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Original Article

Regeneration of Sciatic Nerve Injury by Polyglycolic Acid/ Collagen/Bioglass Conduit

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Abstract

Introduction: The repair process of severed peripheral nerves is conducted by the bridging of the regenerating neural fibers across a gap in the case of the existence of an appropriate route (space) between the proximal and distal severed stumps. The current study aimed to improve the transected sciatic nerve of rats with a 10 mm gap by means of applying electrospun conduits composed of polymer nanocomposites of polyglycolic acid (PGA), collagen, and nanobioglass (NBG). Then, the efficacy of the designed conduits (PGA/collagen/NBG, PGA/collagen, and PGA alone) was histologically and electrophysiologically compared with autograft nerves to determine whether these conduits have superiority over the autograft procedures in the process of nerve regeneration.

Materials and Methods: In this experiment, 50 healthy adult male Wistar rats underwent sciatic nerve transection. After four, eight, and 12 weeks of the surgical procedures, the therapeutic effects of conduits on sensory and motor recovery of transected nerves were evaluated.

Results: The analysis of the functions of motor and sensory nerves showed marked improvement in rats treated with PGA/collagen/NBG conduit. Also, histological staining and immunohistochemical assessment of the expression of NF200, S100, and CD31 proteins revealed newly-formed nerve fibers with micro blood vessels at the proximity of regenerated nerve fibers.

Conclusions: It seems that due to the high surface area of electrospun nerve conduits to adhere the cells, the application of these compounds would be beneficial in clinical practice in the future. The results suggest that PGA/collagen/NBG nanofibrous conduit possesses the highest capability in increasing nerve regeneration following nerve transection in murine models.

Keywords: Biomaterial, Bioglass, Nerve Regeneration, Polyglycolic Acid, Tissue Engineering

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Introduction

The Peripheral Nervous System (PNS) is located outside the Central Nervous System (CNS) and contains the cranial, spinal, and peripheral nerves that conduct impulses from and to the CNS. The Peripheral Nerve Injury (PNI), a global clinical impairment that damages the quality of life of patients, can be caused by mechanical, thermal, chemical, or ischemic injuries, mainly resulting from traumatic accidents or some degenerative disorders. It has been shown that the severity of injuries determines the functional outcome. PNI can also lead to the destruction of neuronal communication in sensory and motor nerves bridging the CNS to peripheral organs.[1] The annual incidence of PNI in the world is estimated to be 13 to 23 cases per 100.000,[2-4] and currently, more than 200.000 individuals are under-treatment of PNI in the United States.[5] Among these patients with PNI, less than half of injured individuals could be fully recovered following the therapeutic procedures.[6]

In contrast to the CNS, peripheral nerves have the ability to regenerate after cell injury; however, in mammalian neural cells, the intrinsic and spontaneous restoration of the peripheral nerve cells is partial. Therefore, seeking therapeutic approaches to boost the process of nerve repair would be requisite.[7,8] Microsurgical nerve autograph is the most frequently used interventional strategies used for the restoration of structural and functional nerve regeneration following PNS injury. Nevertheless, despite much efforts made in this field, several restrictions, including the limited accessibility of the donor site, sensation loss of donor areas, and discrepancies in the dimension of nerves between donor and recipient, are considered major obstacles in the field of regenerative medicine.[9] Biological sciences, tissue engineering, and biomaterial engineering have cooperatively established a new approach to restore and reconstruct the damaged tissues and organs.[10] One of the most favored attitudes in the file of neuroscience is nerve tissue engineering.[11] Natural and synthetic neural guidance channels (conduits), as well as

hollow and tubular catheters that bridge between the two ends of the transected nerves, are designed by tissue engineering, and they contribute to axonal outgrowth, minimizing the formation of scar tissues, and reduction of stretching in repairing regions of lesions.[12,13] PGA is a member of biodegradable aliphatic polyesters with appropriate biocompatibility and high tensile strength, and has the potential to be applied in various tissue engineering applications.[14] The applicability of PGA-based absorbable suture suggested that polymers containing PGA could be safely incorporated into soft tissues.[15] PGA is also useful when utilized in various tissue engineering approaches, such as vascular tissue engineering.[16] Since the purified form of PGA has a rigid structure, this property has limited the applicability of this material in the implantation process. The combination of PGA polymer with the extracellular and natural matrix polymers, such as collagen, could improve the physical properties of this polymer to be employed in animal models and humans. In most studies, the extracellular matrix molecules, such as collagen, are used to increase the rate of cell adhesion to conduits.[17] Nano-bioactive glasses (NBGs) are incorporated into biodegradable polymers to enhance their mechanical properties. NBGs have appropriate features, including biocompatibility, controlled biodegradability, and the ability to release the cells, connect to both soft and hard tissues, and secrete the ions during the degradation process. These types of materials could be used for bone tissue repair and wound healing, and their positive effects on the angiogenesis process have been addressed in some reports.[18] The fidelity of NBGs in tissue repair might be attributed to their mineral structures. Accordingly, ions releasing from NBGs can stimulate tissue repair within the human body, thus facilitating the process of recovery. The released ions could be used as a critical factor in the regeneration of axonal fibers.[19] In this study, we aimed to examine the impact of our fabricated nerve conduits that were made of nanocomposite polymers PGA/collagen/NBG on the repair process of the transected sciatic nerve in a rat model and to compare its properties with PGA and PGA/collagen conduits.

Materials and Methods

Sciatic Nerve Surgery

All of the experimental procedures were confirmed by the University of Science and Research Branch of Islamic Azad University (Approval ID: IR.IAU.SRB.REC.1397.062) and was conducted according to the Animal Care and Use guidelines. In this experiment, 50 healthy adult male Wistar rats (10-12 weeks old, weighing 200-250 g) were purchased from the Pasteur Institute (Tehran, Iran). They were randomly divided into ten groups of five rats as follows: 1) Positive control group in which healthy rats neither received any treatment nor underwent surgery (sciatic nerve transection), 2) Negative control group in which the animals underwent surgery but did not receive any interventions, 3 & 4) PGA conduit groups in which the animals underwent surgery and received PGA conduit for eight and 12 weeks, 5 & 6) PGA/collagen conduit groups in which the animals underwent surgery and received PGA/collagen conduit for eight and 12 weeks, 7 & 8) PGA/collagen/NBG conduit groups in which the animals received PGA/collagen/NBG conduit for eight and 12 weeks, 9 & 10) Autograft groups in which the animals underwent surgery and received nerve autograft for eight and twelve weeks in the absence of any conduits. The animals were anesthetized by intraperitoneal injection of ketamine (60 mg/kg) and xylazine (10 mg/kg) as previously described.[9] The right sciatic nerve was chosen for each animal, and all of the implantation procedures were conducted by inverted microscopy. As shown in Figure 1, the right feet of rats were carefully shaven and then cleaned by alcohol. Under an inverted microscope, the posterior side of the right thigh was gently cut, and the muscle and fascia were pulled away to expose the sciatic nerve. Consequently, in the experimental groups that received different types of conduits, the right sciatic nerve was dissected as proximal and distal segments at the center of the right thigh. Then, the nerve guidance conduit at a length of 12 mm was sutured by 7-0 nylon to bridge the lesion site to the proximal and distal nerve stumps in which both ends entered the conduit by 1 mm, and skin was sutured with 6-0 silk.[20,21]

In the experimental groups that received the nerve autograft, a 10 mm nerve segment was resected, reversed 180°, and re-implanted across the defect. In the negative control group, there was no bridge to connect two stumps. As a routine stage of post-operation, intraperitoneal injection of lidocaine was administered at the end of the surgery, immediately after the incision was closed and before the animals regained consciousness. Finally, all of the animals were housed, fed with chow and tap water, and monitored to analyze the changes in their conventional conditions and motor functions.

Walking-Footprint Analysis

One of the most common methods for evaluating the improvement of the sciatic nerve function in laboratory animals is the calculation of the Sciatic Functional Index (SFI).[22] Following four, eight, and 12 weeks of surgery and implantation of nerve conduits, the rats' footprints were recorded to measure the SFI. To this aim, the hind limbs of rats were soaked in ink, and were allowed to walk inside a walking alley (100 × 20 × 15 cm) lined with a millimeter paper and ended along with a darkened goal box. The SFI values were calculated according to the below equation:

SFI =-38.3 (OPL - NPL)/NPL + 109.5 (OTS - NTS)/ NTS + 13.3(OIT - NIT)/NIT - 8.8

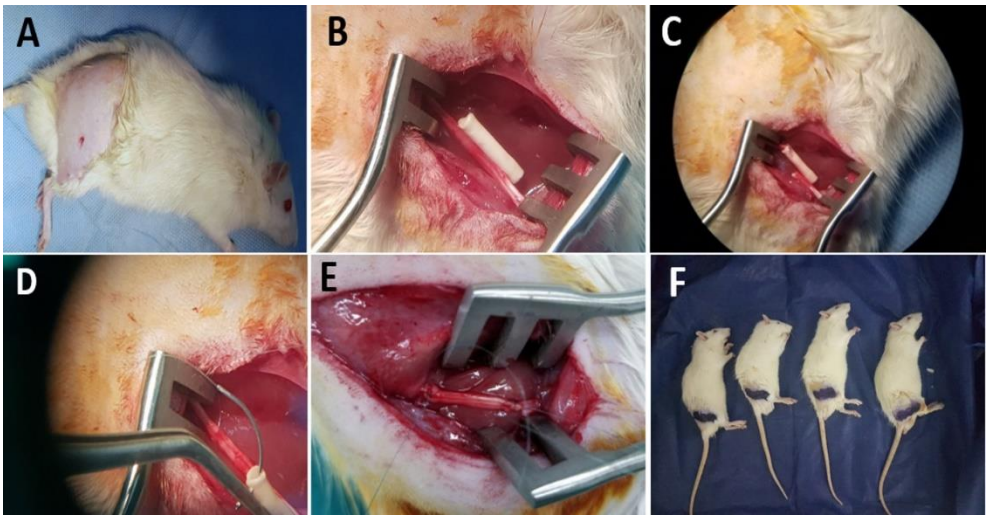

Figure 1. Surgical Implantation of Fabricated Nerve Conduits Bridging a Damaged Sciatic Nerve at a Length of 10 mm in Rats, A) Early stages of the surgical processes, B) Exposed sciatic nerve, (C and D) Nerve guidance conduits sutured to the distal and proximal ends, E) Nerve autograft group: a 10 mm length of the nerve fiber was resected, reversed 180°, and re-implanted across the lesion, F) Rats after the surgical operation.

where PL shows the print length that denotes the distance from the heel to the top of the third toe, TS exhibits the toe spread that implies the distance between the first and the fifth toe, and IT indicates the intermediary toe spread, denting the distance from the second to the fourth toe. NPL, NTS, and NIT were the measured values obtained from non-operated feet, while OPL, OTS, and OIT were the measured values obtained from the operated feet.[23]

Closer values to zero point represent the normal function, while -100 implies the complete loss of function. The values of SFI were expressed as the means of values obtained from all five rats in each group.

Hot-Plate Latency Test

The hot-plate latency test was carried out following four, eight, and 12 weeks of surgery to assess the thermal pain sensitivity. The hot-plate latency test is used to evaluate animals' pain perception by measuring the withdrawal reflex latency (WRL). Throughout the experiment, rats were placed at the center of an open-ended cylinder-shaped space with a floor containing a heated plate. The plate was warmed to 56 °C, causing two behavioral reactions, namely paw licking and jumping in which the duration of each behavioral reaction is recorded. The time elapsed from the onset of hot plate contact to a withdrawal of hind limb was measured by a timer activated by an external switch and recorded as withdrawal reflex latency. The cut off time for their reaction was set at 16 seconds. The values of WRL were presented as the means of all measurements obtained from all rats in each group.

Nerve Conduction Test

Gas chromatography (GC) is an analytical technique to separate compounds based on their volatilities. In this study, Gas chromatography (GC) was used to determine the concentration of each chlorinated product of PCE. This apparatus was equipped with two detectors, Electron Capture Detector (ECD) and Flame Ionization Detector (FID). After taking the samples, it could take a while that equilibration of water samples and headspace of 2 mL vials by use of Solid Phase Micro Extraction (SPME) (Supelco) device would be achieved. SPME was inserted into the inlet of GC for 3 minutes for desorption of the adsorbed volatile compounds at 240 °C. GS-GasPro column (30 mm × 0.31 mm) was used for chromatographic separation. In addition, helium was used as the carrier gas. The GC oven was initially set at 30 °C for 3 minutes, heated at 30 °C/min to 180 °C and 25 °C/min to 230 °C, and kept at 230 °C for 10 minutes.

Histological and Immunohistochemical Evaluation of Regenerated Nerve

After three months of surgery and electromyography, the animals were euthanized by overdose injection of ketamine (200 mg/kg). The distal part of the sciatic nerve was harvested and fixed in a 10% formalin solution at room temperature for six hours, then dehydrated by ascending concentrations of ethanol, embedded in paraffin, sectioned at a thickness of 4 µm, and stained with hematoxylin-eosin (H & E) and toluidine blue (TB). The prepared samples were examined under a light microscope (Carl Zeiss, Thornwood, NY, USA) equipped with a digital camera (Olympus, Tokyo, Japan). Mammalian neuro-filaments are one of the major components of the neuronal cytoskeleton and play essential roles in axonal structure, diameter, and outgrowth. In order to determine the axonal regeneration rate, the anti-NF-200 antibody (Abcam, USA), which specifically binds to neurofilament proteins at a molecular weight of 200-kDa,

was utilized. The presence of Schwann cells and the degree of angiogenesis, primary antibodies against S100 (a specific marker of Schwann cells), and anti-CD31 proteins as a marker of endothelial cells (Duccio-Artiuo, Abcam, USA) were applied. The rabbit anti-rats secondary antibody (Envision Templas Dakumus) was employed for the identification of primary antibodies. Finally, tissues were stained with Diaminobenzidine (DAB), and the immunohistochemical reaction was evaluated.[9,24]

Statistical Analysis

The analysis of the obtained data was conducted by SPSS software (SPSS, Chicago, IL, USA) version 16.0. The difference between the data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The values were expressed as the means ± standard deviation (means ± SD). The level of statistical significance was set at P <0.05.

Results

Walking-Footprint Test

Figure 2 illustrates the average SFI values of all experimental groups. The average SFI for the negative control showed a slight improvement and increased from -88.95 ± 4.91 to -78.58 ± 1.28 after eight weeks, and to -73.38 ± 1.23 after 12 weeks. The autograft group had the means SFI values of -65.13 ± 2.36, -61.83 ± 2.59, and -19.79 ± 0.82 following four, eight, and 12 weeks of surgery, respectively. The SFI values of the PGA/collagen/NBG conduit group were remarkably enhanced from -76.39 ± 1.42 to -62.41 ± 3.12 and -23.71 ± 2.94 after four, eight, and 12 weeks of surgery, respectively. Such values for the PGA/collagen conduit group were -79.14 ± 1.42, -70.23 ± 2.63 and -59.14 ± 2.24 after four, eight, and 12 weeks of surgery, respectively. Similar to the negative control, the PGA conduit group exhibited poor healing conditions, and its SFI values were -80.92 ± 1.98, -76.11 ± 1.64, and -70.24 ± 0.97 following four, eight, and 12 weeks of surgery, respectively. The SFI values of the PGA/collagen/NBG conduit group were significantly (P <0.001) higher than those of PGA conduit, PGA/collagen conduit, and negative control groups at the following 12 weeks of surgery, showing the beneficial role of this conduit in improving the motor function of the sciatic nerve.

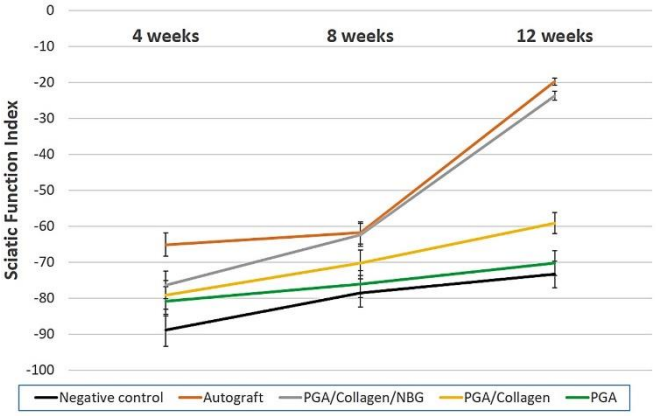

Figure 2. The Comparison of the Sciatic Function Index (SFI) at Four, Eight, and 12 Weeks Post-Implantation of Conduits.

Hot-Plate Latency Test

For the assessment of nociceptive functions of the injured hind limb, the hot-plate latency assay was performed, and the results are presented in Figure 3. Figure 3 indicates that the WRL values of injured feet were markedly altered after sciatic nerve transection, and a significant (P <0.001) difference was found in WRL values between positive and negative control groups. The animals in the negative control group did not withdraw their paws from the hot plate within 16 seconds, whereas the positive control group had the smallest hot plate latency (4.45 ± 0.57 seconds) after 12 weeks of surgery. Following four weeks of surgery, signs of recovery were detectable in the form of the WRL values of feet in all groups except the negative control group, and the healing process continued until week 12 post-surgery. However, a normal value of four seconds was unachievable.[25]

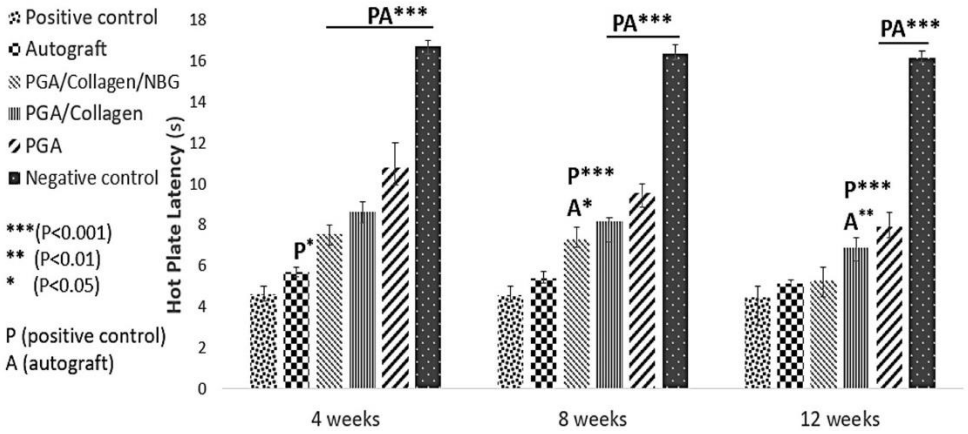

Figure 3. The Comparison of the Hot-Plate Response of the Experimental Groups that Underwent Surgery Four, Eight and 12 Weeks Post-Surgery.

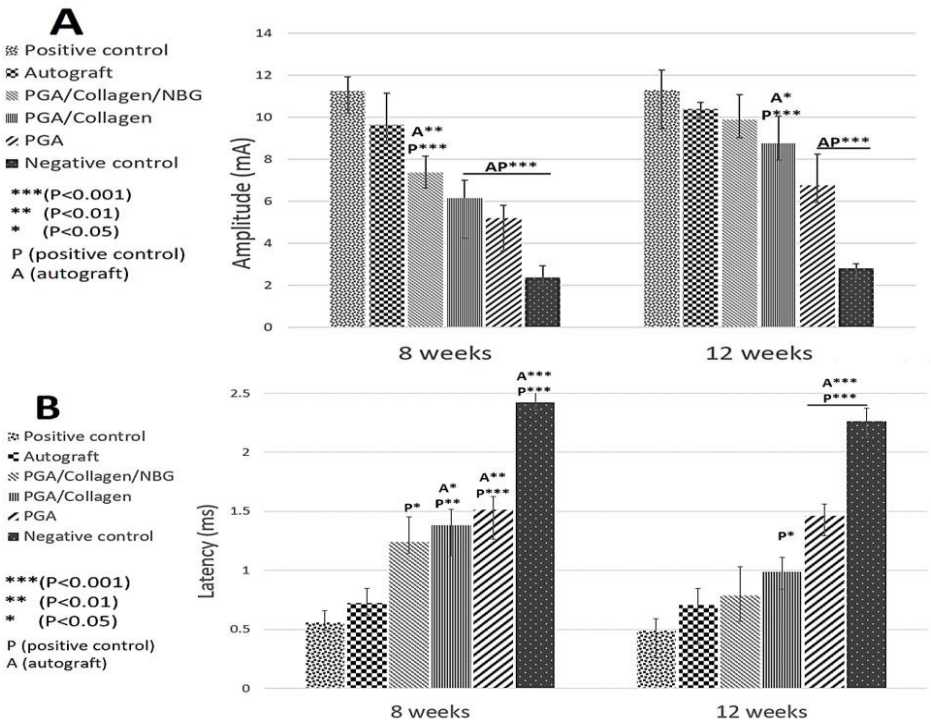

Figure 4. The Comparison of CMAP among Experimental Groups that Underwent Surgery Eight and 12 Weeks Post-Surgery. A) The comparison of the amplitudes of CMAP between conduit groups and positive control and autograft groups, B) The comparison of latency between conduit groups with positive control and autograft groups.

After 12 weeks, all rats receiving conduits (PGA, PGA/collagen, and PGA/collagen/NBG) had significantly smaller WRL time compared with the negative control group (P <0.001). Among the experimental groups that received conduits, the PGA/collagen/NBG group had the lowest rate of WRL after 12 weeks of surgery, and there was no significant difference (P >0.05) between the PGA/collagen/NBG group, autograft and positive control groups.

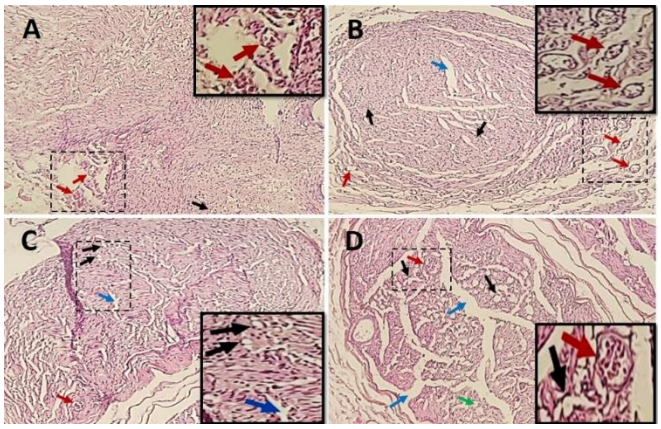

Figure 5. Light Microscopic Images of Nerve Tissues Stained with H & E Staining Obtained 12 Weeks after Surgery, Showing a Cross-Section of Reconstructed Nerves in (A) Autograft, (B) PGA/collagen/NBG conduit, (C) PGA/collagen conduit, and (D) PGA conduit groups (×100). Red arrows: blood vessels; blue arrows: vacuole degeneration; green arrow: endoneurium focal loss; black arrows: Schwann cells.

Nerve Conduction Test

The amplitudes of CMAP and latency time results are displayed in Figure 4. The results demonstrated that, after week 12 post-surgery, the lowest amplitude (2.79 ± 0.22) and maximum latency (2.26 ± 0.48) were found in the negative control group, whereas the highest amplitude (11.29 ± 1.86) and lowest latency (0.49 ± 0.21) were detected in the positive control group. Among the experimental groups that received nerve conduits, the PGA/collagen/NBG group had the highest amplitude (9.88 ± 1.2) and lowest latency (0.78 ± 0.56). Following 12 weeks of surgery, there was no statistically significant difference (P >0.05) in amplitude and latency between PGA/collagen/NBG group and autograft and positive control groups, implying that the rate of muscle innervation in the PGA/collagen/NBG group is similar to the autograft group.

Histological and Immunohistochemical Evaluation

Figures 5 and 6 showed the histological assessments of the longitudinal and cross-sectional tissue slides obtained from the distal part of the regenerated nerve fibers in different groups following 12 weeks of surgery. According to H & E staining, the sciatic nerve was successfully restored in the autograft group (Figure 5A), and the PGA/collagen/NBG group (Figure 5B) had normal nerve fibers with central axons, normal myelin space, a low rate of connective tissue growth, and low degree of vacuolar formation. These findings were further confirmed by toluidine blue staining

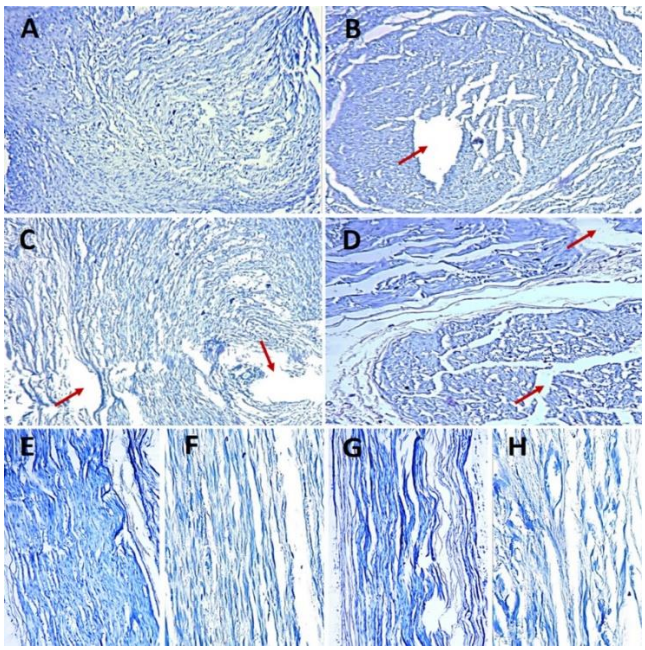

Figure 6. Toluidine Blue Staining of the Nerve Tissue of (A, E) Autograft, (B, F) PGA/collagen/NBG conduit, (C, G) PGA/collagen conduit, and (D, H) PGA conduit groups (×100). Red arrows: vacuole degeneration; A-D: cross-sectional nerve tissue; E-H: longitudinal section of nerve tissue.

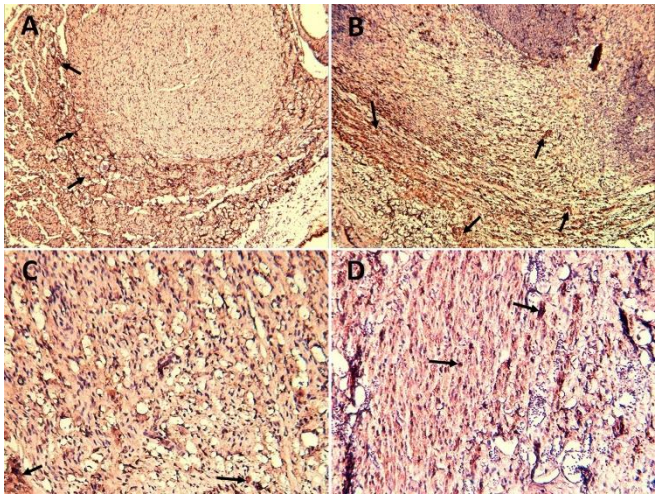

Figure 7. The Immunohistochemical Analysis of Anti-Neurofilament (NF) in the Transverse Sections of the Reconstructed Nerve in (A) Autograft, (B) PGA/collagen/NBG conduit, (C) PGA/collagen conduit, and (D) PGA conduit groups (×100).

that stains the myelin sheath in blue color. Toluidine blue staining showed a higher rate of demyelination in the PGA/collagen/NBG conduit group compared with the other conduit groups. Regenerated nerve fibers in the PGA/collagen/NBG conduit was almost similar to the group autograft. It had the most number of new capillaries regenerated around the lesion, suggesting that the application of PGA/Collagen/NBG conduit has the most efficacy in the nerve regeneration compared with the other conduit groups. This may be due to the role of bioglass in the process of angiogenesis and its presence in the designed conduit. For the determination of the degree of nerve regeneration in the experimental groups, axonal neurofilaments were analyzed qualitatively (Figure 7). Following 12 weeks of nerve grafting, immunohistochemical analysis of the anti-NF200 protein demonstrated a higher rate myelinated nerve fibers (increased immunohistochemical reaction) in autograft and PGA/Collagen/NBG conduit groups (Figure A and B) compared with PGA and PGA/collagen groups. Besides, immunoreactivity of the S100 protein was assessed as a marker of Schwann cells 12 weeks after surgery. The secondary antibody was visualized by DAB staining, in which the intensity of brown-colored protein bands indicated the contribution of Schwann cells in the regenerated nerves. According to Figure 8, the highest incorporation of Schwann cells was observed in autograft (Figure A) and PGA/collagen/NBG conduit groups (Figure B), while the lowest S100 immunoreactivity was detected in the PGA conduit groups (Figure D). The immunohistochemical evaluation of anti-CD31 proteins as a specific marker of the endothelial cells and index of angiogenesis during the nerve regeneration process was evaluated. As shown in Figure 9, following 12 weeks of surgery, the highest rates of blood vessel formation and angiogenesis were observed in the PGA/collagen/NBG conduit group (Figure B) in which the degree of angiogenesis was similar to that of in the autograft group (Figure A).

Discussion

Neural tissue engineering has emerged as one of the most promising applications for the development of ideal substitutes for native tissues with similar properties. The design of these scaffolds must meet several chemical and physical criteria of the normal nervous system, such as having proper biodegradability and biocompatibility, as well as mechanical properties close to that of native nervous tissues.[26] Nanofibrous structures can be fabricated by electrospinning in the form of neural guidance channels whose polymeric components play a pivotal role in nerve regeneration. The optimal neural guidance channels should possess the following features: (a) they must be non-toxic and non-carcinogenic, (b) they should stimulate axonal growth as much as possible, (c) they must be fully absorbed through the normal metabolic pathways in the host's body, (d) the rate of the degradation should be proportional to the axonal growth rate, and (e) they must have good physical properties, and not collapse. They should be flexible to be able to use for in-vivo purposes.[27] Natural and synthetic materials can be employed for the fabrication of neural guidance channels.[28] PGA and collagen are classified as polymers with acceptable biodegradability, biocompatibility, desired cell adhesion, and suitable surface properties, which enabled them to be widely applied in biomedical fields.[29] Bioceramics are usually utilized in combination with

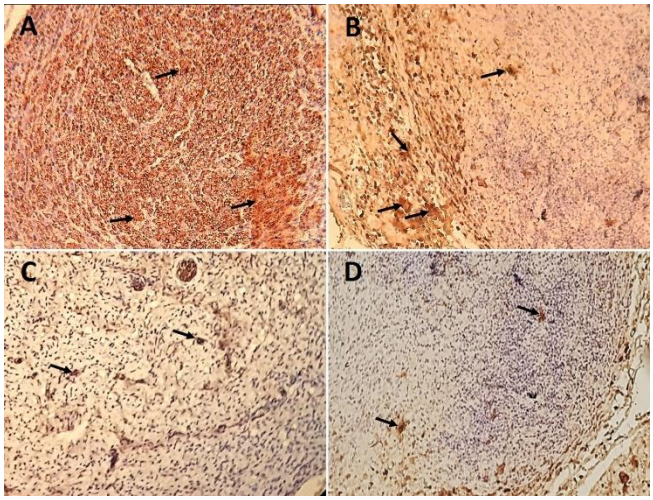

Figure 8. The Immunohistochemical Analysis of Anti-S100 in the Transverse Sections of the Rreconstructed Nerve in (A) Autograft, (B) PGA/collagen/NBG conduit, (C) PGA/collagen conduit, and (D) PGA conduit groups (×100).

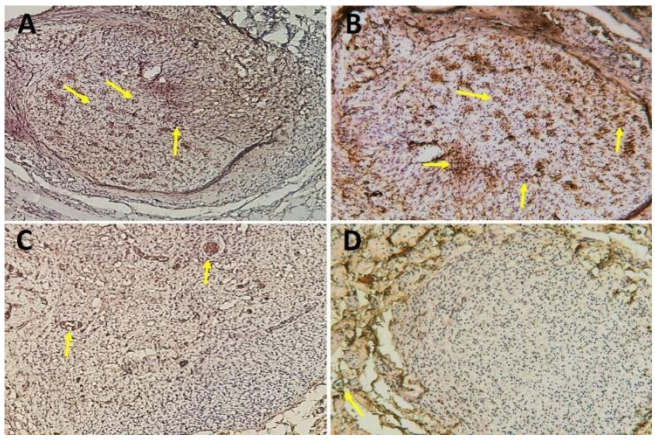

Figure 9. The Immunohistochemical Analysis of Anti-CD31 in the Transverse Sections of the Reconstructed Nerve in (A) Autograft, (B) PGA/collagen/NBG conduit, (C) PGA/collagen conduit, and (D) PGA conduit groups (×100).

biodegradable polymers to achieve optimal biological and mechanical functions.[30] Furthermore, the addition of NBG has a profound effect on the growth of peripheral axons and effective participates in the process of angiogenesis.[10,19,31] In a previous study,[32] it was proved that PGA/collagen/NBG conduits possess suitable mechanical properties and biocompatibility to support the adhesion and proliferation of rat bone marrow mesenchymal stem cells (rbMSCs). This finding was in agreement with the study performed by Reilly et al., who indicated that bone marrow mesenchymal stem cells could proliferate in a culture medium containing bioglass 45S5.[33] A study demonstrated the antibacterial activity of bioglass 45S5, which could confirm the biocompatibility of bioglass nanoparticles used in this study.[34] The walking-footprint analysis, hot-plate latency, and nerve conduction assay are the most popular postoperative examinations to assess the regeneration of the sciatic nerve.[35,36] The evaluation of SFI showed that after four weeks of surgery, the lowest amount of SFI (better motor functional recovery) was observed in the PGA/collagen/NBG and autograft groups (Figure 2). The application of PGA and PGA/collagen conduits had lower effects on functional motor recovery compared with other experimental groups and showed no significant difference when compared with the negative control groups following four weeks of surgery (P >0.05). The SFI values of the PGA/collagen/NBG conduit group were significantly (P <0.001) higher than those of PGA, PGA/collagen conduit and negative control groups after 12 weeks of surgery, which showed the superiority of this conduit on improving the motor function of the sciatic nerve. Our results were in line with the findings of the study performed by Nakamura et al.[17] For the determination of the nociceptive functions of the injured hind limb, the hot-plate latency test was performed.[37] Following four weeks of surgical sciatic nerve transection, the signs of recovery of the WRL of feet were detectable in all groups except the negative control group, and the healing process continued until week 12 post-surgery. The animals in the conduit groups receiving PGA, PGA/collagen, and PGA/collagen/NBG had significantly lower WRL time in comparison with the negative control group (P < 0.001) after 12 weeks. Among conduit groups, the PGA/collagen/NBG group had the lowest rate of WRL after 12 weeks of surgery, and there was no significant difference (P > 0.05) between the PGA/collagen/NBG group, autograft and positive control groups. The improved recovery of response in the heat-exposed groups after 12 weeks could be attributable to enhanced regeneration of sensory axons. The results of the present study were consistent with the study of Matsumoto et al.[38] For the evaluation of the electrophysiological activity of reconstructed nerves, the amplitude of CAMP (a parameter indirectly representing the number of reconstructed motor nerve fibers and the rate of muscle innervation) and latency (an indirect index of the maturation of nerve fibers) were analyzed.[39] Our findings showed a significant difference (P <0.001) between the negative control group compared with conduit, autograft, and positive control groups following the12-week of surgery. In this time, there was no significant difference (P >0.05) between the PGA/collagen/NBG conduit group and autograft and positive control groups, which implied the superiority of PGA/collagen/NBG conduit over other conduits for neural regeneration. Our results were in line with the results obtained in the study of Matsumoto et al.[38] As shown in Figure 10, after complete neural regeneration, PGA/collagen/NBG conduit was entirely absorbed by the metabolic pathway as confirmed by Niimi et al., whose findings showed that PGA conduit could be fully absorbed in the body and a second operation would

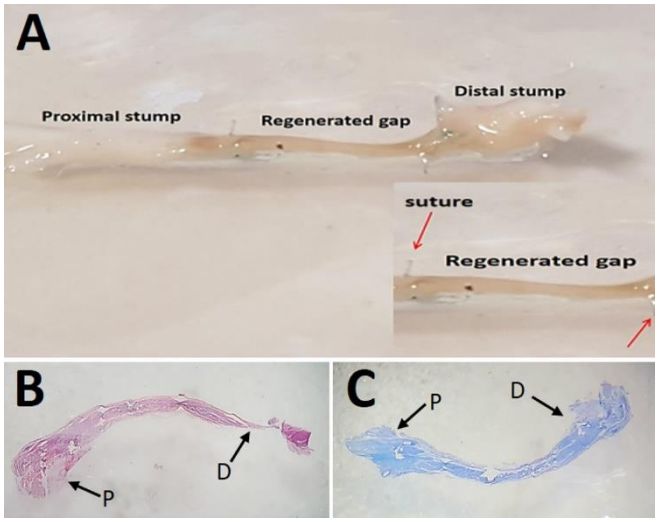

Figure 10. The Regenerated Nerve in Response to the Treatment of Rats with PGA/collagen/NBG Conduit after 12 Weeks of Surgery. (A) Complete absorption of conduits (note the newly regenerated nerve between the two ends of the stitch (red arrows), (B) Hematoxylin-eosin and (C) Toluidine blue staining of the regenerated nerve (×10). (P: Proximal ends of the nerve; D: Distal ends of the nerve).

not be required.[40] The thin layer of the surrounding fibrous tissue and minimal inflammation indicated that PGA/collagen/NBG conduit was biocompatible. Also, in the PGA/collagen/NBG conduit groups, a vast majority of newly-formed capillaries were formed, indicating that PGA/collagen/NBG conduit can induce the angiogenesis process. This could be attributed to the presence of bioglass nanoparticles and their pivotal role in stimulating angiogenesis which is inconsistent with the findings of Day et al., who reported an enhanced secretion of VEGF in response to low concentrations of bioglass 45S5, as well as the formation of the endothelial cells and improved angiogenesis.[41] Furthermore, Zhai et al. showed that the expression of VEGFRs could be increased by silicate-based bioglass in the presence of high concentrations of Si. Besides, they demonstrated an increased rate of angiogenesis in response to silicate-based bioglass, which indicates the essential role of silicate extracts containing high Si concentrations in upregulating the expression of VEGFRs.[42] Although autograft and conduit groups regenerated numerous bundles of sciatic nerve fibers in the distal region of the regenerated tissues, no marked regeneration of axons was detected in the negative control group. The results of the H & E staining method indicated less vacuole formation and higher rate angiogenesis. As depicted in Figure 5, a lower degree of vacuole formation, a higher number of Schwann cells, and an increased rate of angiogenesis was found in autograft and PGA/collagen/NBG conduit groups in comparison with PGA and PGA/collagen conduit groups. These findings were further confirmed by the Toluidine blue staining method (Figure 6). The histological assessments showed that regenerated nerves in response to autograft and PGA/collagen/NBG conduit successfully grew across the gap and connected the proximal and distal nerve stumps. The immunohistochemical analysis of anti-NF200, S100, and CD31 demonstrated higher levels of axonal remodeling, increased number of Schwann cells, and an increased rate of angiogenesis in the PGA/collagen/NBG conduit group in comparison with PGA and PGA/collagen conduit groups.

Conclusion

Since the restoration of PNI following the spontaneous regeneration is partially fellfield after microsurgical procedures, we designed a novel neural guidance channel containing PGA/collagen/NBG by means of the electrospinning method, and its effects on the improvement of the transected sciatic nerve were examined in a rat animal model. Our finding revealed that the fabricated conduit has the potential to improve sciatic nerve regeneration, and its effectiveness on nerve regeneration is higher than PGA and PGA/collagen conduits. Although various examinations proved our claim about the beneficial role of this conduit in peripheral nerve restoration, further studies are warranted to unravel the detailed mechanism(s) of these effects.

Authors' Contributions

All authors contributed to the study conception and design. Sciatic nerve surgery was performed by MRN and ND. The study of neural conduction was performed in collaboration with KM. Material preparation, data collection, and analysis were completed with the cooperation of all authors. The first draft of the manuscript was written by ND and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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