Proceedings of the 4th International Conference on Materials Chemistry and Environmental Engineering
DOI: 10.54254/2755-2721/84/20240844

Review on potential nerve guidance conduits design for spinal cord injuries

Chuqi Xu[1,†], Mengtao Wang[2,4,†], Peiyuan Li[3,†]

[1]The University of Glasgow, Glasgow, G1 1EB, UK
[2]The University of Warwick, Coventry, CV4 7AL, United Kingdom
[3]China Pharmaceutical University, Nanjing, 211198, China

[4]WangMengtao0322@163.com
[†]These authors contributed equally to this work and should be considered co-first authors.

Abstract. The current treatment of spinal cord injuries (SCI) focuses mainly on the elimination of secondary damage to the central nervous system. The performance of treatments targeted at neurological recovery is not satisfactory. Inspired by the treatment of peripheral nerve injuries using tissue engineering, the nerve guidance conduits became one of the potential designs to improve the rate of neurological recovery among patients. With the proper design like multichannel and fabrication techniques like 3D bioprinting, the hybrid biomaterial may provide good guidance for nerves to grow in long gaps under well-picked neuromorphic factors. The new technologies including vascularization and electrical stimulation give more possibilities for the construction of treatment. New materials like shape-memory polymers may improve the fabrication of NGCs. However, the combination of these innovative findings was not performed for lab tests and clinical tests. The commercialization of NGC for SCI is also in need of better standardization and regulation. There may be a comprehensive NGC-based design for SCI emerging in the future which improves the performance of neurological recovery.

Keywords: Neural Tissue Engineering, Spinal cord injuries, Nerve Guidance Conduits.

1. Introduction

Spinal cord injury (SCI), resulting from unfortunate accidents like crashing vehicles, imposes unimaginable burdens on its patients and their families physically and mentally. Since the spinal cord is a significant part of the central nervous system, its damage often leads to total or partial loss of motor or sensory functions, which directly causes the disability of patients [1]. SCI is often categorized into primary and secondary injuries. The primary in most cases is used to describe the mechanical damage. The secondary often refers to the failure of nerve regeneration and complications induced by cell death and infection, which is usually what the treatments are designed for. The traditional treatment of SCI usually aims to eliminate the chance of further damage to the spinal cord while reducing cell death. These treatments, however, cannot help the patients to regain their nerve functions. The glial scar forming after injury contains the inhibitory molecules and cells that build a barrier for neuro regeneration and synaptic transmission [2]. With the development of biomaterials, more approaches to help SCI patients get a neurological recovery were brought up. The cell-based therapy considered the

DOI: 10.54254/2755-2721/84/20240844

differentiation potential of the stem cell which provided a new regenerative nerve to replace the damaged ones. The biomolecular delivery with bioactive molecules focused on eliminating the inhibiting factor caused by the glial scar, which provided an environment for the nerve to regenerate. However, there are about 250000 to 500000 reported cases of SCI around the world per year according to the World Health Organization; only 0.7% of them got a complete neurological recovery after treatment [3]. The current approaches may not have the performance they aimed for. The replacement of damaged nerves faced the challenge of lacking the proper environment and mechanical support for the nerves to grow. The bioactive molecules were successful in eliminating glial scars but failed in growing functional nerves because of the lack of guidance on the growth of nerves. At this point, a similar clinical case called peripheral nerve injuries (PNI) provided ideas on treatment design by using tissue engineering. One of the scaffold designs called the Nerve Guidance Conduit (NGC) is especially promising with its ability to provide both an "inhibitor-free" environment and good mechanical support at the same time. The NGC can be understood as a hollow tubular structure filled with neurotrophic factors and an extracellular matrix (ECM) that is used to bridge the gap of a severed nerve [4]. Although there are proven clinical cases of regaining nerve function in peripheral nervous systems (PNS) using NGCs, the regeneration of nerves in axonal tracts of the central nervous systems (CNS) is more complicated than in the PNS. Therefore, the NGC design for SCI treatment needs to have even better performance than the ones used in PNS injury treatment. This article focused on finding the strengths and flaws of the current NGC designs from the material to the production based on a literature review. There will be three parts reviewing structure design, fabrication, and neurotrophic factors used for NGC construction. There will be a discussion part introducing new techniques in tissue engineering and SCI treatment which potentially contribute to the future development of NGC for SCI treatment.

2. Structure design

The functional recovery after SCI is a complex and long-lasting process requiring the NGCs to provide constant mechanical and biological support. Various studies have shown that both superficial and internal structures are vital to the performance of NGCs. Generally, the outer structure is a hollow cylinder tube that reduces the formation of the glial scar, guides the nerve fibers to extend, and prevents them from being exposed to different stress derived from movements and in vivo environments [4]. Besides, it is expected to recruit different cells (Schwann cells, et al.) and accumulate neurotrophic factors (NTFs), which promote functional regeneration. To acquire these properties, the outer tube should be tough and porous with appropriate diameters [5]. Zhang et al. conducted simulations to measure the performance of three commonly designed NGCs to study the influence of various structural features. The results showed that NGCs with circular pores have higher mechanical strength and lower permeability than those with square pores. In addition, the porosity of 71% and the tensile strength of 8Mpa make the NGCs best suitable for the realization of multiple functions.[6]. It is also found that the diameter bound of the channels is approximately 150-300 μm [7], else it may cause bad mechanical properties of the NGC and dysplasia of the neuron system, especially in spinal cord recovery [8].

According to internal structures, NGCs can be briefly divided into two types, single-channel NGC, and multi-channel NGC. Single-channel NGCs (SNGC) were the first conduits to be applied in nerve repair. Due to their low cost and relatively simple structures, they are still extensively approved and used in clinical treatment [9]. However, the traditional SNGC doesn't show satisfying effects in nerve regeneration as it doesn't provide enough bio-factors and nutrients. Various methods have been applied to improve the functions of SNGC, such as the fabrication of multi-layer pipes and pre-vascularization tissue. Itai et al. designed an SNGC with a chitosan outer layer to provide mechanical support and an inner collagen layer to recruit cells together with bio-factors. The NGC showed high biocompatibility and properly guided the axonal extension of the nerve fibers [10]. Fan et al. co-cultured endothelial cells (ECs) and mesenchymal stromal cells (MSCs) to produce pre-vascularized cell sheets, which were then rolled into NGCs and implanted into transected SCI rats. The results showed that pre-vascularized NGC markedly increased the expression of β-III tubulin (Tuj-1) and reduced glial fibrillary acidic protein

DOI: 10.54254/2755-2721/84/20240844

(GFAP), which means hypoxia and glial scar formation were prevented and the healing process was promoted [11].

Although the performance of SNGC has been greatly improved, its inherent defects, especially the lack of spatial guidance always restrict the axonal regeneration. SCI often causes the formation of a large nerve gap. Unfortunately, studies have shown that SNGC is not effective for gaps longer than 10mm due to the incorrect connection of dispersed nerves. Multi-channel NGCs (MNGC) have aroused great interest in the application of SCI recovery as they are superior to SNGCs in various situations [12]. The main purpose of building multiple channels is to provide physical and topographical guidance for axonal growth, which is proven significant in SCI recovery [13]. He et al. used PLGA to fabricate MNGCs with high porosity. The MNGCs showed fine biocompatibility after the implantation in rats spinal cord allowing stem cells together with Schwan cells to propagate [14]. Another advantage is that MNGCs can better imitate the natural extracellular matrix (ECM). The inner spatial structures of MNGCs serve as scaffolds that allow cells and NTFs to attach, which means MNGCs may provide more biological cues. Wang et al. fabricated a conduit with multiple parallel channels based on ApF/PLCL/GO nanofibers. To study biocompatibility, Schwan cells (SCs) were seeded on the conduit and cultured in vitro. The results demonstrated that SCs can survive for more than 5 days and gradually secrete NTFs. After the implantation of sciatic nerve defects in rats, the MNGC signally accelerated the formation of the myelin sheath and the regeneration of new vessels [15]. Although MNGCs have exhibited superiorities in practical application, several problems still need to be solved, such as the slow degradation rate and relatively high spatial hindrance, which is harmful to axonal regeneration.

3. Fabrication method

Numerous methods have been applied to fabricate NGCs. Injection molding is one of the most widely used methods to produce conduits due to its uncomplicated process.[10] Both SNGCs and MNGCs with simple inner structures can be fabricated by this method, with an adjustable diameter of the channels decided by mold cavities or microwires [16]. In addition, the fabrication process has little influence on the survival of cells, which enables it to build conduits with high bioactivity. Sometimes it is combined with lyophilization to ensure the NGC is fixed and purified [17]. However, injection molding has limited accuracy and resolution, making it difficult to control the porosity and pore size. Other traditional methods such as gas foaming and solvent casting also have this problem, which hinders cell attachment and the interchange of bio-factors inside the conduits [18,19]. Although electrospinning is often used to build complex structures, its cumbersome process and high cost restrict its extensive application in clinical trials [20].

3D printing is a promising method to tailor specific structures. Available technologies include inkjet, micro-extrusion, and laser-assisted processes, all of which can fabricate NGCs with complex microstructures. Recent research shows that 3D printing can use software to control the pore arrangements and diameter or provide the NGC with anisotropic mechanical features [21]. Besides, 3D printing can process bioactive raw materials and build cells-loaded NGCs with various biological functions [22]. In general, the viability of printed cells is higher than 80%, which means the effectiveness of the materials is slightly affected [23]. Due to the development of bioinks, 3D printing is now able to place different cells and bio-factors in precise positions, creating biomimetic NGC that promotes cell-to-cell interactions and neuron differentiation in the spinal cord [7]. Nevertheless, 3D printing is still unable to create intact and contiguous cell sheets due to the low cell density of bioinks [24].

Cell sheet technology (CST) is a potential way to preserve the integrity of bioactive tissues. However, existing research only reports the fabrication of simple inner architectures through this method [25].

The studies about natural and synthetic materials have provided various choices for the fabrication of NGCs. In general, composites can combine the advantages of different materials thus improving the performance of the NGC. For example, collagen/chitosan can balance the strength and biocompatibility [26], while PCL/PLGA can control the degradation rate of the materials [27]. The most significant designs of the NGC materials are the bio-factors and cells loaded on the conduit.

DOI: 10.54254/2755-2721/84/20240844

4. Neurotrophic factors (NFs) & delivery

Neurons in the CNS have a deficiency of endogenous neurotrophic factors for cells to regenerate at the injury site. Therefore, exogenous neurotrophic factors could be used to maintain proper therapeutic concentration for regeneration, combined with scaffolds like NGCs in tissue engineering. Neurotrophic factors could be either delivered directedly by biomaterials, like natural or synthetic polymers or produced by cells implanted with the scaffolds at the SCI site. Nowadays, exogenous neurotrophic factors that are widely used are usually classified as brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), nerve growth factor (NGF), neuropoietic cytokines, and other NTs.

4.1. BDNF

Brain-derived neurotrophic factor (BDNF) is highly expressed in the central nervous system. It promotes the processes of neurogenesis, cell differentiation, neuron survival, and maturation [28]. More axons penetrated the lesion site where BDNF/NT-3 was delivered and less area of atrophy was observed indicating that BDNF could prevent neuronal atrophy [29].

It also serves as a neuroprotective factor against adverse situations. In the aspect of the immune system, BDNF has an immunomodulatory effect between airway inflammatory events and neuronal changes, which could prevent the incidence of inflammation and immune disorders [30].

In Chang, DJ et al. 2021 study, BDNF-overexpressing human neural stem cells are used to treat SCI in rat models. The regeneration of spared myelination is increased, and inflammatory cells are decreased in F3. BDNF-transplanted rats indicate that BDNF can enhance nerve regeneration and modulate inflammatory cells [31].

4.2. GDNF

GDNF is a small protein, which can be produced by glial cells, widely distributed in both PNS and CNS. It has two signaling pathways that lead to different physiological effects [32]. One pathway contributes to the survival of neurons, cell migration, and cell differentiation and the other pathway contributes to the growth of neurites and axons, neuroblast migration, and cytoskeleton reestablishment [33]. GDNF also has neuroprotective effects in a way that reduces secondary damage after SCI. It can reduce the permeability of the blood-spinal cord barrier to prevent further immune response. Moreover, it decreases the expression of nitric oxide synthase to avoid the production of free radicals which can trigger immune defense [34].

4.3. NT-3

Neurotrophin-3 (NT-3) was discovered after the identification of NGF and BDNF as the third NFs. It has become one of the most studied NFs and had promising applications in the treatment of both acute and chronic spinal cord injury. NT-3 can effectively promote survival, growth, and the formation of neuronal synapses, given its more versatile binding capacity with multiple tyrosine kinase neurotrophin receptors [34].

In recent studies, scaffolds like chitosan combined with NT-3 illustrate great therapeutic effects in animals with spinal cord injury. Jia-Sheng Rao et al utilize NT3-chitosan to treat spinal cord injury in monkeys. The results show that NT-3-chitosan triggered de novo neural tissue reestablishment at the 1 cm lesion of SCI, in contrast with the coverage of scar tissues in the LC group [35].

More recently, NT3-chitosan was also reported to be applied to a chronic complete SCI rat model. The study shows that NT3-chitosan was found to retain its ability on neural tissue regeneration when the scar tissue had been removed before introducing NT3-chitosan [36]. Therefore, NT-3 involved tissue engineering treatment may have great potential in the clinical study of subacute or chronic spinal cord injury repair.

DOI: 10.54254/2755-2721/84/20240844

4.4. NGF

Never growth factor (NGF) is a classic member of the NFs. It plays a significant role in neural survival, growth, plasticity, and function recovery. In stem cells of several non-neuronal tissues, a high level of NGF was reported to be expressed, which indicates that NGF reinforces cell proliferation [37]. NGF overexpression has been shown to elicit neurogenesis in several CNS diseases. Moreover, NGF also has a neuroprotective effect against oxidative stress and apoptosis [38]. In Zhang et al. 2014 study [39]. NGF administration reduced endoplasmic reticulum (ER) stress-induced neuronal apoptosis and increased neuron survival in the SCI rat model. The results of the above studies explore a new way for further application of the classic NGF in the treatment of spinal cord injury.

5. Cell implantation

Apart from the direct delivery of neurotrophic factors, cell implantation is a commonly used way to sustainedly secrete multiple favorable proteins. Stem cells can differentiate into specific cells when exposed to certain stimulation, thus it could be perceived as an adaptable “biodegradable” material. It can simultaneously stimulate the regeneration of neural tissues and repair the injured tissue through differentiation. Non-stem cells like Schwann cells and olfactory ensheathing cells could provide a mimic cellular environment for nerve regeneration. Furthermore, gene-editing techniques can help design desirable cells. Varies cells including stem cells, like pluripotent adult stem cells, embryonic stem cells, induced pluripotent stem cells, as well as non-stem cells, like olfactory ensheathing cells, Schwann cells, inhibitory interneurons, can be applied to treat SCI [39]. However, stem cell implantation still must face the issue of uncontrolled cell proliferation and carcinogenesis. The immune response caused by cell implantation is required to be tackled. A safe and effective cell implantation treatment is still anticipated in clinical studies.

6. Discussion

In addition to the structural designs, fabrication techniques, and neurotrophic factors reviewed in previous parts, there is other new biomaterial-based research that may contribute to the clinical treatment level of NGC for SCI. One interesting idea used 3D printing technology to fabricate pre-vascularized scaffolds to promote the regeneration of nerves in the spinal cord with the help of stem cells. Significant functional recovery was found when using the pre-vascularization techniques when producing NGC [25]. Further research on vascularization technology created a link with organs on a chip, which fabricated an in situ neural tissue system. This provided an opportunity to test the performance of NGC before clinical testing on the human body.

Besides vascularization, with the development of 3D bioprinting, the replacement of damaged spinal cords can also be possible through in vitro regeneration. The structure of the spinal cord was mimicked through a multi and continuous channel. The printed scaffold is then used to cultivate the neural tissues in vitro [7]. The biomaterial used for NGC fabrication also had some potential improvement through the development of shape-memory polymers. With its temperature-responsive property, the shape memory polymers can be programmed to form a tubular shape under high temperatures while remaining planar under low temperatures [40]. That made the fabrication of multi-channeled structures as simple as single-channeled tubular structures. Then a specific high temperature triggered the polymer to restore the permanent tubular shape programmed at the beginning, which created channels inside the fabricated tubular structures.

Neurotrophic factors may play an essential role in guiding the growth of nerves biochemically, physical stimulation has not been considered when designing SCI treatment with NGC. In recent years, electrical stimulation was brought up for peripheral nerve regeneration through multiple pieces of research. The stimulation enhances nerve regeneration and sensory axon outgrowth under a certain level of low frequency [41]. Recent work of giving electrical stimulation for NGC-guided nerve regeneration on the rat model showed improved results compared with non-stimulated cases [42]. This further confirmed the enhancing ability of electrical stimulation in the treatment of functional recovery in nervous systems.

DOI: 10.54254/2755-2721/84/20240844

Unfortunately, there are no research cases of electrically stimulated NGC on spinal cord injuries. The number of NGC research designed for peripheral nerve injuries is much more than the ones for central nerve injuries like SCI. The reason for such may lie in the difficulties of transforming lab cases into clinical treatments. The current NGCs on the market found under the FDA regulation can either be used as bridges to cover the defect areas or as wraps that prevent the second damage. Only 21.1% of these products were designed for gaps between 8.9mm and 20mm while most of them were not implemented as primary interventions beyond 20mm. The length of gaps that need to be covered in SCI usually exceeds more than 30mm. For such gaps, most surgeons still prefer autografts [43]. To develop the clinically approved NGCs for SCI, a standard for long-gap NGCs needs to be brought up. The combination of innovative fabrication methods and emerging biomaterials should be tested in clinical trials as the preparation of long-gap nerve regeneration instead of focusing only on generating more small-gap NGCs. The promising prospects of NGC in spinal cord injuries should not be buried by the regulations and standardizations.

7. Conclusion

With the development of biomaterials and fabrication techniques, there has been progress in preparing NGC for spinal cord injuries. From the bionic structure design to top-level fabrication techniques using 3D bioprinting and emerging new materials. The neurotrophic factors also had exciting development which provided a better biochemical environment for the regrowth of nerves in the spinal cord. However, there is still a lack of research cases combining these findings for a comprehensive new NGC design. With more attention on the commercialization of innovative findings in NGC and a new standard for NGC clinical tests, there may be an emerging treatment for SCI based on the proper design of NGC potentially improving the rate of neurological recovery.

Acknowledgments

All authors contributed equally to this work and should be considered co-first authors.

References

[1] Kang, N.-U., Lee, S.-J., & Gwak, S.-J. (2022). Fabrication Techniques of Nerve Guidance Conduits for Nerve Regeneration [; Review]. Yonsei medical journal, 63(2), 114-123.

[2] M. Büyüköz, “Nerve Guidance Conduits for Spinal Cord Injury,” Natural and Applied Sciences Journal, vol. 3, no. Special Issue: Full Papers of 2nd International Congress of Updates in Biomedical Engineering, pp. 20-25, 2021.

[3] Jeong, H. J., Yun, Y., Lee, S. J., Ha, Y., & Gwak, S. J. (2021). Biomaterials and strategies for repairing spinal cord lesions [Article]. Neurochemistry International, 144, 11, Article 104973.

[4] Rodriguez Doblado, L., Martinez-Ramos, C., & Pradas, M. M. (2021). Biomaterials for Neural Tissue Engineering. Frontiers in Nanotechnology, 3, 643507 (643517 pp.)-643507 (643517 pp.).

[5] Lee, S. J., Zhu, W., Heyburn, L., Nowicki, M., Harris, B., & Zhang, L. G. (2017). Development of Novel 3-D Printed Scaffolds With Core-Shell Nanoparticles for Nerve Regeneration [Article]. Ieee Transactions on Biomedical Engineering, 64(2), 408-418.

[6] Zhang, S., Vijayavenkataraman, S., Chong, G. L., Fuh, J. Y. H., & Lu, W. F. (2019). Computational Design and Optimization of Nerve Guidance Conduits for Improved Mechanical Properties and Permeability [Article]. Journal of Biomechanical Engineering-Transactions of the Asme, 141(5), 8, Article 051007.

[7] Joung, D., Truong, V., Neitzke, C. C., Guo, S. Z., Walsh, P. J., Monat, J. R., McAlpine, M. C. (2018). 3D Printed Stem-Cell Derived Neural Progenitors Generate Spinal Cord Scaffolds [Article]. Advanced Functional Materials, 28(39), 10, Article 1801850.

[8] Krych, A. J., Rooney, G. E., Chen, B., Schermerhorn, T. C., Ameenuddin, S., Gross, L., Windebank, A. J. (2009). Relationship between scaffold channel diameter and number of

DOI: 10.54254/2755-2721/84/20240844

regenerating axons in the transected rat spinal cord [Article]. Acta Biomaterialia, 5(7), 2551-2559.

[9] Carvalho, C. R., Oliveira, J. M., & Reis, R. L. (2019). Modern Trends for Peripheral Nerve Repair and Regeneration: Beyond the Hollow Nerve Guidance Conduit [Review]. Frontiers in Bioengineering and Biotechnology, 7, 30, Article 337.

[10] Itai, S., Suzuki, K., Kurashina, Y., Kimura, H., Amemiya, T., Sato, K., Onoe, H. (2020). Cell-encapsulated chitosan-collagen hydrogel hybrid nerve guidance conduit for peripheral nerve regeneration [Article]. Biomedical Microdevices, 22(4), 9, Article 81.

[11] Fan, Z. J., Liao, X. Z., Tian, Y., Xie, X. Z. Z., & Nie, Y. Y. (2020). A prevascularized nerve conduit based on a stem cell sheet effectively promotes the repair of transected spinal cord injury [Article]. Acta Biomaterialia, 101, 304-313.

[12] Yao, L., Billiar, K. L., Windebank, A. J., & Pandit, A. (2010). Multichanneled Collagen Conduits for Peripheral Nerve Regeneration: Design, Fabrication, and Characterization [Article]. Tissue Engineering Part C-Methods, 16(6), 1585-1596.

[13] Yang, B., Zhang, F., Cheng, F., Ying, L. W., Wang, C. G., Shi, K., Chen, Q. X. (2020). Strategies and prospects of effective neural circuits reconstruction after spinal cord injury [Review]. Cell Death & Disease, 11(6), 14, Article 439.

[14] He, L. M., Zhang, Y. Q., Zeng, C. G., Ngiam, M., Liao, S., Quan, D. P., Ramakrishna, S. (2009). Manufacture of PLGA Multiple-Channel Conduits with Precise Hierarchical Pore Architectures and In Vitro/Vivo Evaluation for Spinal Cord Injury [Article]. Tissue Engineering Part C-Methods, 15(2), 243-255.

[15] Wang, J., Cheng, Y., Wang, H. Y., Wang, Y. H., Zhang, K. H., Fan, C. Y., Mo, X. M. (2020). Biomimetic and hierarchical nerve conduits from multifunctional nanofibers for guided peripheral nerve regeneration [Article]. Acta Biomaterialia, 117, 180-191.

[16] Zeng, C. G., Sheng, P. Y., Xie, G. Y., Zhu, J. X., Dong, P., & Quan, D. P. (2011). Fabrication of PLLA nanofibrous multi-channel conduits for neural tissue engineering [Meeting Abstract]. Journal of Controlled Release, 152, E234-E236.

[17] Sun, X. M., Bai, Y., Zhai, H., Liu, S., Zhang, C., Xu, Y. W., Quan, D. P. (2019). Devising micro/nano-architectures in multi-channel nerve conduits towards a pro-regenerative matrix for the repair of spinal cord injury [Article]. Acta Biomaterialia, 86, 194-206.

[18] Fregnan, F., Ciglieri, E., Tos, P., Crosio, A., Ciardelli, G., Ruini, F., Raimondo, S. (2016). Chitosan crosslinked flat scaffolds for peripheral nerve regeneration [Article]. Biomedical Materials, 11(4), 13, Article 045010.

[19] Yang, Y., De Laporte, L., Rives, C. B., Jang, J. H., Lin, W. C., Shull, K. R., & Shea, L. D. (2005). Neurotrophin releasing single and multiple lumen nerve conduits [Article]. Journal of Controlled Release, 104(3), 433-446.

[20] Frost, H. K., Andersson, T., Johansson, S., Englund-Johansson, U., Ekstrom, P., Dahlin, L. B., & Johansson, F. (2018). Electrospun nerve guide conduits have the potential to bridge peripheral nerve injuries in vivo [Article]. Scientific Reports, 8, 13, Article 16716.

[21] Du, J., & Jia, X. F. (2019). Engineering nerve guidance conduits with three-dimenisonal bioprinting technology for long gap peripheral nerve regeneration [Editorial Material]. Neural Regeneration Research, 14(12), 2073-2074.

[22] Cui, H. T., Nowicki, M., Fisher, J. P., & Zhang, L. G. (2017). 3D Bioprinting for Organ Regeneration [Review]. Advanced Healthcare Materials, 6(1), 29, Article 1601118.

[23] Mao, H. L., GU, Z L. (2018). The development and trends of Bio-printing polymers. Materials China, 37(12),949-969+993.

[24] Liu, K., Yan, L. S., Li, R. T., Song, Z. M., Ding, J. X., Liu, B., & Chen, X. S. (2022). 3D Printed Personalized Nerve Guide Conduits for Precision Repair of Peripheral Nerve Defects [Review]. Advanced Science, 9(12), 22, Article 2103875.

DOI: 10.54254/2755-2721/84/20240844

[25] Frisch, A. N., Debbi, L., Shuhmaher, M., Guo, S. W., & Levenberg, S. (2022). Advances in vascularization and innervation of constructs for neural tissue engineering [Review]. Current Opinion in Biotechnology, 73, 188-197.

[26] Sun, Y., Yang, C., Zhu, X., Wang, J. J., Liu, X. Y., Yang, X. P., Li, X. H. (2019). 3D printing collagen/chitosan scaffold ameliorated axon regeneration and neurological recovery after spinal cord injury [Article]. Journal of Biomedical Materials Research Part A, 107(9), 1898-1908.

[27] Kaplan, B., Merdler, U., Szklanny, A. A., Redenski, I., Guo, S., Bar-Mucha, Z., Levenberg, S. (2020). Rapid prototyping fabrication of soft and oriented polyester scaffolds for axonal guidance [Article]. Biomaterials, 251, 10, Article 120062.

[28] Bathina, S., & Das, U. N. (2015). Brain-derived neurotrophic factor and its clinical implications [Article]. Archives of Medical Science, 11(6), 1164-1178.

[29] Brock, J. H., Rosenzweig, E. S., Blesch, A., Moseanko, R., Havton, L. A., Edgerton, V. R., & Tuszynski, M. H. (2010). Local and Remote Growth Factor Effects after Primate Spinal Cord Injury [Article]. Journal of Neuroscience, 30(29), 9728-9737.

[30] Leon, A., Buriani, A., Dal Toso, R., Fabris, M., Romanello, S., Aloe, L., & Levi-Montalcini, R. (1994). Mast cells synthesize, store, and release nerve growth factor. Proceedings of the National Academy of Sciences of the United States of America, 91(9), 3739-3743.

[31] Chang, D. J., Cho, H. Y., Hwang, S., Lee, N., Choi, C., Lee, H., Song, J. (2021). Therapeutic Effect of BDNF-Overexpressing Human Neural Stem Cells (F3.BDNF) in a Contusion Model of Spinal Cord Injury in Rats [Article]. International Journal of Molecular Sciences, 22(13), 17, Article 6970.

[32] Ortmann, S. D., & Hellenbrand, D. J. (2018). Glial cell line-derived neurotrophic factor as a treatment after spinal cord injury [Editorial Material]. Neural Regeneration Research, 13(10), 1733-1734.

[33] Paratcha, G., & Ledda, F. (2008). GDNF and GFR alpha: a versatile molecular complex for developing neurons [Review]. Trends in Neurosciences, 31(8), 384-391.

[34] Rosich, K., Hanna, B. F., Ibrahim, R. K., Hellenbrand, D. J., & Hanna, A. (2017). The Effects of Glial Cell Line-Derived Neurotrophic Factor after Spinal Cord Injury [Review]. Journal of Neurotrauma, 34(24), 3311-3325.

[35] Rao, J. S., Zhao, C., Zhang, A. F., Duan, H. M., Hao, P., Wei, R. H., Li, X. G. (2018). NT3-chitosan enables de novo regeneration and functional recovery in monkeys after spinal cord injury [Article]. Proceedings of the National Academy of Sciences of the United States of America, 115(24), E5595-E5604.

[36] Zhao, C., Rao, J. S., Duan, H. M., Hao, P., Shang, J. K., Fan, Y. B., Li, X. G. (2022). Chronic spinal cord injury repair by NT3-chitosan only occurs after clearance of the lesion scar [Article]. Signal Transduction and Targeted Therapy, 7(1), 13, Article 184.

[37] Xiao, N., & Le, Q. T. (2016). Neurotrophic Factors and Their Potential Applications in Tissue Regeneration [Review]. Archivum Immunologiae Et Therapiae Experimentalis, 64(2), 89-99.

[38] Salinas, M., Diaz, R., Abraham, N. G., de Galarreta, C. M. R., & Cuadrado, A. (2003). Nerve growth factor protects against 6-hydroxydopamine-induced oxidative stress by increasing expression of heme oxygenase-1 in a phosphatidylinositol 3-kinase-dependent manner [Article]. Journal of Biological Chemistry, 278(16), 13898-13904.

[39] Zhang, H. Y., Wu, F. Z., Kong, X. X., Yang, J., Chen, H. J., Deng, L. C., Xiao, J. (2014). Nerve growth factor improves functional recovery by inhibiting endoplasmic reticulum stress-induced neuronal apoptosis in rats with spinal cord injury [Article]. Journal of Translational Medicine, 12, 15, Article 130.

[40] Wang, J., Xiong, H., Zhu, T. H., Liu, Y., Pan, H. B., Fan, C. Y., Lu, W. W. (2020). Bioinspired Multichannel Nerve Guidance Conduit Based on Shape Memory Nanofibers for Potential Application in Peripheral Nerve Repair [Article]. Acs Nano, 14(10), 12579-12595.

DOI: 10.54254/2755-2721/84/20240844

[41] Gordon, T. (2016). Electrical Stimulation to Enhance Axon Regeneration After Peripheral Nerve Injuries in Animal Models and Humans [Review]. Neurotherapeutics, 13(2), 295-310.
[42] Hasiba-Pappas, S., Kamolz, L. P., Luze, H., Nischwitz, S. P., Holzer-Geissler, J. C. J., Tuca, A. C., Winter, R. (2023). Does Electrical Stimulation through Nerve Conduits Improve Peripheral Nerve Regeneration?-A Systematic Review [Review]. Journal of Personalized Medicine, 13(3), 13, Article 414.
[43] Parker, B. J., Rhodes, D. I., O'Brien, C. M., Rodda, A. E., & Cameron, N. R. (2021). Nerve guidance conduit development for primary treatment of peripheral nerve transection injuries: A commercial perspective [Review]. Acta Biomaterialia, 135, 77-99.