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Evaluation of the Use of Nerve Allograft Preserved in Glycerol

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Background: We aimed to evaluate the use of nerve allograft preserved in glycerol. We compared the efficiency of glycerol-preserved allografts with autogenous nerve grafting, cryopreserved grafts, and detergent-processed grafts in the axonal regeneration. Secondarily, we evaluated the effectiveness of each preservation method in maintaining the extracellular matrix free of cellular components.
Methods: This was a prospective experimental, longitudinal, unblinded, nonrandomized, controlled animal model study. Three different allograft preservation techniques for the repair of sciatic nerve injuries were compared, including cold preservation, glycerol preservation, and detergent preservation. Functional assessment was performed, and histomorphometric analyses were further performed, which enabled the allograft structure evaluation and an estimation of the nerve regeneration efficacy based on the myelinated axons count and on their diameters.
Results: After the 14th week, all groups were already balanced and similar (P = 0.265): all groups present near-zero SFIs, thus confirming their efficiency in promoting nerve regeneration. In the histomorphometric evaluations, all groups were equivalent, presenting a similar efficiency in nerve regeneration (P = 0.716 and P = 0.577, respectively). Similarly, histomorphometric evaluations showed a reduction in the number of axons and in their diameters, but none of them effectively eliminated all cellular debris. Comparing the groups with each other, the groups preserved in glycerol and detergent solution were similar, both presenting better results than the cooled group.
Conclusion: By evaluating the presence of cell debris after the treatment using glycerol, it was found to be similar to the treatment using detergent and significantly better than the cold-preservation treatment. *(Plast Reconstr Surg Glob Open 2021;9:e3514; doi: 10.1097/GOX.0000000000003514; Published online 15 April 2021.)*

INTRODUCTION

Peripheral nerves, as extensions of the central nervous system, are responsible for integrating sensory and motor activities of the locomotor apparatus, and injuries are extremely frequent. Peripheral nerve injuries occur in between 3% and 5% of traumatic limb injuries[1,2] and, though more commonly associated with direct trauma, they may also occur as surgery and tumor invasion sequelae, resulting in costs of approximately $150 billion in annual healthcare in the United States.[3,4]

The ideal nerve repair is primary neurorrhaphy without tension. According to Millesi et al,[5,6] tensionless nerve coaptation results in a good axonal regeneration and in the return of function, especially if the denervation time is <6 months, and the patient is under 50 years of age.

However, increased tension at the repair site negatively affects the regenerating axons and results in poor function, pain, and neuroma formation. Therefore, termino-terminal primary neurorrhaphy should not be used in gaps <1 cm.[5–7]

When there is a loss in nerve substance and there is no possibility of primary suture without tension, nerve autograft is the surgical technique of choice. The autologous graft has its own Schwann cells assisting the injured nerve cells in the production of growth factors and cellular signaling. The presence of a natural extracellular matrix of the nervous tissue also provides a support framework for nerve sprouting.[8,9]

Although autograft is the technique of choice in injuries with substance loss, the need to sacrifice a healthy nerve from another body part correlates with numerous disadvantages. Donor-site infections (10%), delayed wound healing (12%), and chronic pain (5%)[10] are possible complications of autologous grafts. Loss of sensation

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in the lateral portions of the foot and ankle (44%), paresthesia (42%), and persistent calf pain (16%) are consequences found in patients who had their sural nerve removed.[11] Further disadvantages include the limitation in the number or portions of the nerve to be reconstructed and incompatibility of diameter between the injured and donor nerve.

Numerous alternative conduits have been proposed to avoid the autograft complications. Biological conduits such as vein and artery showed no benefit when compared with the autologous graft and present the same problem of morbidity at the donor site.[12,13]

The allograft is one of the most promising alternatives to the use of autologous graft in the treatment of peripheral injuries. Allogeneic grafts obtained from cadaveric donors are abundantly available and have potential size and length, as well as motor and sensory specificity. They have Schwann cells and an endoneurial microstructure that allows the same potential of regeneration presented by autografts.[14–18] Unfortunately, the use of fresh cadaver grafts requires systemic immunosuppression, which predisposes the occurrence of opportunistic infections, neoplasms, and toxicity.[19,20]

Allogeneic grafts processed to remove cellular components offer an interesting alternative to immunosuppression problems. Despite the differences in each process, they all aim to reduce immunogenicity by eliminating cellular components and maintaining the regenerative capacity while preserving the native extracellular matrix.[21]

There are many methods of acellular nerve graft preparation, including lyophilization, cold preservation, use of detergents, and irradiation.[22–25] There is little consensus as to which allograft processing technique best preserves the regenerative capacity, maintaining the extracellular matrix with lower tissue immunogenicity and with maximum functional recovery in vivo.

The use of glycerol occurs more commonly in the preservation of homogeneous cadaver skin for temporary grafts use in the treatment of burns, through the organization and maintenance of skin and tissue banks.[26–31] Glycerol is able to dehydrate the tissue, removing most of the intracellular water but without changing the cells' ionic concentration, being an efficient fixator and protector of the tissue matrix, as it makes the local cells unviable, maintaining the local architecture.[32]

The absence of acute inflammatory reactions to the use of the implants indicates the transplants' low antigenicity obtained through this type of preservation.[32,33] Glycerol preservation also has antibacterial and antiviral properties,[34–36] including for the elimination of the HIV virus.[37] Regarding glycerol preservation of nerve allografts, there are few references in the literature.

Objective

We will compare the efficiency of glycerol-preserved allografts with autogenous nerve grafting, cold-preserved grafts, and detergent-processed grafts in the axonal regeneration of the sciatic nerve in rats. Secondarily, we will evaluate the effectiveness of each preservation method in maintaining the extracellular matrix free of cellular components.

MATERIALS AND METHODS

Forty male, isogenic Wistar rats aged between 120 and 140 days and weighing between 250 and 330 g were operated on. This study was conducted after receiving authorization by the ethics committee.

Four groups of rats properly identified by numbers were created. Each group was submitted to a different type of treatment for previously severed sciatic nerve injury.

Additionally, 3 subgroups—A, B, and C—were created, consisting of sciatic nerves of rats submitted to the treatments of cold preservation, glycerol preservation, and detergent preservation. A 5-mm section was produced in the sciatic nerve in the right hind paw of each rat, 5 mm proximally to the sciatic nerve bifurcation into the tibial nerve and the common fibular nerve. Nerve repair was performed immediately after the injury, always by the same surgeon. Group 0 (control group) corresponded to autogenous graft; Group 1, to cold-preserved allograft stored in Celsior solution for 14 consecutive days at 4°C—the same procedure was performed in Group A (10 nerves treated only); Group 2, to allograft preserved in 98% glycerol solution for seven consecutive days at 4°C—the same procedure was performed in Group B (10 nerves treated only); and Group 3, to allograft preserved in detergent solution according to the protocol of Hudson et al[21] for a period of 4 days, stored at 4°C—the same procedure was performed in Group C (10 nerves treated only).

Aiming to use as few rats as possible, the paws were not operated on at the same time. The groups were operated upon sequentially.

In Group 0, a 5-mm nerve fragment was grafted from the right paw to the same paw and sutured using 4 10.0 nylon stitches. Fourteen weeks after the functional evaluations, a 2-mm thick nerve fragment was removed from the same paw, 3 mm distally to the suture, and sent for histopathological evaluation.

A 5-mm nerve fragment was removed from the left paw for subsequent preparation. This graft was cold preserved, forming Group A, to serve as a graft for Group 1.

In Group 1, a 5-mm nerve fragment was removed from the right paw and preserved in glycerol to be used as a graft for Group 2. At the same time, a cold-preserved nerve fragment obtained from Group 0 was grafted to the segmental defect. At the final time point, fragments from the right paw were removed to evaluate the reinnervation, and fragments from the left paw were removed to evaluate the effectiveness of the preservation method in eliminating cellular debris through histopathological analysis.

The subsequent groups followed the same pattern as shown in Figure 1.

The functional recovery degree associated with the obtained neural regeneration was assessed by walking track analysis in rats preoperatively and postoperatively (immediate: 3, 6, 12 weeks; at the final time point: 14 weeks), according to a method previously described by de Medinaceli et al[38] and modified by Bain et al.[14]

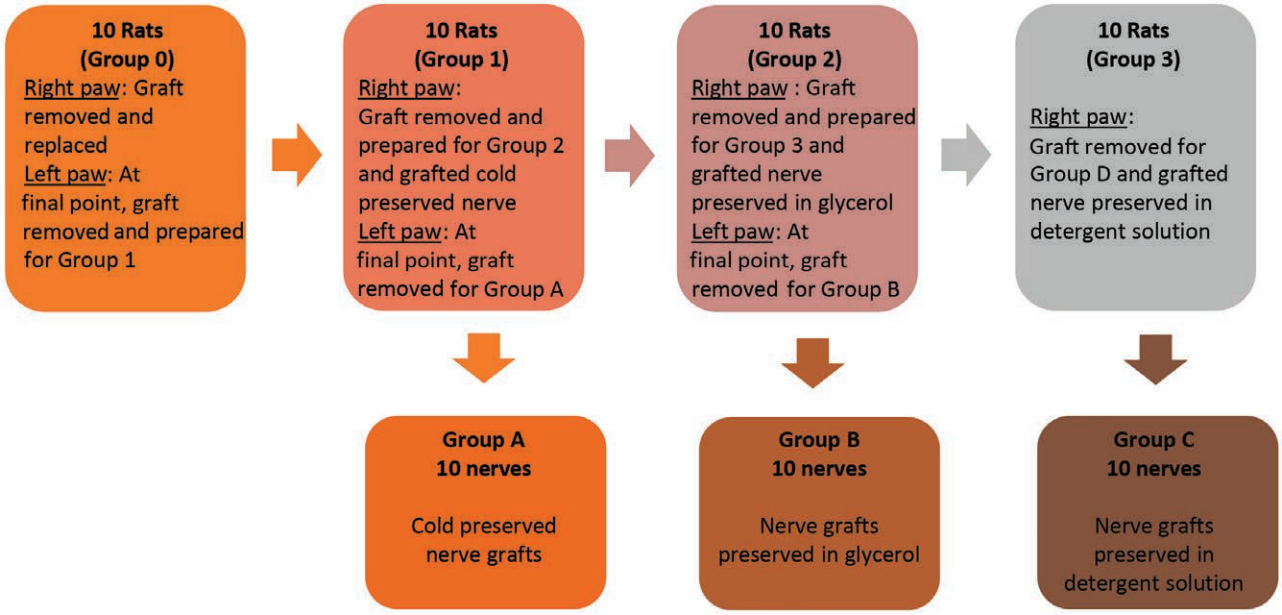

Fig. 1. Flow of groups' formation.

The material collection for histomorphometric examination in the operated groups (0, 1, 2, and 3) was performed at the 14th week after grafting, before the animals' euthanasia. A 2-mm thick sample was removed from the sciatic nerve of each rat 3 mm distally to the graft distal suture site, avoiding suture areas with possible fibroses and other tissue reactions.

In the allograft groups only treated and not operated on and reinnervated (A, B, and C), a 2-mm thick sample was removed from the nerve and histomorphometric analysis was performed (Figs. 2–5).

Based on the obtained data, the total number of reinnervated myelin fibers in a standardized area of 10,995 μm² was counted. After the reinnervated myelin fibers count, the nerve fiber cross-sectional areas (smaller diameter) were counted, and the average of these areas was calculated by summing the total measured areas and dividing the result by the number of fibers counted in the standardized field (Groups 0, 1, 2, 3, A, B, and C).

RESULTS

By individually assessing the sciatic functional index (SFI) values of each operated group (Group 0–3), we observe that all groups modified their results over each time point evaluated in all comparisons (Fig. 6) ($P < 0.05$).

By comparing the operated groups over the weeks, in the preoperative evaluation, there was no difference between the groups. In the first postoperative evaluation, the sciatic function index was negative, thus proving the effective denervation in all groups. After 3 postoperative weeks, the similarity between groups was maintained. It was only at week 6 that we observed a difference between the control and detergent groups and the control and cooling groups, with a worse SFI response in the control group (autologous graft) in relation to both groups.

There was no difference between the control and glycerol groups. Perhaps this can be explained by the greater presence of cellular remnants in the autologous graft, which must be resorbed before the regeneration process.

In the 12-week evaluation, the glycerol group was already similar to the cooling group ($P = 1.00$), but still different from the detergent group ($P = 0.001$). Nevertheless, after the 14th week, all groups were already balanced and similar ($P = 0.265$): all groups presented near-zero SFIs, thus confirming their efficiency in promoting nerve regeneration.

In the histomorphometric evaluations, all groups were equivalent, presenting a similar efficiency in nerve regeneration ($P = 0.716$ and $P = 0.577$, respectively) (Figs. 7, 8).

Similarly, histomorphometric evaluations in groups A, B, and C showed a reduction in the number of axons and in their diameters, but none of them effectively eliminated all cellular debris. Comparing the groups with each other, the groups preserved in glycerol and detergent solution were similar, both presenting better results than the cooled group (Figs. 9, 10).

DISCUSSION

The interest in nerve regeneration studies dates back to past times. The concepts established by Millesi[6] still prevail in the present, determining the primary and unstressed suture as the treatment of choice. However, a primary and unstressed suture is not possible in segmental lesions.

Despite the evolving techniques and numerous experimental and clinical studies on injuries with more extensive losses, no treatment has been able to achieve fully satisfactory results. Even autologous grafting, which is the treatment of choice in segmental loss, has its own disadvantages. This encourages studies such as the present one, aiming at developments toward the best nerve allograft

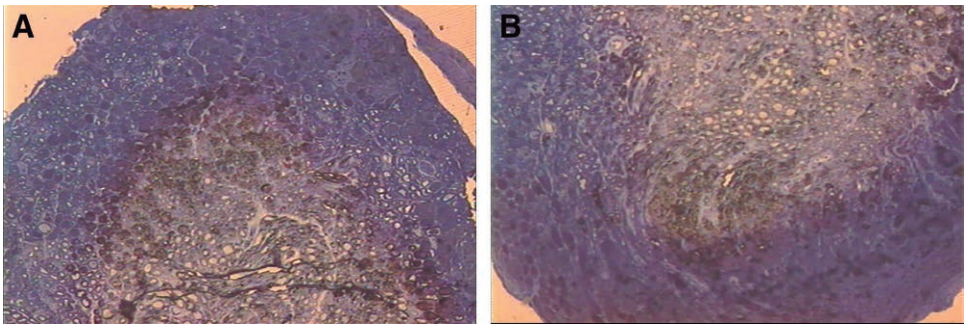

Fig. 2. Histomorphometric analysis of autografts (A) before operations, and (B) after its reinnervation, at 14 weeks (Group 0).

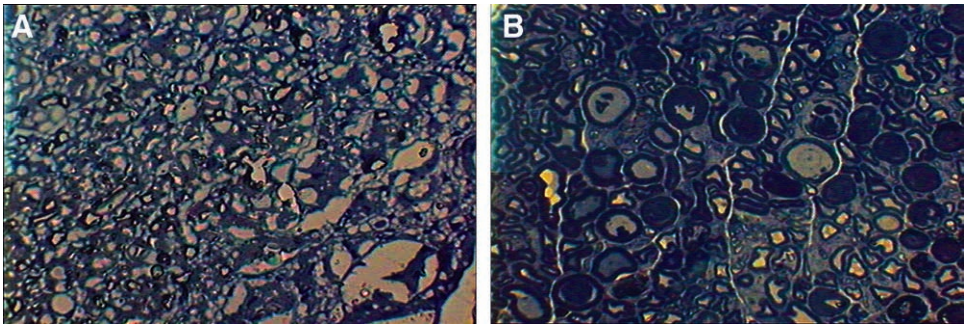

Fig. 3. Histomorphometric analysis of (A) the graft treated only by cold preservation in celsior solution (Group A), and (B) the reinnervated tissue with a cold-preserved allograft (Group 1).

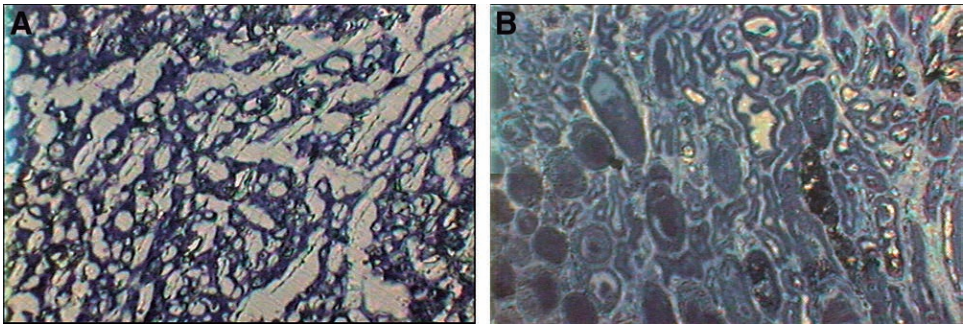

Fig. 4. Histomorphometric analysis of (A) the graft treated only with glycerol solution (Group B), and (B) the reinnervated tissue with an allograft preserved in glycerol solution (Group 2).

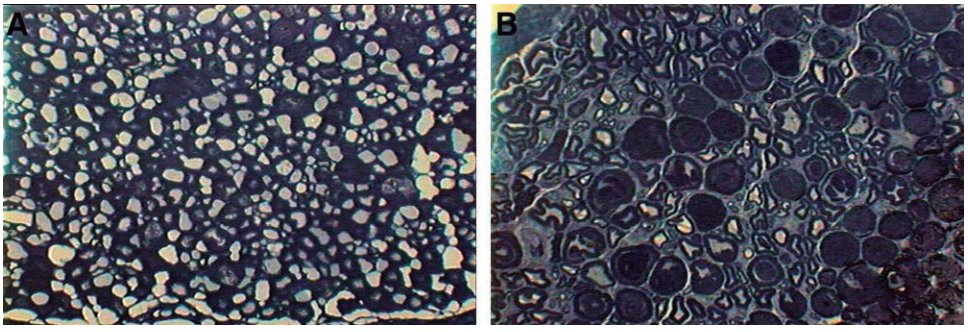

Fig. 5. Histomorphometric analysis of (A) the graft treated only with detergent solution (Group C), and (B) the reinnervated tissue with an allograft preserved in detergent solution (Group 3).

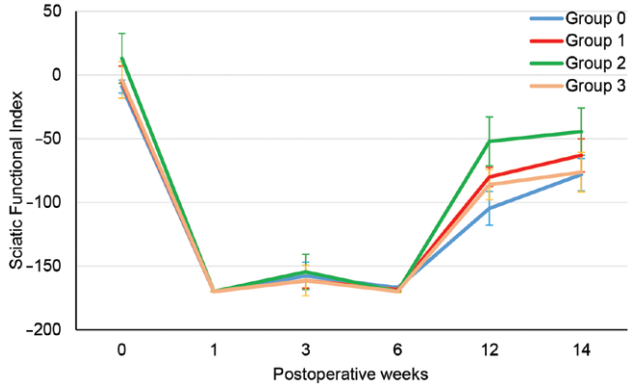

Fig. 6. SFI per assessment performed—overview of groups (mean and SD).

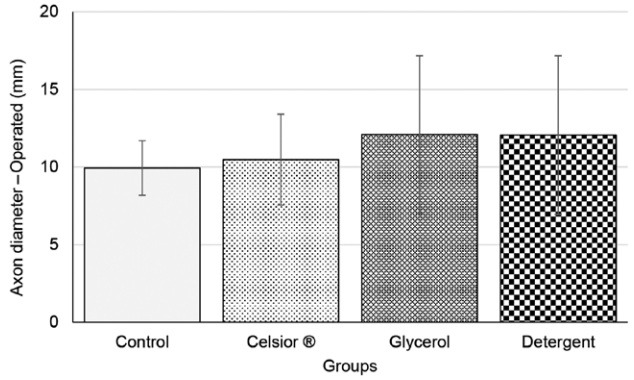

Fig. 7. Operated groups' axon diameter—groups overview (mean and SD): Groups 0, 1, 2, and 3.

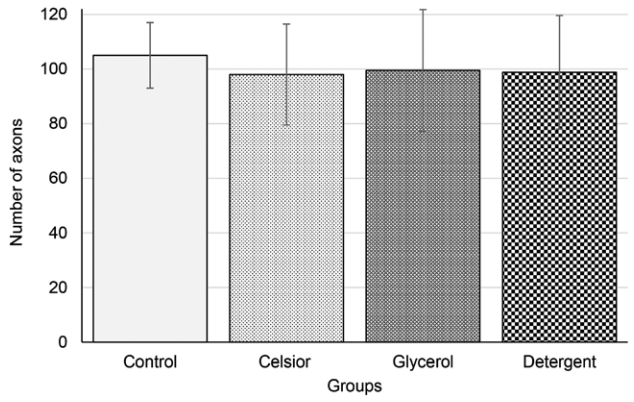

Fig. 8. Operated groups' axons—groups overview (mean and SD): Groups 0, 1, 2, and 3.

preservation alternatives. Initially, they should assume an experimental form, possibly leading to further clinical use.

Nerve allograft reconstructions have the advantage of minimizing sequelae at possible donor sites, in addition to the possibility of obtaining grafts of various sizes and lengths.

Processed allografts should not induce an immune response. Immunogenicity reduction can be achieved by eliminating cellular constituents, which leads to a low reaction or to the absence of reaction.[25] The permanence

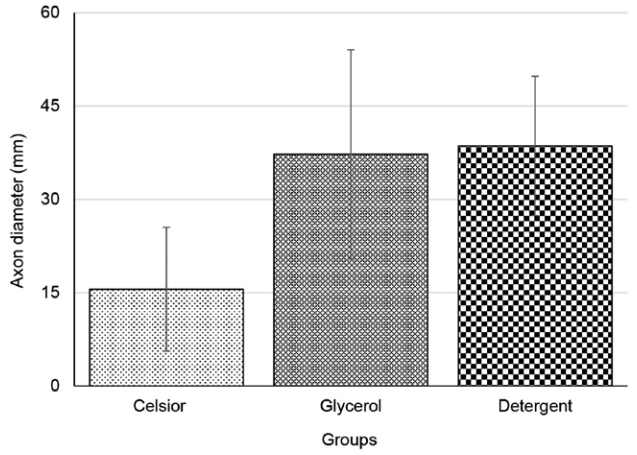

Fig. 9. Nonoperated groups' axon diameter—groups overview (mean and SD): Groups A, B, and C.

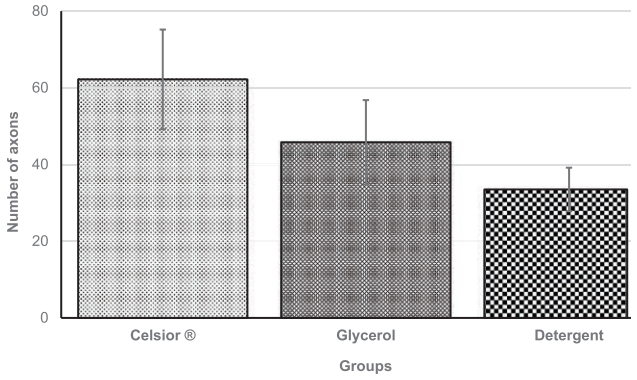

Fig. 10. Nonoperated groups' axons—groups overview (mean and SD): Groups A, B, and C.

of cellular debris also reduces or inhibits the nerve regeneration capacity by promoting an intraluminal healing barrier.

Further enhancement of the graft regenerative capacity will also be achieved by preserving the native extracellular matrix. Hence, the general objective of nerve decellularization is to remove all cellular elements except the basal lamina, thus excluding the possibility of any tissue immunogenicity. More aggressive processes will decrease immunogenicity, but will also alter structural properties with the aggression to the extracellular matrix. The ideal allograft will be the one that presents a balance between removal of cellular debris and maintenance of structural properties.[39]

The present study sought to compare 2 of the most popular methods for acellular allograft preparation, which are the cold and detergent processing[40] with the glycerol graft preservation. Glycerol is the skin preservation method for the use of temporary grafts in the treatment of burns,[26–31] being widely used in tissue banks.

A 14-week follow-up period was implemented following the most recent works. The increase in the observation period is due to the fact that, in the early stages of regeneration, many sprouts are generated, but only the axonal fibers that effectively reinnervate the target organ

will remain viable; fibers that have failed in the reinnervation tend to disappear.[41,42] Thus, after 14 weeks, we may have a later and consolidated functional assessment.

In the histomorphometric analysis, we chose to count the total number of axonal fibers in the transverse sections distal to the suture, besides measuring the myelinated fibers diameter following authors such as Hayashi et al[43] and Salles et al.[44]

Using glycerol was a cheaper preservation method when compared with the solutions used in cold and detergent preservation; besides, its management was simpler because it would not take more than 7 days of preservation nor complementary management.[45,46]

The effectiveness of the preservation methods in creating an environment free of cellular debris while maintaining the extracellular matrix was also compared. In addition to stimulating autoimmune reactions, the presence of cellular debris also inhibits the nerve regenerative capacity because it forms an intraluminal occlusive blockade.[39]

Functional assessments were made by walking track analysis and subsequent calculation of the sciatic function index. By individually assessing SFI values of each operated group (Groups 0–3) during the evaluation weeks, we observed that all groups modified their results over each time point they were evaluated; in all comparisons, therefore, the postoperative evaluation was different from the preoperative evaluation, the 3-week evaluation was different from the postoperative evaluation, and so on, until the last 14-week evaluation ($P < 0.05$). Nevertheless, after the 14th week, all groups were already balanced and similar ($P = 0.265$), showing near-zero SFI, thus confirming the efficiency in promoting the nerve regeneration.

Regarding the histomorphometric analyses, we tried to demonstrate the reinnervation in each group in the last evaluation made on the animals, at the 14th week. Therefore, we determined the number of myelinated axons present in a 3-mm section distal to the neurorrhaphy site, thus avoiding possible areas of suture fibrosis. This evaluation was aimed at showing the cell viability and functions, representing the regenerative capacity of such nerves.

We also evaluated the diameter of nerve fibers to analyze axon structural changes, including sheath thickness and the amount of intracellular collagen and myelin, as sprouts that can reinnervate the target organ and consequently reconduct nerve impulses receive layers of myelin deposited by Schwann cells and gradually increase and restore the diameter of fibers.[47,48] Thus, fiber diameter evaluation is also justified and becomes an important factor for histological evaluation.

In the histomorphometric evaluations, both regarding the fiber diameter (Graph 4) and myelinated fiber count (Graph 5), all groups were equivalent, showing a similar efficacy in nerve regeneration ($P = 0.716$ and $P = 0.577$, respectively).

A secondary objective of our study was, in addition to compare the nerve regeneration effectiveness in these three treatments, to assess whether they are effective in removing all cellular debris, thus maintaining only the extracellular matrix and making the graft truly acellular. To achieve this purpose, nerve grafts were taken and preserved according to the 3 methods studied: cold preservation, glycerol preservation, and detergent solution preservation, respectively called Groups A, B, and C.

The same histomorphometric evaluation was performed in the operated groups. The objective was to evaluate the microscopic structure of nerves and whether the preservation actually eliminated cellular debris, consequently favoring a more effective regeneration with a lower immune response. The evaluation method was not intended to qualify cells or proteins, as this would require immunohistochemical evaluations, DNA quantification, hydroxyproline assays, 2-dimensional protein electrophoresis, among others.[49] By comparing the groups, all showed a reduction in axon numbers and also in their diameters, but none of them effectively eliminated all cellular debris.

By analyzing and comparing the results of this study with the indexed literature, we believe that the nerve allograft preservation in glycerol can be useful in possible treatments of segmental lesions because it presents results similar to those of the two most studied preservation methods in functional analysis and a possibly better result in the decrease of cellular debris, than the cold-preserved group.

Although nearly all academic studies are conducted in rats, this is a particularly poor model for repairing critical human defects due to its small size and species-specific neurobiological regenerative profile. The transfer of experimental models to clinical practice in humans has shown to be unreliable for nerve regeneration, as in many other applications. Finally, like most nerve regeneration data generated in experimental models, it distorts treatment results and leads to an inadequate assessment of the risks and benefits in clinical practice.[50]

Despite possible criticism, a satisfactory nerve regeneration evolution of glycerol-preserved allografts was evident. Perhaps, combining this preservation method with others may, in future, improve the quality of these grafts to further decrease cellular debris and provide more safety in clinical practice.

CONCLUSIONS

We observed that glycerol nerve graft preservation allowed functional results and nerve regeneration with nerve fiber numbers and myelinated fiber diameters similar to autografts and to cold-preserved and detergent-preserved grafts. By evaluating the presence of cell debris after the treatment using glycerol, it was found similar to the treatment using detergent and significantly better than the cold-preservation treatment.

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REFERENCES

1. Isaacs J, Browne T. Overcoming short gaps in peripheral nerve repair: Conduits and human acellular nerve allograft. *Hand (N Y).* 2014;9:131–137.
2. Taylor CA, Braza D, Rice JB, et al. The incidence of peripheral nerve injury in extremity trauma. *Am J Phys Med Rehabil.* 2008;87:381–385.
3. Faroni A, Mobasseri SA, Kingham PJ, et al. Peripheral nerve regeneration: Experimental strategies and future perspectives. *Adv Drug Deliv Rev.* 2015;82-83:160–167.
4. Griffin JW, Hogan MV, Chhabra AB, et al. Peripheral nerve repair and reconstruction. *J Bone Joint Surg Am.* 2013;95:2144–2151.
5. Millesi H, Meissl G. Consequences of tension at the suture site. In: Gorio A, Millesi H, Mingrino S, eds. *Post-Traumatic Peripheral Nerve Regeneration.* New York: Raven Press; 1981:277.
6. Millesi H. Forty-two years of peripheral nerve surgery. *Microsurgery.* 1993;14:228–233.
7. Terzis J, Faibisoff B, Williams B. The nerve gap: Suture under tension vs. graft. *Plast Reconstr Surg.* 1975;56:166–170.
8. Lundborg G. A 25-year perspective of peripheral nerve surgery: Evolving neuroscientific concepts and clinical significance. *J Hand Surg Am.* 2000;25:391–414.
9. Streppel M, Azzolin N, Dohm S, et al. Focal application of neutralizing antibodies to soluble neurotrophic factors reduces collateral axonal branching after peripheral nerve lesion. *Eur J Neurosci.* 2002;15:1327–1342.
10. Staniforth P, Fisher TR. The effects of sural nerve excision in autogenous nerve grafting. *Hand.* 1978;10:187–190.
11. Rappaport WD, Valente J, Hunter GC, et al. Clinical utilization and complications of sural nerve biopsy. *Am J Surg.* 1993;166:252–256.
12. Colen KL, Choi M, Chiu DTW. Nerve grafts and conduits. *Plast Reconstr Surg.* 2009;124(6 Suppl):e386–e394.
13. Chiu DT, Strauch B. A prospective clinical evaluation of autogenous vein grafts used as a nerve conduit for distal sensory nerve defects of 3 cm or less. *Plast Reconstr Surg.* 1990;86:928–934.
14. Bain JR, Mackinnon SE, Hudson AR, et al. The peripheral nerve allograft: An assessment of regeneration across nerve allografts in rats immunosuppressed with cyclosporin A. *Plast Reconstr Surg.* 1988;82:1052–1066.
15. Strasberg SR, Hertl MC, Mackinnon SE, et al. Peripheral nerve allograft preservation improves regeneration and decreases systemic cyclosporin A requirements. *Exp Neurol.* 1996;139:306–316.
16. Midha R, Mackinnon SE, Evans PJ, et al. Comparison of regeneration across nerve allografts with temporary or continuous cyclosporin A immunosuppression. *J Neurosurg.* 1993;78:90–100.
17. Brenner MJ, Moradzadeh A, Myckatyn TM, et al. Role of timing in assessment of nerve regeneration. *Microsurgery.* 2008;28:265–272.
18. Mackinnon SE, Doolabh VB, Novak CB, et al. Clinical outcome following nerve allograft transplantation. *Plast Reconstr Surg.* 2001;107:1419–1429.
19. Mackinnon SE, Novak CB. Nerve transfers. New options for reconstruction following nerve injury. *Hand Clin.* 1999;15:643–66, ix.
20. Porayko MK, Textor SC, Krom RA, et al. Nephrotoxic effects of primary immunosuppression with FK-506 and cyclosporine regimens after liver transplantation. *Mayo Clin Proc.* 1994;69:105–111.
21. Hudson TW, Liu SY, Schmidt CE. Engineering an improved acellular nerve graft via optimized chemical processing. *Tissue Eng.* 2004;10:1346–1358.
22. Myckatyn TM, Mackinnon SE. A review of research endeavors to optimize peripheral nerve reconstruction. *Neurol Res.* 2004;26:124–138.
23. Hiles RW. Freeze dried irradiated nerve homograft: A preliminary report. *Hand.* 1972;4:79–84.
24. Johnson PC, Duhamel RC, Meezan E, et al. Preparation of cell-free extracellular matrix from human peripheral nerve. *Muscle Nerve.* 1982;5:335–344.
25. Sondell M, Lundborg G, Kanje M. Regeneration of the rat sciatic nerve into allografts made acellular through chemical extraction. *Brain Res.* 1998;795:44–54.
26. Granger HJ. Physicochemical properties of the extracellular matrix. In: Hargens AR, ed. *Tissue Fluid Pressure and Composition.* Baltimore: Williams & Wilkins; 1981:43–61.
27. Friedmann PS. The skin as a permeability barrier. In: Thody AJ, Friedmann PS, eds. *Scientific Basis of Dermatology.* New York: Churchill Livingston, 1986:89–112.
28. Doughty MJ, Aakre BM, Patel S. Hydration (water binding) of the mammalian corneal stroma ex vivo and vitro: Sample mass and error considerations. *Optom Vis Sci.* 1996;73:666–675.
29. Hansbrough JD. *Wound Coverage With Biologic Dressings and Cultured Skin Substitutes.* Georgetown, Tex.: RG Lands Company Austin. 1992;1–8.
30. Ghosh MM, Boyce S, Layton C, et al. A comparison of methodologies for the preparation of human epidermal-dermal composites. *Ann Plast Surg.* 1997;39:390–404.
31. Farrington M, Wreghitt T, Matthews I, et al. Processing of cardiac valve allografts: 2. Effects of antimicrobial treatment on sterility, structure and mechanical properties. *Cell Tissue Bank.* 2002;3:91–103.
32. Richters CD, Hoekstra MJ, van Baare J, et al. Morphology of glycerol-preserved human cadaver skin. *Burns.* 1996;22:113–116.
33. Richters CD, Mayen I, Havenith CE, et al. Rat monocyte-derived dendritic cells function and migrate in the same way as isolated tissue dendritic cells. *J Leukoc Biol.* 2002;71:582–587.
34. van Baare J, Buitenwerf J, Hoekstra MJ, et al. Virucidal effect of glycerol as used in donor skin preservation. *Burns.* 1994;20(Suppl 1):S77–S80.
35. Marshall L, Ghosh MM, Boyce SG, et al. Effect of glycerol on intracellular virus survival: Implications for the clinical use of glycerol-preserved cadaver skin. *Burns.* 1995;21:356–361.
36. van Baare J, Ligtvoet EE, Middelkoop E. Microbiological evaluation of glycerolized cadaveric donor skin. *Transplantation.* 1998;65:966–970.
37. Cameron PU, Pagnon JC, van Baare J, et al. Efficacy and kinetics of glycerol inactivation of HIV-1 in split skin grafts. *J Med Virol.* 2000;60:182–188.
38. 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;77:634–643.
39. Hundepool CA, Nijhuis TH, Kotsougiani D, et al. Optimizing decellularization techniques to create a new nerve allograft: An in vitro study using rodent nerve segments. *Neurosurg Focus.* 2017;42:E4.
40. Moore AM, MacEwan M, Santosa KB, et al. Acellular nerve allografts in peripheral nerve regeneration: A comparative study. *Muscle Nerve.* 2011;44:221–234.
41. Cragg BG, Thomas PK. The conduction velocity of regenerated peripheral nerve fibres. *J Physiol.* 1964;171:164–175.
42. Fawcett JW, Keynes RJ. Peripheral nerve regeneration. *Annu Rev Neurosci.* 1990;13:43–60.
43. Hayashi A, Yanai A, Komuro Y, et al. Collateral sprouting occurs following end-to-side neurorrhaphy. *Plast Reconstr Surg.* 2004;114:129–137.
44. Salles GS Jr, Faria JC, Busnardo FF, et al. Evaluation of nerve regeneration in diabetic rats. *Acta Cir Bras.* 2013;28:509–517.
45. Wolff KD, Walter G, Zimmer C. Nerve reconstruction with glycerol-preserved allogenic grafts in the rat. *Microsurgery.* 1993;14:315–322.

46. Souza Lemos SP, Hayashi I, Cunha AS, et al. Nervo alógeno conservado em glicerol. Estudo experimental em ratos. *Acta Bras Ortop.* 2008;16:133–137.
47. Lundborg G. Nerve regeneration and repair. A review. *Acta Orthop Scand.* 1987;58:145–169.
48. Sanders FK, Young JZ. The degeneration and re-innervation of grafted nerves. *J Anat.* 1942;76(Pt 2):143–166.7.
49. Hrebikova H, Diaz D, Mokry J. Chemical decellularization: A promising approach for preparation of extracellular matrix. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub.* 2015;159:12–17.
50. Kaplan HM, Mishra P, Kohn J. The overwhelming use of rat models in nerve regeneration research may compromise designs of nerve guidance conduits for humans. *J Mater Sci Mater Med.* 2015;26:226.