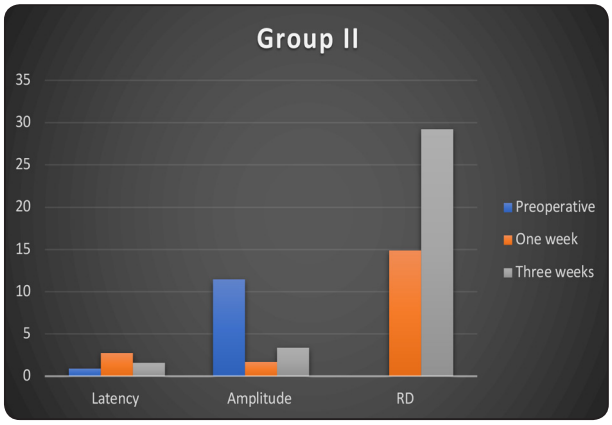

Fig. (7) Chart shows results of the three parameters within group II.

TABLE (3): The mean values and the P scores of results Group I and Group II, preoperatively and after one and three weeks follow-up postoperative periods respectively.

	Parameter	G I		G II		P value	Significance
		Mean	SD	Mean	SD		
Preoperative	Latency	1	0.11	0.95	0.21	0.2	Not significant
	Amplitude	10.15	1.24	11.47	2.5	0.15	Not significant
One weeks Postoperatively	Latency	7.1	2.1	2.79	1.32	0.0003	extremely significant
	Amplitude	0.4	0.11	1.69	0.51	0.0002	extremely significant
	RD* *(mV)*	3.93	0.94	14.93	3.12	0.0002	extremely significant
Three weeks Postoperatively	Latency	4.35	0.92	1.63	0.57	0.0001	extremely significant
	Amplitude	1.5	0.62	3.38	1.37	0.0006	extremely significant
	RD* *(mV)*	14.55	4.73	29.24	9.0	0.0002	extremely significant

Mann Whitney- test ***RD*: Reaction of degeneration. Statistical significance if P value ≤ 0.05***

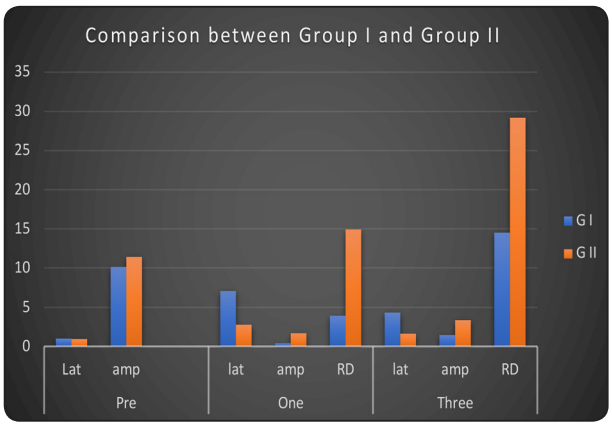

Fig. (8) Chart shows results of the three parameters between the two groups.

DISCUSSION

According to many studies, end-to-end neurorrhaphy is considered superior technique to end-to-side in surgical repair of peripheral nerves with direct repair procedure and quicker healing response.[9, 17-19] On the other hand, Viterbo et al.,[20] found that there are no functional or histological differences between end-to-end and end-to-side techniques of neurorrhaphy. Many repair techniques have been developed and improved to enhance and increase the potentiality of healing with function restoration of the injured nerve.[21]

After introduction of platelet-rich fibrin (PRF) in maxillofacial surgery, it was found that it is superior to PRP regarding the potentiality to produce growth factors for a longer period, yet few research concerning nerve regeneration was performed on it.[22]

In the present study, we used PRF as a wrap membrane to be able to gain double advantage, the first is the conduit-like form that directs and guides the growth of nerve fibers during nerve regeneration and the second is the presence of growth factors that stimulate and enhance the regeneration. The same concept was supported by Kokkalas[23] who found that PRF membrane is promising and can improve function outcome of the sciatic nerve.

Evaluation of the effect of PRF was performed through using of electromyography examination of the sciatic nerve where three parameters were evaluated, conduction latency in milliseconds, amplitude in millivolt, and reaction of degeneration. We find that electromyography as an objective assessment method of nerve repair, this is matching with what reported by Senses et al., and others,[10, 14], beside that many studies used latency and amplitude as an assessment parameters.[17, 24, 25]

On studying the perioperative records between the two groups we did not find a significant difference at the initial level regarding any of the three parameters of the study. Comparing the obtained results between group I and group II, we found a very significant differences between the results of the three parameters, we can attribute this significance to the healing potentiality of PRF and enhancement in nerve regeneration associated with the usage of PRF.

Our results are contradicting with what reported by Senses et al.,[10] who found that, the results obtained by simple suturing technique is better than that obtained from adding PRF to neurorrhaphy regarding latency and nerve conduction speed despite that difference is not significant. However other authors[13] found that application of PRF is associated with improvement in functional assessment.

In this study one week and three weeks follow-up periods were selected, this is matching with a study conducted by Czapla.[14] Our results focused on the period of early regeneration of the nerve which is the first three to four weeks postoperatively, this is the same concept followed by Torul et al. and others. [26, 27] On the other hand, other studies find that 12 weeks is not enough for nerve to reach the complete regeneration.[13, 28]

We can attribute improved results with using of PPF to the presence of growth factors that play an essential role in initiation of nerve regeneration and maintenance of healing, this concept was reported by many authors in several studies.[29-31] On addition, Chuang et al.,[32] find that PRF also has a curative potentiality due to the presence of platelet-derived protein and other biologically active substances that are essential in healing mechanism. On other hand, others still feel that PRF effect is still controversial and should be subjected for more investigations.[33, 34]

CONCLUSION

Based on results obtained from using of electromyography in nerve conduction measurements regarding latency in conduction, amplitude, and reaction of degeneration, we can conclude that using of PRF with end-to-end neurorrhaphy of sciatic nerve in rat model is superior to the end-to-end neurorrhaphy and is associated with improvement in healing and nerve regeneration.

REFERENCES

1. Aberg M., Ljungberg C., Edin E., Millqvist H., Nordh E., Theorin A., Terenghi G., Wiberg M. Clinical evaluation of a resorbable wrap-around implant as an alternative to nerve repair: a prospective, assessor-blinded, randomised clinical study of sensory, motor and functional recovery after peripheral nerve repair. J Plast Reconstr Aesthet Surg, 2009. 62(11): p. 1503-9.

2. Stocco E., Barbon S., Macchi V., Tiengo C., Petrelli L., Rambaldo A., Borean A., Capelli S., Filippi A., Romanato F., Parnigotto P.P., Grandi C., De Caro R. New bioresorbable wraps based on oxidized polyvinyl alcohol and leukocyte-fibrin-platelet membrane to support peripheral nerve neurorrhaphy: preclinical comparison versus NeuraWrap. Sci Rep, 2019. 9(1): p. 17193.

3. Allan C.H., Functional results of primary nerve repair. Hand Clin, 2000. 16(1): p. 67-72.

4. Cohen SP., Mao J. Neuropathic pain: mechanisms and their clinical implications. BMJ. 2014 Feb 5;348

5. Hovgaard C., Angermann P., Hovgaard D. The social and economic consequences of finger amputations. Acta Orthop Scand, 1994. 65(3): p. 347-8.

6. Zhu X., Wei H., Zhu H. Nerve wrap after end-to-end and tension-free neurorrhaphy attenuates neuropathic pain: A prospective study based on cohorts of digit replantation. Sci Rep, 2018. 8(1): p. 620.

7. Battiston B, Geuna S, Ferrero M, Tos P. Nerve repair by means of tubulization: literature review and personal clinical experience comparing biological and synthetic conduits for sensory nerve repair. Microsurgery, 2005. 25(4): p. 258-67.

8. Martins RS, Siqueira MG, da Silva CF, Plese JP. Correlation between parameters of electrophysiological, histomorphometric and sciatic functional index evaluations after rat sciatic nerve repair. Arquivos de neuro-psiquiatria, 2006. 64: p. 750-6.

9. Sherif M.A., Ashraf N.M, Ayman M.M, Moamen S.K., Mohamed H.A., Bahaaeldin S. A., Omar A.S., Reda A.S. An Analytic Study of Nerve Regeneration between End-to-End and End-to-Side Neurorrhaphy: An Experimental Study in Rats. The Medical Journal of Cairo University, 2018. 86(December): p. 4595-4599.

10. Şenses F., Önder M.E., Koçyiğit I.D., Kul O., Aydin G., Inal E., Atil F., Tekin U. Effect of Platelet-Rich Fibrin on Peripheral Nerve Regeneration. J Craniofac Surg, 2016. 27(7): p. 1759-1764.

11. Bielecki T, Dohan Ehrenfest DM. Platelet-rich plasma (PRP) and Platelet-Rich Fibrin (PRF): surgical adjuvants, preparations for in situ regenerative medicine and tools for tissue engineering. Curr Pharm Biotechnol, 2012. 13(7): p. 1121-30.

12. Bayram B, Akdeniz SS, Diker N, Helvacioğlu F, Erdem SR. Effects of Platelet-Rich Fibrin Membrane on Sciatic Nerve Regeneration. J Craniofac Surg. 2018 May; 29(3):e239-e243.

13. Lichtenfels M., Colomé L., Sebben A.D., Braga-Silva J. Effect of Platelet Rich Plasma and Platelet Rich Fibrin on sciatic nerve regeneration in a rat model. Microsurgery, 2013. 33(5): p. 383-90.

14. Czapla N., Bargiel P., Petriczko J., Kotrych D., Krajewski P., Prowans P. Electromyography as an intraoperative test to assess the quality of nerve anastomosis – experimental study on rats. Open Medicine, 2020. 15(1): p. 556-562.

15. Kimura J. Long and short of nerve conduction measures: reproducibility for sequential assessments. J Neurol Neurosurg Psychiatry, 2001. 71(4): p. 427-30.

16. Fidancı H., Öztürk İ. The relationship between nerve conduction studies and neuropathic pain in sciatic nerve injury due to intramuscular injection. (2005-9159 (Print)).

17. Liao W.C., Chen J.R., Wang Y.J., Tseng G.F. The efficacy of end-to-end and end-to-side nerve repair (neurorrhaphy) in the rat brachial plexus. J Anat, 2009. 215(5): p. 506-21.

18. Jaeger M.R., Braga-Silva J., Gehlen D., Pereira-Filho Gde A., Zettler C.G., de Souza M.A., Veas J.R., Sebben A. End-to-end versus end-to-side motor and sensory neurorrhaphy in the repair of the acute muscle denervation. Ann Plast Surg, 2011. 67(4): p. 391-6.

19. Bontioti E., Kanje M., Lundborg G., Dahlin L.B. End-to-side nerve repair in the upper extremity of rat. J Peripher Nerv Syst, 2005. 10(1): p. 58-68.

20. Viterbo F., Brock R.S., Maciel F., Ayestaray B., Garbino J.A., Rodrigues C.P. End-to-side versus end-to-end neurorrhaphy at the peroneal nerve in rats. Acta Cir Bras, 2017. 32(9): p. 697-705.

21. Ganga M.V., Coutinho-Netto J., Colli B.O., Marques Junior W., Catalão C.H., Santana R.T., Oltramari M.R., Carraro K.T., Lachat J.J., Lopes Lda S. Sciatic nerve regeneration in rats by a nerve conduit engineering with a membrane derived from natural latex. Acta Cir Bras. 2012 Dec;27(12):885-91.

22. Roth F., Fernandes M., Valente S.G., Santos J.B.G., Furukawa R.B., Fernandes C.H., Leite V.M., Faloppa F. Platelet-Rich Fibrin Conduits as an Alternative to Nerve Autografts for Peripheral Nerve Repair. J Reconstr Microsurg. 2017 Oct;33(8):549-556.

23. Kokkalas N, Kokotis P, Diamantopoulou K, Galanos A, Lelovas P, Papachristou DJ, Dontas IA, Triantafyllopoulos

IK. Platelet-rich Plasma and Mesenchymal Stem Cells Local Infiltration Promote Functional Recovery and Histological Repair of Experimentally Transected Sciatic Nerves in Rats. Cureus. 2020 May 24;12(5):e8262.

24. Martins R.S., Siqueira M.G., Silva C.F., Godoy B.O., Plese J.P. Electrophysiologic assessment of regeneration in rat sciatic nerve repair using suture, fibrin glue or a combination of both techniques. Arq Neuropsiquiatr, 2005. 63(3A): p. 601-4.

25. English A.W., Chen Y., Carp J.S., Wolpaw J.R., Chen X.Y. Recovery of electromyographic activity after transection and surgical repair of the rat sciatic nerve. J Neurophysiol, 2007. 97(2): p. 1127-34.

26. Torul D., Bereket M.C., Onger M.E., Altun G. Comparison of the Regenerative Effects of Platelet-Rich Fibrin and Plasma Rich in Growth Factors on Injured Peripheral Nerve: An Experimental Study. J Oral Maxillofac Surg, 2018. 76(8): p. 1823 e1-1823 e12.

27. Vela F.J., Martínez-Chacón G., Ballestín A., Campos J.L., Sánchez-Margallo F.M., Abellán E. Animal models used to study direct peripheral nerve repair: a systematic review. Neural regeneration research, 2020. 15(3): p. 491-502.

28. Gravvanis A.I., Tsoutsos D.A., Tagaris G.A., Papalois A.E., Patralexis C.G., Iconomou T.G., Panayotou P.N., Ioannovich J.D. Beneficial effect of nerve growth factor-7S on peripheral nerve regeneration through inside-out vein grafts: an experimental study. Microsurgery. 2004;24(5):408-15.

29. de Boer R., Knight A.M., Borntraeger A., Hébert-Blouin M.N., Spinner R.J., Malessy M.J., Yaszemski M.J., Windebank A.J. Rat sciatic nerve repair with a poly-lactic-co-glycolic acid scaffold and nerve growth factor releasing microspheres. Microsurgery, 2011. 31(4): p. 293-302.

30. Barmpitsioti A., Konofaos P., Ignatiadis I., Papalois A., Zoubos A.B., Soucacos P.N. Nerve growth factor combined with an epineural conduit for bridging a short nerve gap (10 mm). A study in rabbits. Microsurgery. 2011 Oct;31(7):545-50.

31. Karagoz H., Ulkur E., Kerimoglu O., Alarcin E., Sahin C., Akakin D., Dortunc B. Vascular endothelial growth factor-loaded poly(lactic-co-glycolic acid) microspheres-induced lateral axonal sprouting into the vein graft bridging two healthy nerves: nerve graft prefabrication using controlled release system. Microsurgery. 2012 Nov;32(8):635-41.

32. Chuang M.H., Ho L.H., Kuo T.F., Sheu S.Y., Liu Y.H., Lin P.C., Tsai Y.C., Yang C.H., Chu C.M., Lin S.Z. Regenerative Potential of Platelet-Rich Fibrin Releasate Combined with Adipose Tissue-Derived Stem Cells in a Rat Sciatic Nerve Injury Model. Cell Transplant. 2020 Jan-Dec;29:963689720919438.

33. Bastami F., Vares P., Khojasteh A.. Healing Effects of Platelet-Rich Plasma on Peripheral Nerve Injuries. J Craniofac Surg. 2017 Jan;28(1):e49-e57.

34. Stocco E., Barbon S., Macchi V., Tiengo C., Petrelli L., Rambaldo A., Borean A., Capelli S., Filippi A., Romanato F., Parnigotto P.P., Grandi C., De Caro R., Porzionato A. New bioresorbable wraps based on oxidized polyvinyl alcohol and leukocyte-fibrin-platelet membrane to support peripheral nerve neurorrhaphy: preclinical comparison versus NeuraWrap. Sci Rep 9, 17193 (2019).