Comparison of Nerve Clamping Time-Based Animal Models of Acute Peripheral Nerve Crush Injury: A Narrative Review
Short title: Acute Crush Peripheral Nerve Injury

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Abstract

Introduction: Peripheral nerve regeneration needs research. Studying nerve regeneration requires animal models. Acute peripheral nerve crush injury animal models are not standard. This study compares nerve clamping time-based animal models of peripheral nerve crush injury. There is no standard method for generating acute nerve crush injury, and nerve clamping time can vary between investigations. Many studies employ animal models to explore peripheral nerve regeneration. This work reveals the best nerve clamping period for a peripheral nerve crush injury animal model, which can help researchers design more uniform and similar experiments. This study compares nerve clamping time-based animal models of peripheral nerve crush injury.
Methods: PubMed, google scholar, Cochrane, NCBI articles from 2017–2022. PICO question: How long should nerve clamping be for acute peripheral nerve crush injury animal models? Animal models; Nerve clamping duration; Nerve clamping durations; Acute peripheral nerve crush damage in animal models. Acute peripheral nerve crush injury animal models were included. The articles were evaluated by year, experimental animal, nerve, clamping duration, and outcome.
Results: Sixteen articles were examined. Clamping lasted 5–3 minutes, with 10 seconds being the most common. Functional index and nerve microscopic features showed that the smallest clamping period of 5 seconds caused acute crush injury to peripheral nerves. A

prolonged clamping time than 30 seconds with serrated forceps may cause neurotmesis and prevent animal model production.

Conclusion: This review suggests that 5 seconds of clamping can cause peripheral nerve crush injury in animal models. This work emphasizes standardized animal models of peripheral nerve crush injury. This study can inform future research.

Keywords: peripheral nerve, acute nerve crush injury, animal model, clamping duration.

Introduction

Peripheral nerve injuries are mostly caused by trauma factors and cause a fairly high level of disability worldwide.(1) The therapeutic approach taken is adjusted to the degree of nerve damage that occurs. Between 2000 and 2018, preclinical research has shifted a lot from the development of direct neural repair to indirect neural repair.(2) This of course requires the support of making animal models that are in accordance with the required research. One of the common types of peripheral nerve injury is acute crush injury in which the cause of nerve damage is caused by ischemia and deformation of the nerves. The method of making animal models used is usually done by compression of the peripheral nerves which is the focus of research with the aim of interrupting the continuity of the axon but without breaking the continuity of the supporting connective tissue (especially the epineurium) so that the nerve trunk is still generally connected.(3)

Nerve deformation that occurs due to compression is influenced by the magnitude of the pressure and the duration of compression. The standard amount of pressure on the nerves for non-serrated clamps is 8.98 – 22.5 MPa, and this can be used as a reference for making animal models.(4) The use of several variations of forceps has also been carried out in modeling.(5) The challenge is that most studies cannot determine exactly how much pressure is produced by the clamp tool used, because of the large variety of each tool, so the researchers only rely on previous researchers. And the clamping duration is a very influential factor, namely the minimum duration needed for nerve clamping which is considered sufficient to produce nerve damage for the acute crush injury model and the maximum duration that can cause damage to the epineurium until nerve trunk are severed.

Based on several published studies, the available data can be used as a basis for formulating the appropriate clamping duration for acute crush injury animal models. And for that purpose, this study was made.

Methods

Information source and search

This study was conducted by searching the PubMed electronic database between 2017 and 2022, in English, using the “acute nerve injury model” and “acute nerve compression model” as the keywords.

Study selection

The titles and abstracts of the filtered articles were then evaluated according to the inclusion and exclusion criteria. Inclusion criteria included: English language, original article,

using experimental animal subjects, and peripheral nerve clamping in the treatment group. The flow diagram (Figure 1) shows the flow of this study.

Data collection process

The data collected from the articles collected are summarized in table (Table 1) for further analysis. Information taken from the article includes: first author, title, year of publication, experimental animals used, type of nerve, clamping tool, duration of clamping, and measures.

Statistical analysis

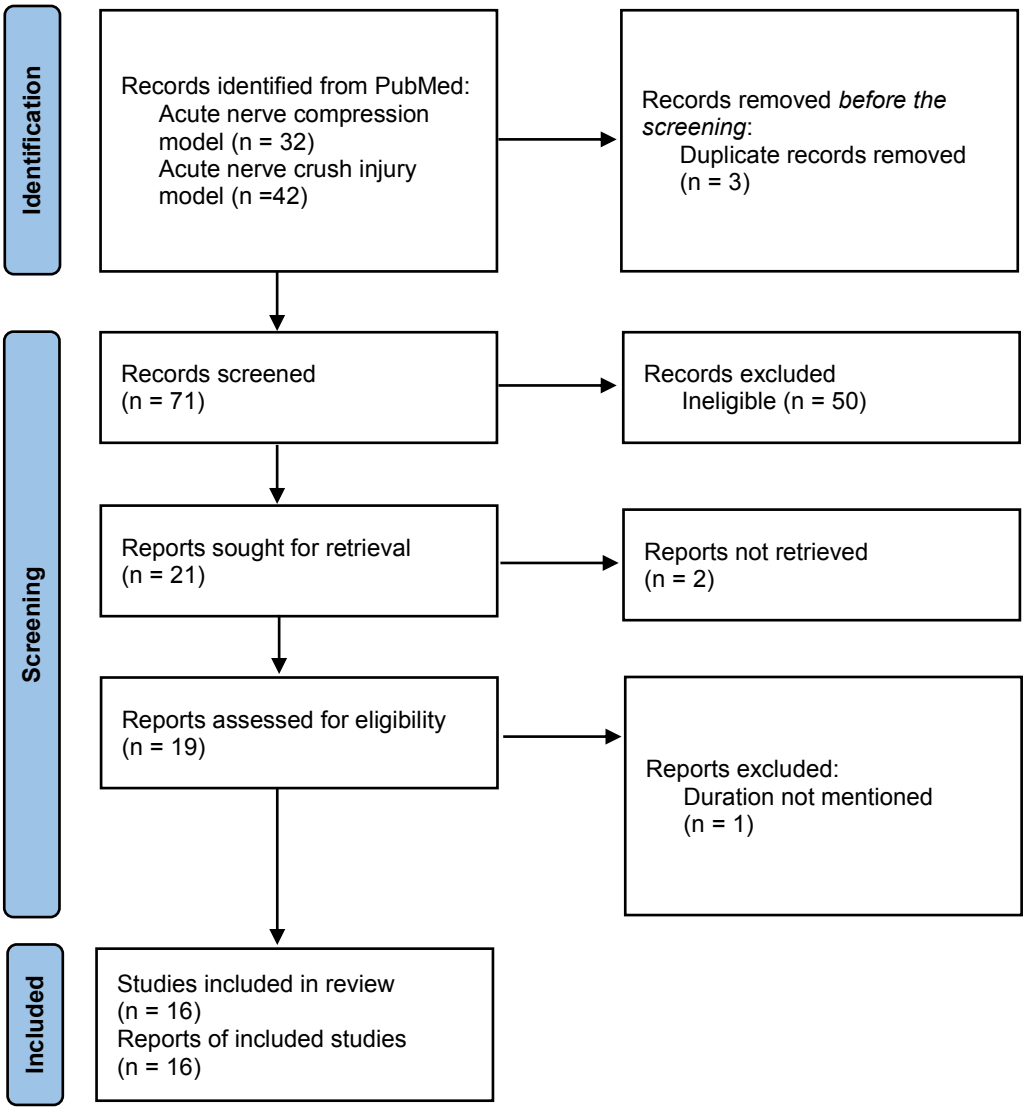
In this study, no statistical analysis was carried out.

Table 1 Summary of the studies included in this review

No.	Animal	Nerve	Tools	Duration	Measures
1.	8-week-old Sprague-Dawley rat	Sciatic nerve	Clamps (not specified)	60s	SFI is decreased (from day 0 to 7) and recovery is visible from day 7 to 14(6)
2.	7-week-old Sprague-Dawley rat	Sciatic nerve	Small forceps (not specified)	5s	Magnetic Resonance is more visible on day 10 than on day 3, Histologic neurofilament is destroyed in the injury site, and decreased in the distal portion of the injury(7)
3.	11-week-old Sprague-Dawley rat	Cavernosal nerve	Microsurgical vascular clamp	60s	At 2-week postinjury, erectile response is decrease(8)
4.	54-week-old mice	Sciatic nerve	Smooth forcep	30s	SFI is decreased (from day 0 to 7) and recovery is visible from day 7 to 15(9)
5.	6 to 8-week-old mice	Sciatic nerve	Haemostatic forcep (not specified)	20s at 3rd click	Compound muscle action potential (CMAP) is decreasing on day 3 and recovery is visible on day 18 to 36(10)
6.	4 to 6-month-old mice	Optic nerve	Dumont forcep No. 5	3 to 5s	Intrinsic apoptotic signaling is detected after 6 hours post-injury(11)
7.	6 to 10-month-old Wistar rat	Optic nerve	Dumont forcep No. 7	10s	Retinal nerve fiber layer thickness is decreased from week 1 to 5 post-injury(12)
8.	Rat	Sciatic nerve	Sugita clip	3 min	Phosphatase and tensin homolog (PTEN) was reduced from day 3 and began to increase from day 14 postinjury(13)
9.	3-month-old mice	Optic nerve	Dumont forcep No.5	10s	TNF upregulated at 6 hours and remained at 24 hours(14)

No.	Animal	Nerve	Tools	Duration	Measures
10.	6 to 8-week-old mice	Ventral roots spinal nerve L4, L5, and L6	Forcep No. 4 (not specified)	3x10s	Neuronal survival rate decreased on day 7, 14 and 28 postinjury(15)
11.	3 to 6-month-old mice	Optic nerve	Self-closing forcep No. 7 (Fine science tools or Roboz surgical instrument)	5s	Retinal ganglion cells loss by 3 weeks postinjury(16)
12.	Sprague-Dawley adult rat	Sciatic nerve	Needle holder	10s	SFI is decreased at day 7 and increased significantly from day 14 to 42(17)
13.	10-week-old mice	Sciatic nerve	Integra Miltex 18-1107	30s	SFI is at its lowest level on day 1, and starts to increase from day 7 to 28(18)
14.	6 to 8-week-old mice and Sprague-Dawley rat	Optic nerve	Curved forcep (not specified)	10s	4-sulfated (19)glycosaminoglycans are elevated on day 1, 3 and 7 postinjury(20)
15.	10-week-old mice	Sciatic nerve	Integra Miltex 18-1107	30s at a preassure of 4.4 MPa	SFI is decreased at day 1 and start to increase significantly from 12 weeks postinjury(21)
16.	Adult Wistar rat	Sciatic nerve	Non-serrated hemostatic forcep	20s	Functional deficit of injured limb is detected on day 3 and visible recovery from day 15 to 30(22)

Figure 1 Flow diagram of the study



Results

Study selection

As shown in Figure 1, there are a total of 74 articles obtained from the PubMed database using two keywords. After removing duplicate articles, there were 71 articles which were then screened based on title and abstract. Of the 71 articles, there were 50 articles that did not meet the inclusion criteria, and the remaining 21 articles were searched for the full text for further screening. Of the 21 articles, there were 2 articles that fail in obtaining full text, leaving 19 articles. After further screening, there was 1 article that did not include the duration of clamping, Ultimately, 16 articles were selected for this review.

Study characteristics

Year of publication

The analysis was carried out in the year of publication, in 2017 there are 2 articles, in 2018 there are 4 articles, in 2019 there are 3 articles, in 2020 there are 5 articles, in 2021 there are 1 articles, and in 2022 there are 3 articles.

Animals

Of the 16 studies, 9 of them used rats and 9 others used mice.

Nerve

Studies included in this review, 9 studies used the sciatic nerve, 7 studies used the optic nerve, 1 study used the cavernosal nerve, and 1 study used the spinal nerve.

Clamping tools

Of the 16 studies, there were 11 studies using smooth/non-serrated forceps, 6 studies did not specify the type of forceps used, and 1 study used serrated forceps.

Duration

Out of 16 studies, 4 studies used clamping duration 5s, 5 studies used 10s duration, 2 studies used 20s duration, 4 studies used 30s duration, 2 studies used 60s duration, and 1 study used 3 minutes duration.

Outcomes

In studies using the sciatic nerve, the SFI measurement results will be at a low level on the 3rd day of measurement and will only show improvement on the 7th day and thereafter. Observation of the histological features also showed damage to the neurofilament around injury and reduced in the distal part of the injury. The results shown were consistent both in rats with a clamping duration of between 5s to 3 minutes, and mice with a clamping duration of 20s to 30s.

In studies using the optic nerve, the measurement results showed evidence of an intrinsic signal that could already be detected from 6 hours post-injury, and the damage or differences in nerve cell characteristics could still be found up to 5 weeks post-injury. In rats, the clamping duration is 10s and in mice the clamping duration is 5s – 10s.

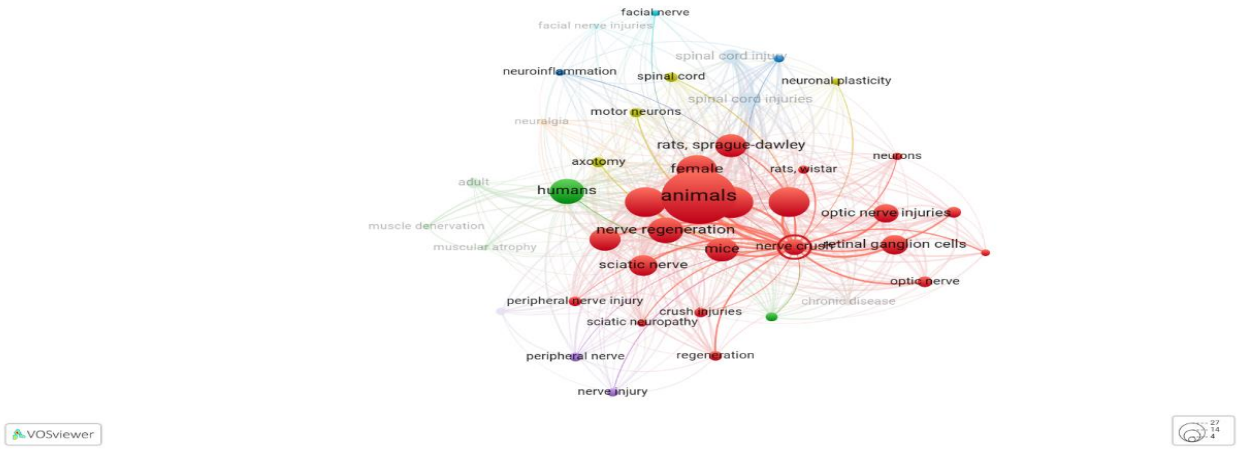
In a study using rat cavernosal nerve, a decrease in erectile function was found in the second week post-injury, with the clamping duration being 60s. In a study using mice spinal nerves, a reduction in neuronal survival rate was found on days 7, 14 and 28 post injury, with the clamping duration was 30s (3x10s).

Discussion

Summary of evidence

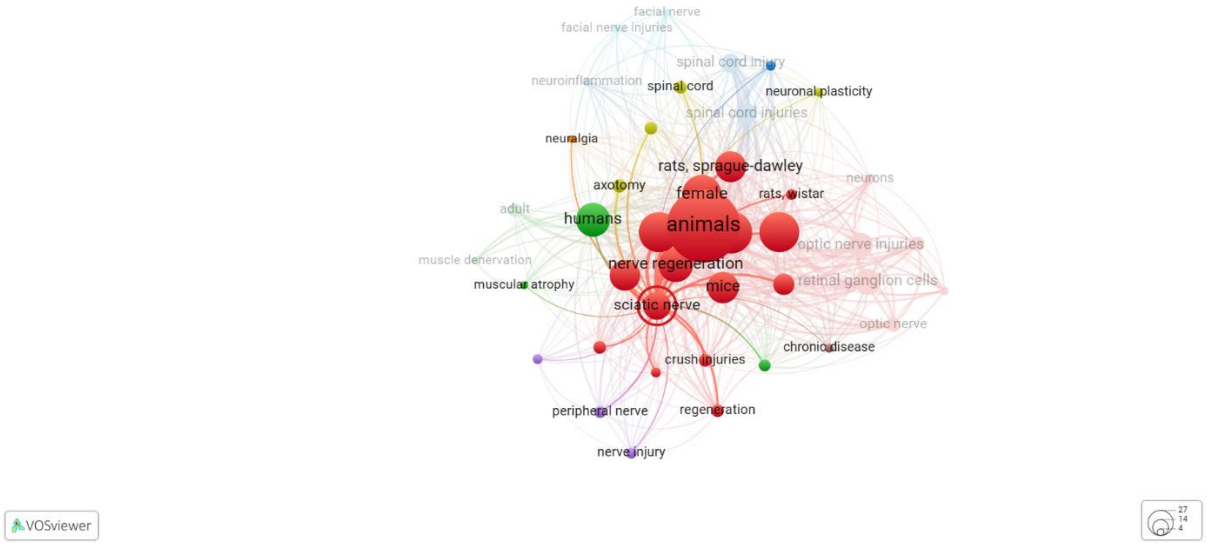
The current research situation is that studies on acute nerve crush injury in rats have been common, and the model has been used in various studies. (3) In terms of selection of experimental animals, rat and mice are still the main choice, the main basis for this selection is probably due to the low cost required, but it still needs to be considered because the mimic with actual clinical conditions is very minimal, and when compared to humans these animals have a faster nerve regeneration capacity (Fig. 2). (23)

Figure 2 Map based on nerve crush data from PubMed 2017 – 2022



For the types of nerves that are the focus of research, the sciatic nerve is still the main choice (Fig. 3) along with SFI as a hindlimb function analysis tool.(24) In the last five years, preclinical research on acute nerve crush injury trend still uses rat models, but the method of making animal models is still used in smooth/non-serrated forceps. This is still following many previous studies as it has been postulated in the table 1 summary of the studies.(4)

Figure 3 Map based on sciatic nerve data from PubMed 2017-2022



The average clamping duration is close to 30s, with the least duration being 5s, the longest duration being 3 minutes, and the most used duration being 10s. From the data of the duration obtained, and according to the results of the measurements carried out, with a least duration of 5s it can be obtained evidence that axonotmesis has occurred, different when compared to the previous study that the minimum duration is 15s,(24) and there is evidence that the use of pressure is less than 8MPa can also produce axonotmesis for acute nerve crush injury models .(4) The longest clamping duration of 3 minutes that included in this study, the evidence of neurotmesis or transection of nerves has not been found. Based on the dominant use of smooth or non-serrated forceps and the average duration used is 30s, it can be made as a reference that the clamping duration should not exceed 30s, especially for serrated forceps.

Several research studies have been conducted to improve nerve regeneration after acute nerve crush injury. The improvement can be achieved by optimizing nerve crush injury methods and using nerve repair materials. This study demonstrates the use of non-serrated forceps for acute nerve crush injury. The results show that this method makes nerve crush injury models more effective than serrated forceps. One of the main reasons for using serrated forceps is that they can exert a greater force, thus reducing the possibility of incomplete injury.(25) This study shows that using non-serrated forceps with a small diameter of the tip is more effective for making the nerve crush injury model than using serrated forceps. This is because the smaller the diameter of the forceps tip, the easier it is to apply pressure on the nerve.

The results of this review indicate that many researchers have been using the nerve crush method to evaluate nerve regeneration in preclinical studies. However, the selection of experimental animals, types of nerves, and methods of nerve crush injury are still varied.(25) The findings in this study also indicate that the acute nerve crush injury model is still frequently used to evaluate the effect of regenerative therapy. This is because of the ease of making the animal model and the low cost required as reviewed in table 1 above. In this review, the nerve crush injury model was most often used to analyze the effect of nerve regeneration therapy in rats and mice, which is still under previous studies. There are still some limitations in this study. One is the need for more ability to capture all the data since this study only uses a single database. This means that there is a possibility that other studies have been carried out using this acute nerve crush injury model method but have yet to be included in this database. Another possibility is that in some studies that have used this method of nerve injury, the information about this model needs to be explicitly stated in the title or abstract of the study. This occurs because the authors only mention the methods of nerve crush injury in the text, which is placed in the methods section.(24,25)

In the acute nerve crush injury model, the nerve damage that occurs is primarily a direct mechanical force on the nerve. In this way, the acute nerve crush injury model is similar to the clinical situation that often occurs in humans. However, this acute nerve crush injury model has several limitations, including the direct mechanical force that occurs in the nerve only at the site of compression (crushed), so the nerve damage that occurs is still very minimal. The acute nerve crush injury model is also unsuitable for nerve reconstruction procedures; therefore, this acute nerve crush injury model is still prevalent in preclinical research. The acute nerve crush injury model has long been a favorite preclinical research model, particularly in nerve regeneration. The acute nerve crush injury model has a great deal of interest in preclinical research because it has a similar mechanism to the clinical situation

that often occurs in humans. However, this acute nerve crush injury model still has several limitations. The acute nerve crush injury model is still very common in preclinical research. The acute nerve crush injury model is still very common in preclinical research because it has a similar mechanism to the clinical situation that often occurs in humans.(25)

Mechanism of acute nerve injury explained.

Acute nerve injury refers to damage to the nerve that results in immediate and severe pain. This can be caused by a variety of factors, including trauma, inflammation, or disease. The most common type of acute nerve injury is traumatic, which can occur due to a direct blow to the nerve or a sudden stretch or tear. Inflammatory conditions, such as Guillain-Barré syndrome, can also cause acute nerve injury. In some cases, the cause of the injury is unknown. The symptoms of acute nerve injury depend on the location of the damage. Common symptoms include severe pain, numbness, tingling, and weakness. The pain is often described as sharp, shooting, or burning. It may be constant or come and go. The symptoms may be worse at night. Acute nerve injury can be diagnosed with a physical examination and nerve conduction studies. Treatment typically includes rest, ice, and anti-inflammatory medications. In some cases, surgery may be necessary to repair the damage. After an acute nerve injury, it is important to rest the affected area and avoid activities that worsen the symptoms. Ice can help reduce inflammation and pain. Anti-inflammatory medications, such as ibuprofen, can also be helpful. Physical therapy may be recommended to help preserve range of motion and prevent muscle weakness. Low-intensity physical exercise can improve the injured nerve function.(26) In pre-clinical setting, the improvement of the injured nerve function can be proven using walking analysis.(27,28) Surgery may be necessary to repair a severe nerve injury. Acute nerve injuries can be extremely painful and debilitating. With proper treatment, most people recover fully. However, some people may experience long-term pain, numbness, or weakness

Figure 3 Successful nerve regeneration

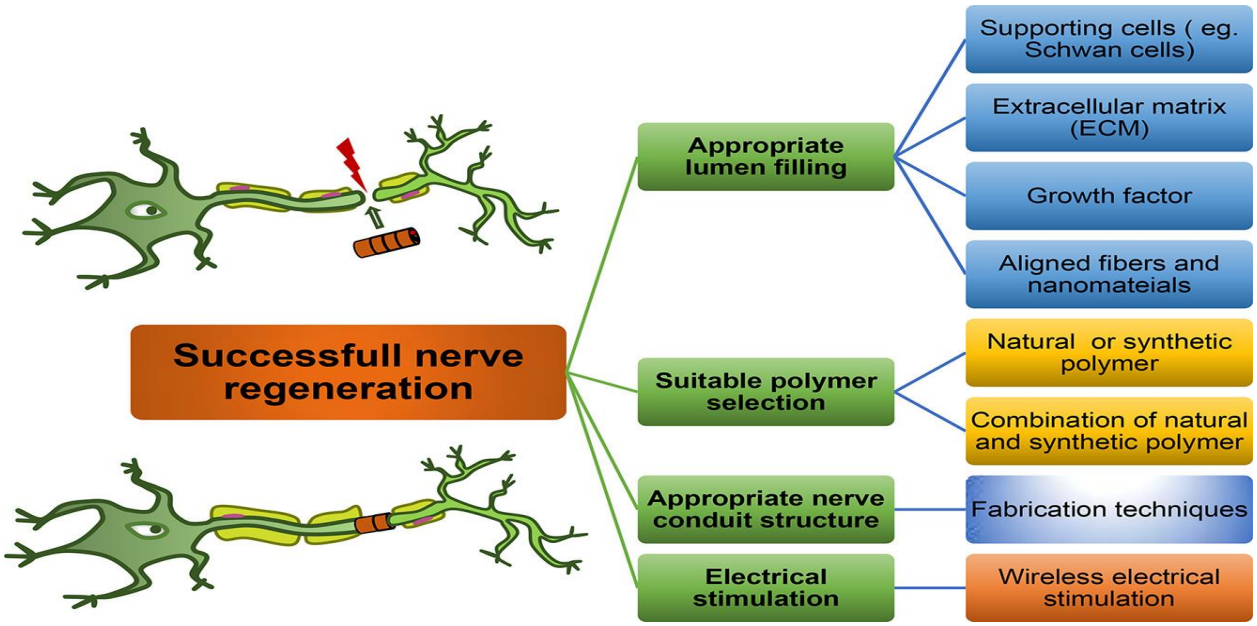


Table 2 Findings of the study conducted

Nerve	**Clamping duration**	**Results**
Sciatic nerve	5s-3min	A low level on the third day, an improvement on the seventh day. Damage to the neurofilament around the injury is reduced in the distal part of the injury.
Optic Nerve	6h-5wks	Intrinsic signal already detectable from 6 hours post-injury. Damage or differences in nerve cell characteristics still present up to 5 weeks post-injury
Cavernosal Nerve	60s	Decrease in erectile function in second-week post-injury
Spinal Nerve	30s	Reduction in neuronal survival rate on days 7, 14, and 28 post-injury

Conclusion

For the making of animal models of acute nerve crush injury with the expected end result being axonotmesis, the duration of clamping can be done at least 5s and not exceeding 30s for serrated forceps. The parameters of pressure and duration need to be emphasized in studies with rats and mice for the acute nerve crush injury model to provide a more measurable assessment.

Conflict of Interest

There is no indication that the researcher has any personal or financial interests that could affect their research or publishing, hence this study does not contain any conflicts of interest.

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References

1. Robinson LR. Chapter 19 Traumatic injury to peripheral nerves. In 2004. p. 173–86.
2. Vela F, Martínez-Chacón G, Ballestín A, Campos J, Sánchez-Margallo F, Abellán E. Animal models used to study direct peripheral nerve repair: a systematic review. Neural Regen Res. 2020;15(3):491.
3. Geuna S. The sciatic nerve injury model in pre-clinical research. J Neurosci Methods. 2015 Mar;243:39–46.

4. Beer GM, Steurer J, Meyer VE. Standardizing Nerve Crushes with a Non-Serrated Clamp. J Reconstr Microsurg. 2001;17(07):531–4.

5. Bridge PM, Ball DJ, Mackinnon SE, Nakao Y, Brandt K, Hunter DA, et al. Nerve Crush Injuries—A Model for Axonotmesis. Exp Neurol. 1994 Jun;127(2):284–90.

6. Kappos EA, Baenziger-Sieber P, Tremp M, Engels PE, Thommen S, Sprenger L, et al. Epineural adipose-derived stem cell injection in a sciatic rodent model. Brain Behav. 2018 Jul;8(7):e01027.

7. Kim BR, Ha DH, Kim JK, Kim YH. Comparison of MR findings of acute traumatic peripheral nerve injury and acute compressive neuropathy in a rat model. PLoS One. 2020 Nov 19;15(11):e0240911.

8. Song W, Son H, Kim S, Paick JS, Cho M. Role of Jun amino-terminal kinase (JNK) in apoptosis of cavernosal tissue during acute phase after cavernosal nerve injury. Asian J Androl. 2018;20(1):50.

9. Clark A, Hsu C, Talukder MH, Noble M, Elfar J. Transdermal delivery of 4-aminopyridine accelerates motor functional recovery and improves nerve morphology following sciatic nerve crush injury in mice. Neural Regen Res. 2020;15(1):136.

10. Geerdink N, Pasman JW, Rotteveel JJ, Roeleveld N, Mullaart RA. Compound muscle action potentials in newborn infants with spina bifida. Dev Med Child Neurol. 2008 Sep;50(9):706–11.

11. Schmitt HM, Fehrman RL, Maes ME, Yang H, Guo LW, Schlamp CL, et al. Increased Susceptibility and Intrinsic Apoptotic Signaling in Neurons by Induced HDAC3 Expression. Investigative Opthalmology & Visual Science. 2021 Aug 16;62(10):14.

12. Huang XR, Kong W, Qiao J. Response of the Retinal Nerve Fiber Layer Reflectance and Thickness to Optic Nerve Crush. Investigative Opthalmology & Visual Science. 2018 Apr 19;59(5):2094.

13. Ding J, Guo J, Yuan Q, Yuan F, Chen H, Tian H. Inhibition of Phosphatase and Tensin Homolog Deleted on Chromosome 10 Decreases Rat Cortical Neuron Injury and Blood-Brain Barrier Permeability, and Improves Neurological Functional Recovery in Traumatic Brain Injury Model. PLoS One. 2013 Nov 28;8(11):e80429.

14. Wang ZY, Bjorling DE. Tumor necrosis factor-α induces expression and release of interleukin-6 by human urothelial cells. Inflammation Research. 2011 Jun 30;60(6):525–32.

15. Cartarozzi LP, Perez M, Kirchhoff F, Oliveira ALR de. Role of MHC-I Expression on Spinal Motoneuron Survival and Glial Reactions Following Ventral Root Crush in Mice. Cells. 2019 May 21;8(5):483.

16. Donahue RJ, Maes ME, Grosser JA, Nickells RW. BAX-Depleted Retinal Ganglion Cells Survive and Become Quiescent Following Optic Nerve Damage. Mol Neurobiol. 2020 Feb 31;57(2):1070–84.

17. Zeng L, Cen Y, Chen J, Lei L, Zhang L, Qin X, et al. Effects of electroacupuncture on functional indices and pS6 expression following acute sciatic nerve crush injury in rats. Acupuncture in Medicine. 2020 Jun 30;38(3):181–7.

18. Lin T, Qiu S, Yan L, Zhu S, Zheng C, Zhu Q, et al. Miconazole enhances nerve regeneration and functional recovery after sciatic nerve crush injury. Muscle Nerve. 2018 May;57(5):821–8.

19. Ahn S, Chantre CO, Ardoña HAM, Gonzalez GM, Campbell PH, Parker KK. Biomimetic and estrogenic fibers promote tissue repair in mice and human skin via estrogen receptor β. Biomaterials. 2020 Oct;255:120149.

20. Yi JH, Katagiri Y, Susarla B, Figge D, Symes AJ, Geller HM. Alterations in sulfated chondroitin glycosaminoglycans following controlled cortical impact injury in mice. J Comp Neurol. 2012 Oct 15;520(15):3295–313.

21. Lee J, Talukder MH, Karuman Z, Gurjar A, Govindappa P, Guddadarangaiah J, et al. Functional recovery and muscle atrophy in pre-clinical models of peripheral nerve transection and gap-grafting in mice: effects of 4-aminopyridine. Neural Regen Res. 2023;18(2):439.

22. Demyanenko S V., Pitinova MA, Kalyuzhnaya YN, Khaitin AM, Batalshchikova SA, Dobaeva NM, et al. Human Multipotent Mesenchymal Stromal Cell–Derived Extracellular Vesicles Enhance Neuroregeneration in a Rat Model of Sciatic Nerve Crush Injury. Int J Mol Sci. 2022 Aug 2;23(15):8583.

23. Wu P, Spinner RJ, Gu Y, Yaszemski MJ, Windebank AJ, Wang H. Delayed repair of the peripheral nerve: A novel model in the rat sciatic nerve. J Neurosci Methods. 2013 Mar;214(1):37–44.

24. de Medinaceli L, Freed WJ, Wyatt RJ. An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. Exp Neurol. 1982 Sep;77(3):634–43.

25. Li C, Zhu X, Lee CM, Wu Z, Cheng L. A mouse model of complete-crush transection spinal cord injury made by two operations. Ann Transl Med. 2020 Mar;8(5):210–210.

26. Margiana R, Alawiyah K, Ima K, Fitriana R, Widodo AR, Wibowo TD. Improvement of Walking Analysis using the Sciatic Function Index for Sciatic Nerve Function in Injured Rat Model Treated with Low-Intensity Aerobics. Open Access Maced J Med Sci. 2021 Nov 27;9(A):1162–8.

27. Margiana R, Jusuf AA, Aman RA, Liem IK, Pawitan JA. A New Method in Walking Analysis Using the Angles around the Midpoint between Print Length and Toe Spread by Four Different Color Footprints. International Journal of Sciences: Basic and Applied Research (IJSBAR) International Journal of Sciences: Basic and Applied Research [Internet]. 2015;21(1):117–28. Available from: http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

28. Margiana R, Aman RA, Pawitan JA, Jusuf AA, Ibrahim N, Wibowo H. The effect of human umbilical cord-derived mesenchymal stem cell conditioned medium on the

peripheral nerve regeneration of injured rats. Electronic Journal of General Medicine. 2019;16(6).