RABMS Journal of Research in Applied and Basic Medical Sciences

The Effect of Human Amniotic Fluid in Decellularized Human Umbilical Vein Guide Channel on Sciatic Nerve Regeneration in Rats

Gholam Hossein Farjah* [1], Parisa Hadavi[2], Masoumeh Zirak Javanmard[2], Bagher Pourheidar[2], Behnam Heshmatiyan[3], Zahra Nozhat[2]

[1] Neurophysiology Research Center, Department of Anatomy, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

[2] Department of Anatomy, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

[3] Department of Physiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

***Corresponding authors:** Gholam Hossein Farjah, **Address**: Neurophysiology Research Center, Department of Anatomy, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran, **Email**: hfarjah@hotmail.com

Tel: +984432770698

Abstract

***Background & Aims*:** The nerve autograft is the clinical gold standard in bridging nerve injury gaps, but it has severe disadvantages. The human umbilical vein (HUV) is suitable for multiple vascular reconstructive usages. The purpose of the present survey was to assess nerve regeneration by human amniotic fluid (AF) in the HUV channel.

Materials& Methods: In this study, 32 adult male rats (250-300g) were randomly divided into four groups: HUV+AF, HUV+normal saline (NS), Autograft, sham surgery. A centimeter gap in the sciatic nerve was grafted by autograft or HUV. Nerve regeneration was examined on days 28 and 90 after surgery by sciatic function index (SFI), electrophysiological assessments, histology, and immunohistology staining.

***Results*:** On days 60 and 90 after surgery, the SFI in the groups of autograft and HUV+AF was more than HUV+NS group ($p < 0.05$). On the 90th day, the average nerve conduction velocity (NCV) and the number of myelinated axons in autograft and HUV+AF groups were significantly more than HUV+NS group ($p < 0.05$).

***Conclusion*:** The results of this study display that the HUV+AF may have beneficial effect for the treatment of peripheral nerve damages.

***Keywords*:** Nerve Regeneration, Human umbilical vein, Nerve guidance channel, Amniotic Fluid, Rat

Received 19 January 2020; accepted for publication 13 April 2020

Introduction

Damage to the peripheral nerve common and may progress to permanent disability and neuralgia (1). The consequence of damage may depend on many different factors, including the type and level of damage, cellular and molecular changes in the central nervous system and end organ, as well as surgery (2).

Epineural repair is a most suitable surgical manner when the peripheral nerve is cut off transversal or the gap is small between the two ends of the nerve (3). When the distance between nerve endings increases, the repair is impossible without a graft or guide channel (1). Although nerve autograft is considered as a golden standard method (4), the results of this method are not completely satisfactory (5). Therefore, the studies are under way to determine the suitable alternative to the autograft method (6). Using the nerve guidance channel (NGC) has several benefits, including the formation and maintenance of the matrix to support the growth of axons, prevention of penetration and formation of scar tissue, and maintenance of secreted growth factors from the nerve endings (7). Ideally, NGCs must be made of biocompatible materials, able to withstand the pressure from surrounding tissues, and the suitability of the inner diameter of the channel with the diameter of the nerve for nerve growth and its guidance (8). In the previous studies, a number of natural and synthetic materials have been developed for the preparation of the NGCs, such as eggshell membrane (6), collagen (9), vein (10), and artery (11).

Previous studies have shown that vein grafts are impressive as NGCs in animals (12), and humans (13). On the other hand, it has been reported that connective tissue and fibrosis formed in vein graft are harmful to axon regeneration (14). In addition, the collapse of the vein is a subject that frequently occurs for longer nerve damages (10).

The umbilical cord is a biological tissue and easily obtained immediately after delivery (15). Normally, the umbilical cord contains 2 arteries and one vein. The human umbilical vein (HUV) has similar structure to natural artery, and it has been used in vascular (with small-caliber) grafts (16, 17), as well as ligament, tendon, and peripheral nerve regeneration (18, 19). The use of HUV for tissue engineering stimulates the immune system. Gui et al. showed that decellularization of vessel minimizes the immunogenicity of the grafts, and allows for long-term storage (20).

Amniotic fluid (AF) is secreted from embryonic and maternal tissues (21), and possess important factors such as nerve growth factor (NGF), which is essential for the development of the fetus (22).

The purpose of this experimental study was to evaluate the effect of decellularized HUV filled with human AF in sciatic nerve regeneration.

Materials and Methods

Animals:

Thirty-Two male Sprague- Dawley rats (200-250 g) were randomized into four groups: HUV + AF, HUV + normal saline (NS), Autograft, sham surgery. For the present study, ethical approval for research was obtained from Urmia University of Medical Sciences.

Preparation of the Human Umbilical Vein:

Umbilical cords (from full-term fresh placentas) were separated from the delivery suite (Shahid Motahari Hospital, Urmia, Iran). The veins were separated from umbilical cords (in a sterile condition) (16), and stocked in phosphate-buffered saline (PBS) containing penicillin and streptomycin (100U/ml) at 4ºC (20).

Decellularization HUV:

A glass bar (1.8 mm OD) was placed inside the HUV lumen. Then, the HUV was immersed in sodium dodecyl sulfate (SDS) 1% (for 24 hours at 4ºC). After that, the HUV was rinsed (three times) in PBS (pH 7), and washed for 24 hours in ethanol 75% (to remove lipid substances) (23). Then, the HUV was divided into 12-mm long pieces and washed (three times) in PBS before use (16).

Human AF Collection:

Almost 50 ml of AF was collected from 10 healthy women (25-35 years old) with vaginal or caesarean deliveries (38 to 42 weeks) under sterile conditions (24).

Surgery Procedure:

Anesthesia was done with xylazine and ketamine and (10 mg/kg; 90 mg/kg, respectively) via intraperitoneal (i.p.). On the left side, the sciatic nerve was displayed under aseptic situations. In the sham group, the surgery was completed at this stage. A nerve piece (10 mm) was removed at the proximal position of the sciatic nerve bifurcation. In HUV groups, the proximal and distal nerve ends were placed inside the channel and each end of the nerve was sutured to the channel wall by 10-0 nylon. Before placing the distal nerve end inside the channel, the space inside the channels was filled with AF or NS. Sterile paraffin was used to seal the nerve entry points into the channel. At the autograft transplant, the nerve segment was sutured at the site. At the end of the surgery, muscle and skin were sutured. After surgery, animals were housed in separate cages.

Functional Evaluation:

The functional evaluation was performed on days 7, 21, 35, 49, 60, and 90 after surgery. For this purpose, the plantar surface of the animal's hind feet was immersed in black ink. Then, the animals were allowed to walk on white paper. The footprints were used for functional evaluation (sciatic functional index or SFI) based on the formula previously described (25). SFI value varies between 0 (normal) and -100 (complete nerve transaction).

Electrophysiological Evaluation:

The animals were anesthetized (urethane, 1 g/kg, i.p.) for electrophysiological evaluation (Narco bio-system 320-3760 A trace 80, USA) on days 28 and 90 after surgery. Four rats from each group were exposed to electrophysiological studies. The body temperature of animals was maintained at 36.5- 37°C by a heat lamp. Initially, the sciatic nerve was re-exposed in the surgical hind limb. Then stimulating (intensity of 2.7 mA) and recording electrodes were placed on the sciatic nerve in the proximal area in the channel and the belly of the gastrocnemius muscle, respectively. Latency, amplitude of action potentials, and nerve conduction velocity (NCV) were determined (26).

Histological Evaluation:

After electrophysiology, middle section of the nerve in the HUV, autograft, and sham surgery groups was removed, fixed (10% buffered formalin), embedded in paraffin, cut into 5 µm transverse sections, and stained with haematoxylin-eosin. Then the number of all myelin axons was counted.

Immunohistochemistry:

The samples were post-fixed (4% paraformaldehyde), embedded in paraffin, cut into 5 µm transverse sections, and stained with anti-S-100 (Dako, 1:200 dilution). Briefly, after blocking non-specific immunoreactions, the specimens were incubated in S-100 protein antibody solutions, and biotinylated goat anti-mouse/rabbit IgG solution, respectively. Then, the sections were incubated in secondary antibody solution (Horseradish peroxidase-labelled) for 20 min at room temperature and they were examined with an optical microscope (9).

Statistical Analysis:

Data were presented in means ± standard error and analyzed using SPSS 16.0 (one-way ANOVA followed by Tukey's post hoc test), and $p<0.05$ was considered significant.

Results

Sciatic Funcional Index:

The wound was well restored and the infection was not detected.

Preoperative SFI values (a day before surgery) for all experimental groups did not differ simultaneously (-2.41 ± 3.94). At days 60 and 90 after surgery, the mean SFI was - 46.64 ± 2.8, - 37.85 ± 2.5 for HUV+AF group, and -54.65 ± 5.34, - 45.22 ± 2.06 for HUV+NS group, respectively ($p< 0.05$), while there was no difference between HUV+AF and Autograft groups ($p> 0.05$) (Fig. 1).

Electrophysiological Evaluation:

The mean NCV on days 28 and 90 after surgery in the HUV+AF and Autograft groups was 15.42 ± 1.76, 31.73± 1.34 m /Sec, and 18.59± 2.43, 39.07± 3.2 m/sec, respectively (p> 0.05). At the same time, the comparison of the amplitude (AMP) between the HUV+AF and autograft groups was 14.9 ± 0.66, 22.8± 3.62 mV and 16.74 ± 0.46, 24.78± 2.2 mV, respectively (p>0.05). (Fig. 2, 3).

Histology and Immunohistochemistry Evaluation:

Microscopic observations showed that the nerve tissue inside the HUV channel contained was covered by a thin neoepineurium and contained numerous Schwann cells and myelinated fibers. The axons were observed in small and delicate fascicles with a smaller amount of connective tissue in the HUV+AF and Autograft groups than the HUV+NS group. On the 90th day after surgery, the number of myelinated fibers in HUV+AF group (4676 ± 114) was greater than HUV+NS group (3016 ± 104) (p < 0.05), while this difference was not significant with the Autograft group (p> 0.05) (Fig. 4). At the same time, the expression of the S-100 protein in the HUV+AF and autograft groups was more apparent than HUV+NS group, and showed the presence of Schwann cells around the axons. So that the anatomy of the sciatic nerve in HUV+AF group was more similar to the normal than the HUV+ NS group (Fig. 5).

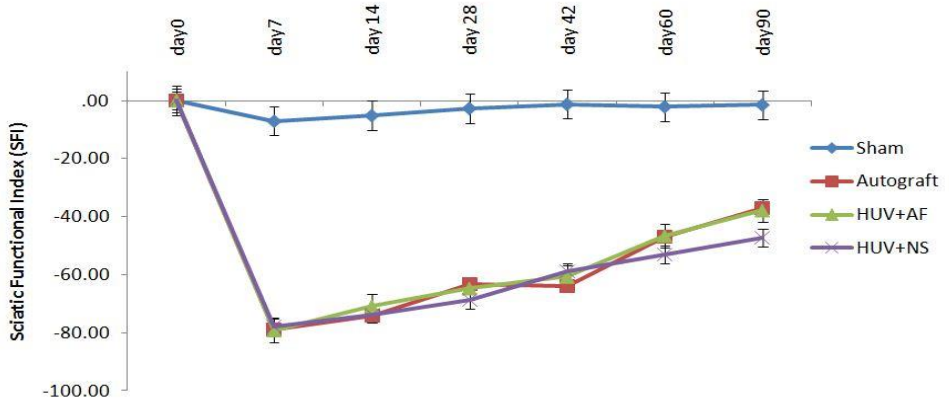

Fig. 1. Sciatic nerve index. * Difference between the HUV+NS with Authograft and HUV+AF at 28, 60, and 90 days after surgery (p<0.05); mean±SEM.

human umbilical vein (HUV), normal saline (NS), amnion fluid (AF)

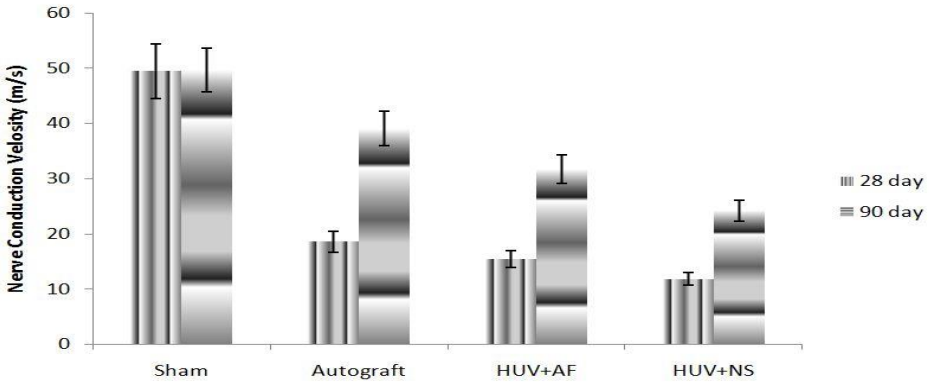

Fig. 2. Mean nerve conduction velocity (m/s). *Difference between the HUV+NS and HUV+AF at 28, and 90 days after surgery (p<0.05); mean±SEM; mean ± SEM.

human umbilical vein (HUV), normal saline (NS), amnion fluid (AF)

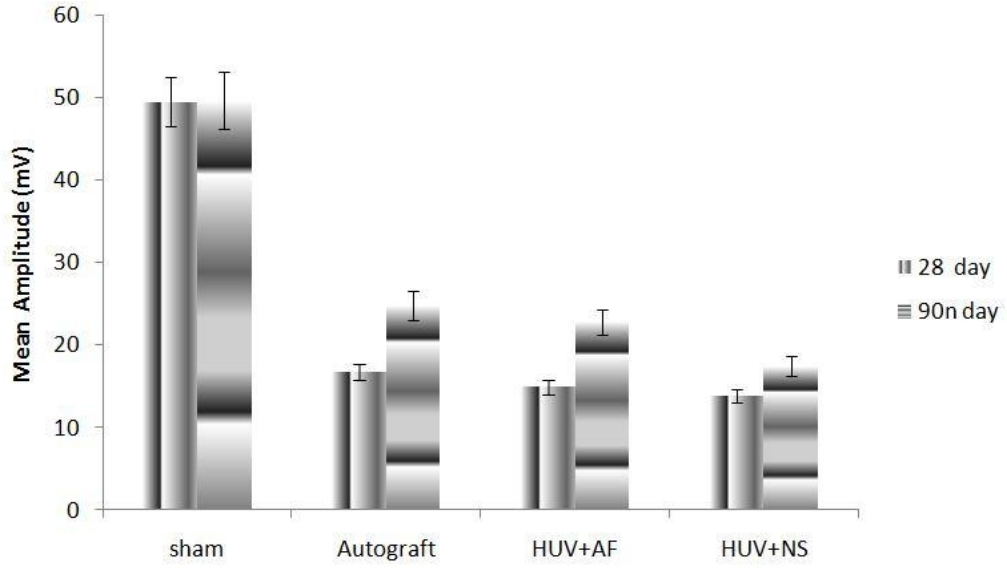

Fig. 3. Mean amplitude (mV) of sciatic nerve. *Difference between the HUV+NS and HUV+AF at 90 days after surgery ($p<0.05$); mean±SEM.

human umbilical vein (HUV), normal saline (NS), amnion fluid (AF)

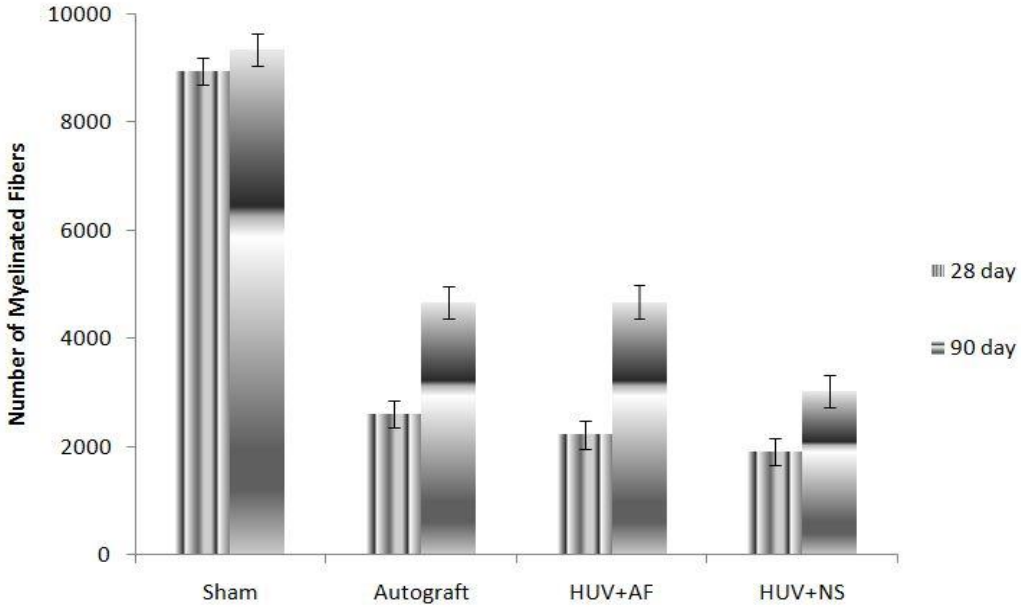

Fig. 4. Mean number of regenerated myelinated fibers.*Difference between the HUV+NS with HUV+AF and Autograft at 28 and 90 days after surgery ($p<0.05$); mean±SEM.

human umbilical vein (HUV), normal saline (NS), amnion fluid (AF)

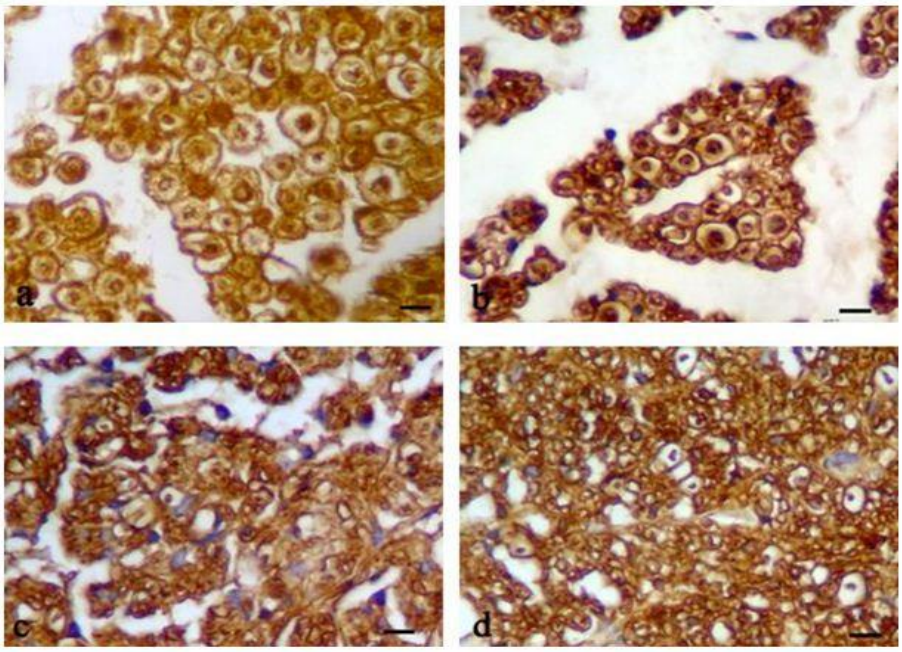

Fig. 5. Micrograft of positive staining of the myelin sheath-associated protein S-100 (Immunohistochemical analysis) 90 days after surgery. (a) Sham surgery, (b) Autograft, (c) HUV+NS, and (d) HUV+AF. Myelinated axons, Schwann cells and blood vessels were present in transverse sections of sciatic nerve (scale bar 20 µm).

human umbilical vein (HUV), normal saline (NS), amnion fluid (AF)

Discussion

The finding of this study indicated that AF in the decellularized HUV can be effective in the regeneration of the peripheral nerve. The benefits of using the vein as the nerve guide channel include leaving fewer complications than removal of the nerve as an autograft tissue, the proper adaptation to the new environment (27), and allowing for diffusion of beneficial nutrients and oxygen into the vein lumen (28).

In the vein wall, the endothelial and media layers contain laminin, and the adventitia layer is rich in collagen (29). The previous studies showed that be vein may be effective in peripheral nerve regeneration (14, 29). However, vein collapse is an important subject that is commonly found in the repair of longer nerve injuries (5).

In this study, histological and functional results revealed that HUV+AF effectively enhances nerve regeneration. The human umbilical cord contains a vein and two arteries and a proteoglycan-rich matrix (Wharton's jelly) (8), and its components are suitable as the origin of donated tissue (18). Since the diameter of the HUV is almost the same throughout the umbilical cord (20), it is used as a grafting material (18). The Wharton's jelly that surrounds the HUV is rich in growth factors, glycosaminoglycans, and proteoglycans (30). It promotes cell proliferation (18) and may have beneficial effects on nerve tissue growth (17).

In the present study, the nerve tissue within the HUV channel was covered by an neoepineurium. Wharton's jelly that covers the HUV in the umbilical cord contains laminin, heparin sulfate, proteoglycans, growth factors (30), collagen (type I, III, IV, V, IV), and hyaluronic acid (31). Laminin and type IV collagen have a major role in Schwann cell adhesion and its proliferation (32). Heparin sulfate supports synaptic plasticity (33), and may be effective in saving the nerve growth factor (34). In addition to this, hyaluronic acid may inhibit the formation of scar tissue and is effective in axonal regeneration (35). Also, the nerve growth factor (NGF) in the vein wall may have a significant role in modulating the nerve injury (36).

In the present study, sodium dodecyl sulfate (SDS) was used to decellularize HUV. SDS effectively

removes immunogenic particles of the vein wall, enhances venous permeability (8), and maintains the basement membrane anatomy (37).

In addition, the size of the inner diameter of the HUV is adjustable in the decellularization phase. So that the dimensions of the channel are very important when using the channel for nerve regeneration (7). A small inner diameter can prevent cell migration and nerve regeneration. The thickness of the channel wall plays an important role in the nerve regeneration. If it is thin, the growth factors leak out of the channel, and the channel may be blocked (7).

The results show that, for the first time, nerve regeneration in the human amniotic fluid group is higher than the normal saline group. The human AF is easily available and can be stored. This study showed that the human amniotic fluid in the channel improved SFI, NCV, and the number of myelinated fibers. The results of a previous study indicated that injection of chick amniotic fluid can enhance the regeneration of the crushed peripheral nerve (26). The fatty acids that are present in the AF can improve the neuronal function through the action of the neuron, and adjustability of changes in ion channels (38). In addition, AF contains important factors such as nerve growth factor (NGF) (39), insulin-like growth factor (IGF) (40), mesenchymal stem cells (41), vascular endothelial growth factor (42), and transforming growth factor –β1 (43) that can be effective in the nerve regeneration. The SFI indicates that the effective parameters in the functional evaluation of the hind limbs are related to the intrinsic muscle function of the feet (44). Also increase the NCV and amplitude in the HUV + AF and autograft groups reflected the thickness of the myelin sheath and the number of the myelinated axons, respectively.

One of the limitations of our study was the accumulation of AF at the end of a normal pregnancy. So that the concentration of growth factors in the AF varies in different months of pregnancy (45).

Conclusion

In conclusion, these data suggest that human amniotic fluid injected into the lumen of human umbilical vein (as the nerve guide channel) may be beneficial for regeneration of the peripheral nerve of the rat. However, further studies are needed to determine and clarify the mechanisms and efficiency of the umbilical vein and amnion fluid in peripheral nerve regeneration.

Acknowledgment

Funding for this research has been extracted from an MSc thesis in the Urmia University of Medical Sciences, Urmia, Iran.

References

1. Mohammadi J, Delaviz H, Mohammadi B, Delaviz H, Rad P. Comparison of repair of peripheral nerve transaction in predegenerated muscle with and without a vein graft. BMC Neurol 2016; 16(1): 237-44.
2. Meaney DF, Smith DH. Cellular biomechanics of central nervous system injury. Handb Clin Neurol 2015; 127:105-14.
3. Griffin MF, Malahias M, Hindocha S, Khan WS. Peripheral nerve injury: principles for repair and regeneration. Open Orthop J 2014; 8:199-203.
4. Evans GR. Challenges to nerve regeneration. Semin Surg Oncol 2000; 19 (3):312-8.
5. Geuna S, Tos P, Titolo P, Ciclamini D, Beningo T, Battiston B. Update on nerve repair by biological tubulization. J Brachial Plex Peripher Nerve Inj 2014; 9(1):3-8.
6. Farjah GH, Heshmatian B, Karimipour M, Saberi A. Using eggshell membrane as nerve guide channels in peripheral nerve regeneration. Iran J Basic Med Sci 2013; 16(8):901-5.
7. Hudson TW, Evans GR, Schmidt CE. Engineering strategies for peripheral nerve repair. Orthop Clin North Am 2000; 31(3):485-98.
8. Crouzier T, McClendon T, Tosun Z, McFetridge PS. Inverted human umbilical arteries with tunable wall thicknesses for nerve regeneration. J Biomed Mater Res A 2009; 89(3):818-28.
9. Farjah GH, Dolatkhah MA, Pourheidar B, Heshmatian B. The effect of cerebro-spinal fluid in collagen guide channel on sciatic nerve regeneration in rat. Turk Neurosurg 2017; 27(3):453-9.

10. Konofaos P, Ver Halen JP. Nerve repair by means of tubulization: past, present, future. J Reconstr Microsurg 2013; 29 (3): 149-64.
11. Liao IC, Wan H, Qi S, Cui C, Patel P, Sun W, et al. Preclinical evaluations of cellular biological conduits for peripheral nerve regeneration. J Tissue Eng 2013; 4:2041731413481036.
12. Roque JS, Pomini KT, Buchaim RL, Buchaim DV, Andreo JC, Roque DD, et al. Inside-out and standard vein grafts associated with platelet-rich plasma (PRP) in sciatic nerve repair. A histomorphometric study. Acta Cir Bras 2017; 32(8): 617-25.
13. Wang X, Luo E, Li Y, Hu J. Schwann-like mesenchymal stem cells within vein graft facilitate facial nerve regeneration and remyelination. Brain Res 2011; 1383:71-80.
14. Steger CM, Bonaros N, Bonatti J, Schachner T. Vein graft disease in a knockout mouse model of hyperhomocysteinaemia. Int J Exp Pathol 2016; 97(6): 447-56.
15. Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, et al. Mesenchymal stem cells in the Wharton·s jelly of the human umbilical cord. Stem. Cells 2004; 22(7): 1330-7.
16. Daniel J, Abe K, McFetridge PS. Development of the human umbilical vein scaffold for cardiovascular tissue engineering applications. ASAIO J 2005; 51(3):252-61.
17. Hoenicka M, Lehle K, Jacobs VR, Schmid FX, Birnbaum DE. Properties of the human umbilical vein as a living scaffold for a tissue-engineered vessel graft. Tissue Eng 2007; 13(1):219-29.
18. Abousleiman RI, Reyes Y, McFetridge P, Sikavitsas V. The human umbilical vein: a novel scaffold for musculoskeletal soft tissue regeneration. Artif Organs 2008 32(9):735-42.
19. Farjah GH, Hadavi P, Pourheidar B, Zirak Javanmard M, Heshmatian B. Using human umbilical vein as nerve guide channels in sciatic nerve regeneration of rat. J North Khorasan Uni Med Sci 2016; 7(4):885-95.
20. Gui L, Muto A, Chan SA, Breuer SA, Niklason LE. Development of decellularized human umbuilical arteries as small-diameter vascular grafts. Tissue Eng Part A 2009; 15(9): 2665-76.
21. Underwood MA, Gilbert WM, Sheman MP. Amniotic fluid: not just fetal urine anymore. J Perinatol 2005; 25(5):341-8.
22. Shaw SW, David AL, De Coppi P. Clinical applications of prenatal and postnatal therapy using stem cells retrieved from amniotic fluid. Curr Opin Obstet Gynecol 2011; 23(2):109-16.
23. McFetridge PS, Daniel JW, Bodamyali T, Horrocks M, Chaudhuri JB. Preparation of porcine carotid arteries for vascular tissue engineering applications. J Biomed Mater Res A 2004; 70(2):224-34.
24. Gutiérrez-García AG, Contreras CM, Vásquez-Hernández DI. Amniotic fluid or its fatty produce actions similar on lateral septal neurons firing rate. ScientificWorld Journal. 2013; 534807.
25. Bain JR, Mackinnon SE, Hunter DA. Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. Plast Reconstr Surg 1989; 83(1): 129-38.
26. Farjah GH, Fazli F. The effect of chick embryo amniotic fluid on sciatic nerve regeneration of rats. Iran J Vet Res 2015; 16(2):167-71.
27. Lu DY, Chen EY, Wong DJ, Yamamoto K, Protack CD, Williams WT, et al. Vein graft adaptation and fistula maturation in the arterial environment. J Surg Res 2014; 188(1):162-73.
28. Loesch A, Dashwood MR. Vasa vasorum inside out/outside in communication: a potential role in the patency of saphenous vein coronary artery bypass grafts. J Cell Common Signal 2018; 12(4):631-43.
29. Allet MA, Leite VM, Albertoni WA, Fernandes FAS, Fernandes M, Faloppa F. Graft versus graft covered with vein conduits in nerve repairs. Rev Bras Ortop 2003; 38:193-200.
30. Sobolewski K, Malkowski A, Bankowski E, Jaworski S. Wharton´s jelly as a reservoir of peptide growth factors. Placenta 2005; 26:747-52.
31. Bankowski E, Sobolewski K, Romanowicz L, Chyczewski L, Jaworski S. Collagen and glycosaminoglycans of wharton´s jelly and their alterations in EPH-gestosis. Eur J Obstet Gynecol Reprod Biol 1996; 66:109-17.
32. Pierucci A, Duek EA, de Oliveira AL. Expression of basal lamina components by Schwann cells cultured on

poly(lactic acid) (PLLA) and poly(caprolactone) (PCL) membranes. J Mater Sci Mater Med 2009; 20(2):489-95.

33. Minge D, Senkov O, Kaushik R, Herde MK, Tikhobrazova O, Wulff AB, et al. Heparan sulfates support pyramidal cell excitability, synaptic plasticity, and context discrimination. Cereb Cortex 2017; 27(2):903-18.
34. Rickard SM, Mummery RS, Mulloy B, Rider CC. The binding of human glial cell line-derived neurotrophic factor to heparin and heparan sulfate: importance of 2-O-sulfate groups and effect on its interaction with its receptor, GFRalpha1. Glycobiology 2003; 13(6):419-26.
35. Li R, Liu H, Huang H, Bi W, Yan R, Tan X, et al. Chitosan conduit combined with hyaluronic acid prevent sciatic nerve scar in a rat model of peripheral nerve crush injury. Mol Med Rep 2018; 17(3):4360-8.
36. Levine MH, Yates KE, Kaban LB. Nerve growth factor is expressed in rat femoral vein. J Oral Maxillofac Surg2002; 60(7):729-33.
37. Schaner PJ, Martin ND, Tulenko TN, Shapiro IM, Tarola NA, Leichter RF, et al. Decellularized vein as a potential scaffold for vascular tissue engineering. J Vasc Surg 2004; 40(1):146-53.
38. Leaf A, Xiao YF, Kang JX. Interactions of n-3 fatty acids with ion channels in excitable tissues. Prostaglandins Leukot Essent Fatty Acids 2002; 67(2-3):113-20.
39. Chan JR, Watkins TA, Cosgaya JM, Zhang C, Chen L, Reichardt LF, et al. NGF controls axonal receptivity to myelination by Schwann cells or oligodendrocytes. Neuron 2004; 43(2):183-91.
40. González Porto SA, Domenech N, Blanco FJ, Centeno Cortés A, Rivadulla Fernández Álvarez Jorge Á, Sánchez IbáñJ, et al. Intraneural IGF-1 in cryopreserved nerve isografts increase neural regeneration and functional recovery in the rat sciatic nerve. Neurosurgery 2019; 85(3):423-31.doi:10.1093/neuros/nyy339.
41. Yan ZJ, Hu YQ, Zhang HT, Zhang P, Xiao ZY, Sun XL, et al. Comparison of the neural differentiation potential of human mesenchymal stem cells from amniotic fluid and adult bone marrow. Cell Mol Neurobiol 2013; 33(4):465-75.
42. Muratori L, Gnavi S, Fregnan F, Mancardi A, Raimondo S, Perroteau I, et al. Evaluation of vascular endothelial growth factor (VEGF) and its family member expression after peripheral nerve regeneration and denervation. Anat Rec (Hoboken) 2018; 301(10):1646-56.
43. Sulaiman W, Nguyen DH. Transforming growth factor beta 1, a cytokine with regenerative functions. Neural Regen Res 2016; 11(10):1549-52.
44. Pan HC, Yang DY, Chiu YT, Lai SZ, Wang YC, Chang MH, et al. Enhanced regeneration in injured sciatic nerve by human amniotic mesenchymal stem cell. J Clin Neurosci 2006; 13(5):570-5.
45. Xia XY, Huang XH, Xia YX, Zhang WH. Changes of nerve growth in amniotic fluid and correlation with ventriculomegaly. Chin Med Sci J 2011; 26(2):109-12.