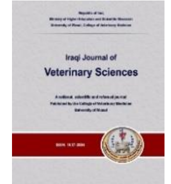
Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com



Efficacy of chitosan nanoparticles and mesenchymal stem cells in rabbit models for sciatic nerve regeneration

D.H. Al-Haideri[1] and H.A. Al-Timmemi[2]

[1]Department of Clinical Science, Faculty of Veterinary Medicine, University of Kufa, Najaf, [2]Departments of Surgery and Obstetrics, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

Article information

Article history:
Received 16 August, 2023
Accepted 13 October, 2023
Published online 06 February, 2024

Keywords:
Conduit
CS NPs
ASCs
PNI
Rabbit

Correspondence:
D.H. Al-Haideri
dhurghamh.alhaideri@uokufa.edu.iq

Abstract

Peripheral nerve injuries (PNI) are frequently caused by several circumstances, including trauma and iatrogenic operations, and there are limited therapy options to help with functional recovery. This study aimed to compare in vivo adipose-derived stem cells (ASCs) to chitosan nanoparticles (CS NPs) in rabbit sciatic nerve defect regeneration. Twenty healthy rabbits of local breed randomly enrolled in two equal groups split into two groups at random: The critical size defect was bridged with a 14 mm conduit from acellular bovine urinary bladder impacted with chitosan nanoparticles (CS NPs) semi-gel 1mg/ml as the first group, and the critical size defect was bridged with mesenchymal stem cells (MSCs) $5x10^6$ in the second group. At the end of 56 and 112 days, assessment of the nerve regeneration, motor and sensory function, and histopathological assessment of the sciatic nerve were investigated. According to the findings, the motor and sensory capabilities of the sciatic nerve increased faster in the mesenchymal stem cells (MSCs) group. In the mesenchymal stem cells group repairing a 10mm sciatic nerve defect, histopathological examination revealed basophilic nuclei of proliferated Schwann cells with thick myelin sheath, suitable orientation nerve fibers, dense axon population, large axon diameter, nerve fiber myelination, and low fibrous tissue at the epineurium compared to chitosan nanoparticles (CS NPs). It is concluded that effect mesenchymal stem cells (MSCs) promote regeneration of the sciatic nerve defect compared to chitosan nanoparticles (CS NPs).

DOI: 10.33899/ijvs.2023.142572.3186, ©Authors, 2024, College of Veterinary Medicine, University of Mosul.
This is an open access article under the CC BY 4.0 license (http://creativecommons.org/licenses/by/4.0/).

Introduction

Peripheral nervous injuries (PNI) are common causes of disabilities, like these injuries that occur from the wrong injection during treatment. Treatment for PNS injuries remains a challenging clinical issue, with many proposed strategies yielding unsatisfactory outcomes, highlighting the need for novel treatment approaches (1,2). Cell therapy shows great promise as a potential approach to repairing damaged peripheral nerves. These cells can create a conducive environment that limits damage and fosters regeneration through immunoregulatory mechanisms via direct cellular interaction and releasing dissolved substances, including TGF-β, IL-10, and others (3). Several cell types have been studied for their potential to promote healing of damaged peripheral nerves, including embryonic stem cells (ESCs), neural stem cells (NSCs) (4), and mesenchymal stem cells (MSCs) (5). Another approach for peripheral nerve regeneration involves using biomaterials to repair damaged tissues (6). Chitosan, a cationic polysaccharide derived from chitin through deacetylation, has been extensively studied as a scaffold for cell growth, anti-adhesive substances, differentiation, integration, immunomodulators, and action against microorganisms (7-9). Numerous studies have reported the beneficial effects of chitosan on nerve regeneration (10,11). Researchers used a nerve conduit containing lyophilized human umbilical cord powder seeded with human umbilical cord mesenchymal

stem cells in a canine sciatic nerve model with a 10mm lesion, indicating the efficiency of this technique for peripheral nerve injury (12).

This work looks at the ability of mesenchymal stem cells and CS NPs to regenerate sciatic nerve lesions in rabbit models using a decellularized urine bladder matrix conduit.

Materials and methods

Ethical approval

The work was approved by Baghdad University's College of Veterinary Medicine's Scientific Ethic Committee (no: 1305/PG on Jan 18, 2023).

Experimental design

Twenty healthy adult male local breed rabbits between 5 and 7 months old and weighing between 1.3 and 1.9 Kg were collected from the animal house of the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq. Animals were kept in specially designed cages, one rabbit in each cage, for fifteen days before the experiment. The rabbits were fed a typical diet and water ad libitum. The animals were randomly divided into two groups, each consisting of ten individuals. A critical size defect (CSD) was created in the left sciatic nerve using a 10 mm transaction. The defect was then repaired by connecting a 14 mm conduit to each proximal and distal end of the nerve defect using 6.0 nylon epineural simple interrupted sutures. In the first group of CS NPs, the CSD was impacted with a semi-gel solution containing CS NPs at 1mg/ml concentration mesenchymal stem cells (MSCs) from the second group, with a quantity of $5x10^6$.

Anesthetic protocol

Before the surgical protocol, the rabbit was denied food for two hours. Rabbits were anesthetized with xylazine 5mg/kg (Holland, Xylazine 2%, Alfasan™) and ketamine 35mg/kg (Holland, ketamine 10%, Alfasan™) from alfasan company after an intramuscular injection of acepromazine 1 mg/kg (Holland, Neurotranq, Alfasan™) (13,14).

Surgical protocol

Prepare the lateral side of the thigh for aseptic surgery and make longitudinal skin incision length parallel posterior-lateral thigh about 3 cm from the caudolateral to the greater trochanter to expose the sciatic nerve splitting the biceps femoris and semitendinosus muscle by blunt dissection (Figure 1). A wooden tongue depressor was gently placed under the nerve, and the nerve was severed using a scalpel blade. A critical size defect to the nerve was created by a 10 mm transaction (Figure 1) and coaptated with a 14 mm conduit connected in each nerve segment with 6.0 nylon epineural simple interrupted sutures (Figure 1). The subcutaneous tissue and skin were closed routinely, and a broad-spectrum antibiotic injection of ceftriaxone 20mg/kg was given IM twice daily for five days.

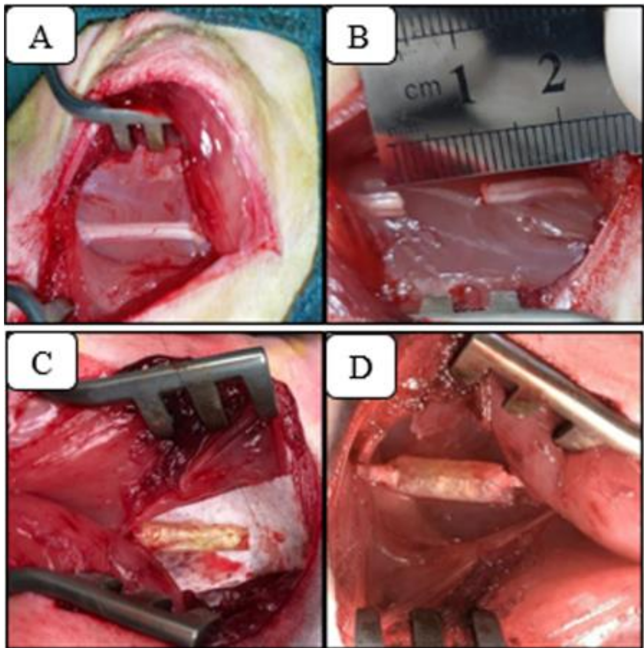


Figure 1: The procedure for doing sciatic nerve neurotmesis. A. The sciatic nerve is isolated from the surrounding tissues. B. A 10mm section of the sciatic nerve is cut. C. The proximal end of the conduit was secured to the stump using 6-0 nylon sutures. D. The conduit end was sutured to the distal site of the nerve.

Preparation of chitosan nanoparticles (CS NPs)

The preparation method of nanomaterials uses the chemical process using the sol-gel technique with certain modifications mentioned by Ghadi *et al.* (15), which entailed dissolving 1 g of chitosan (Sigma-Aldrich) in 200 ml deionized distilled water and subjecting it to ultrasonic bath for 30 min. The pH of the solution was then raised to 10 with NaOH (1N) drop by drop and agitated for 60 minutes at room temperature using a magnetic stirrer on a rotor. The pH was then decreased to 4 using HCL (1N), added drop by drop, and stirred using a magnetic stirrer on a rotor for 60 minutes at room temperature to form a clear solution. Finally, the pH was adjusted to 7 using NaOH (1N) drop by drop and stirred using a magnetic stirrer on a rotor for 60 minutes at room temperature.

Characterization of prepared nanoparticles

Based on Van der Waals or other attracting and repulsive forces, Atomic Force Microscopy (AFM) provides two- and three-dimensional surface topography (16). After being dried in an incubator throughout the previous night, chitosan nanoparticles FT-IR spectra were examined (17).

Preparation and characterization of allogeneic mesenchymal stem cells

The ASCs collected from the adipose tissue from the nape of the neck of a male indigenous breed rabbit were prepared according to Arana *et al.* (18), identified, differentiated following the procedure described in (19).

Conduit fabrication from bovine urinary bladder matrix (UBM)

In creating the urine bladder matrix (UBM), fresh urinary bladders were collected in their whole from slaughtered cows at a local abattoir using the procedure described (20). The conduit was made using procedure (21), and it was cut into appropriate sizes before being wrapped around ECM with a stainless-steel pin that was doubled up depending on the diameter of the tube, which had a 0.18 mm wall thickness. A basic digital caliper measured it. The tube's two edges were joined with a biological adhesive of egg albumen (22).

Evaluation of motor functions

All experimental animals were examined daily to determine their clinical signs or abnormal clinical signs such as walking style, which includes crouching, crawling on the heel, normal, and knuckling. The severity of these behaviors was classified as severe, moderate, mild, or common. The force of muscle contraction was assessed on a grad ranging from mild to moderate to strong (23).

Evaluation of sensory functions

The sensory functions of the conducting sciatic nerves were tested weekly throughout the study. The presence or absence of a toe spreading reflex, the presence or absence of toe pinch, toe prick, and lateral aspect leg sensation were evaluated. Additionally, the lateral aspect of leg sensation was assessed using foot withdrawal and vocalization activities. Positive responses to toe pinching and pricking have been observed, indicating recovery and improved function (24).

Preparing and assessing histology

The nerve tissue specimens were fixated using a 10% neutral buffered formalin solution, and the specimens were dehydrated using a series of ethanol, followed by clearing in xylene. The specimens were then embedded in paraffin and cut into sections with a thickness of 5 µm. Finally, these sections were stained using hematoxylin and eosin. Microscopic examination of nerve tissue sections was used to determine the excellent orientation of nerve fibers, basophilic nuclei of proliferated Schwann cells with thick myelin sheath, presence of vacuolated degenerate nerve fibers, dense axon population, nerve fiber myelination, and low fibrous tissue.

Statistical data analysis

The data was analyzed, and the results were presented as means and standard errors. One-way ANOVA and post hoc tests were used for statistical comparisons between groups, and the Statistical Package for the Social Sciences (SPSS) version 26 software was used, with a significance level of P<0.05.

Results

Chitosan nanoparticles (CS NPs) group

The animals Returned to normal walking abilities on day 80 post-operation (PO), which was a significant discovery. Mild knuckling remained until the end of the study. The muscular contraction force was strong (Table 1). Skin feeling eventually advanced from the foot to the fetlock joint over time. There was no toe spreading reflex, lateral leg feeling, toe pinch, or toe prick sensation (Table 2).

Mesenchymal stem cells (MSCs) Group

On the 56th post-operation, all animals walked usually, which was a significant discovery. Furthermore, the disappearance of knuckling was observed on the 62 days, and there was a notable increase in muscle force contraction on the 63 days post-operation (Table 1). Sensory improvements were also observed, with the sensation of toe spread becoming evident on day 90 post-operation, the appearance of lateral aspect leg response on day 81 post-operation, and the presence of the pinch on day 73 post-operation, as well as toe prick on day 67 post-operation (Table 2).

Sensory clinical functional tests

Sensory clinical indicators such as toe spread, lateral leg sensation, toe pinch, and toe prick did not show sensory responses on day 112 PO in the CS NPs and MSCs groups (Table 3).

Table 1: Meantime rates of motor functional tests on 112 in CS NPs and MSCs groups

Groups	Onset	Walking	Type of gait			Knuckling				Muscle contraction Force		
			Crouch	Crawl	Nor.	Severe	Mod.	Mild	Nor.	Weak	Mod	Strong
CS NPs	4.6	5.8	14.4	27	56.8	14.5	37.2	62.2	74	12.6	28.1	64.2
MSCs	2.6	3.8	11	24	53	10.2	34.2	55.6	62.2	7.8	23.2	62.8

Nor: normal. N: None. Mod: Moderate.

Table 2: Sensory Function tests during 112 days in CS NPs and MSCs groups

Groups/days	Toe spread	Lateral Aspect Leg Sensation	Toe Pinch	Toe prick
CS NPs	+98	+87	+78	+72
MSCs	+90	+81	+67	+73

Table 3: The mean time (days) during 112 sensory clinical observations in CS NPs and MSCs groups

Group	Toe spread	Lateral Aspect Leg Sensation	Toe Pinch	Toe prick
CS NPs	98.2 ± 0.96^a	87.4 ± 1.82^a	78.2 ± 0.86^a	72.2 ± 1.31^a
MSCs	89.8 ± 1.59^b	80.6 ± 0.92^b	67.4 ± 1.36^b	73.4 ± 0.97^b
LSD Value	4.63	4.29	2.94	3.86

At a significance level of $P<0.05$, a, b (Mean SE) with separate superscripts within the same row indicate statistically significant differences.

Histopathological study of the proximal portion of the sciatic nerve in the CS NPs group 56 days postoperatively showed vacuolated nerve fibers in degeneration. Also, collagen fibers impeded the nascent nerve development. The stem cell group showed that the remyelinated new nerve was arranged as a longitudinal neural fiber with angiogenesis in the affected area. However, a small vacuolation was observed (Figure 2).

Histopathological study of the middle segment of the sciatic nerve in the CS NPs group at 56 days postoperatively showed incomplete remyelination of the injured nerve. In the impacted region, vast voids were seen. The stem cells group showed complete remyelination of the middle area injured. However, small vacuoles were observed in the nerve axon (Figure 3).

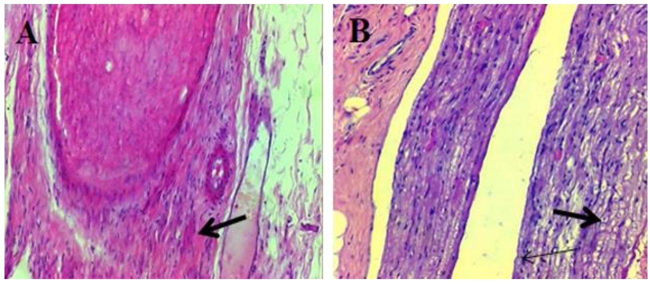


Figure 2: A photomicrograph of the proximal segment of 56 days post sciatic nerve defect. A. Chitosan nanoparticles group, the healing process of the affected nerve shows vacuolation within the neural axon. Collagen fibers (arrow) also disrupt the nascent nerve development. B. Stem cell group, remyelinated new nerve, was structured as a longitudinal neural fiber (thin arrow) with angiogenesis activity in the afflicted location. However, small vacuolation (thick arrow) was observed in these new nerves. H&E, X100.

Histopathological examination of the distal section of the sciatic nerve in the CS NPs group at 56 days postoperatively showed insufficient growth of the injured nerve. Collagen fiber tissue surrounds the new growth nerve's terminal and interrupts the new growth nerve (Figure 4). The stem cells group showed complete growth of the injured nerve. However, spaces between nerve fibers were observed (Figure 4).

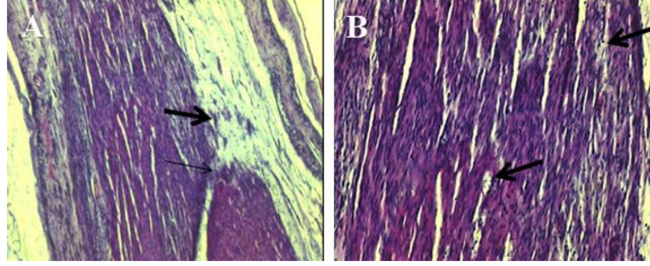


Figure 3: A photomicrograph of the middle segment of 56 days post sciatic nerve defect. A. Chitosan nanoparticles group, incomplete remyelination process area of the injured nerve. Large spaces (thick arrow) were observed in the affected area and unaligned the regenerative fiber (thin arrow). B. Stem cells group, complete remyelination of the middle area injured. However, small vacuoles (arrows) were observed in the nerve axon. H&E, X100.

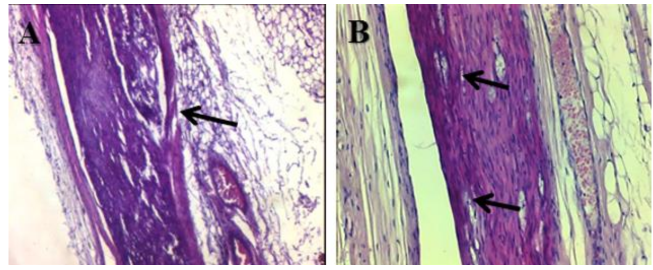


Figure 4: A photomicrograph of the distal segment of 56 days post sciatic nerve defect. A. Chitosan nanoparticles group, insufficient nerve growth. The collagen fiber tissue (arrow) that surrounds the terminal of the new growth nerve severed the new growth nerve. B. Stem cell group, degenerated nerve fibers of the affected area (arrow) and enhanced parallel of the nerve fibers. H&E, X100.

Histopathological study of the proximal segment of the sciatic nerve in the CS NPs group on 112 days postoperatively showed a poor healing process of the sciatic nerve was observed, where low density of neural axons with the presence of large spaces internodal and scar in the affected area was noted (Figure 5). The stem cells group showed Schwann cells at the epineurium and perineurium. However, collagen fibers were observed in the healing area that occupied some nerve areas. Also, the angiogenesis activity was noted (Figure 5).

Histopathological study of the middle segment of the sciatic nerve in the CS NPs group on 112 days postoperatively showed vacuoles in new growth nerve that were discovered in neural fibers of the posterior section of the sciatic nerve, indicating a poor healing process in the wounded sciatic nerve (Figure 6). In the stem cells group, the complete healing process of the sciatic nerve was observed: a large space (black arrow) that separated neural fibers (Figure 6).

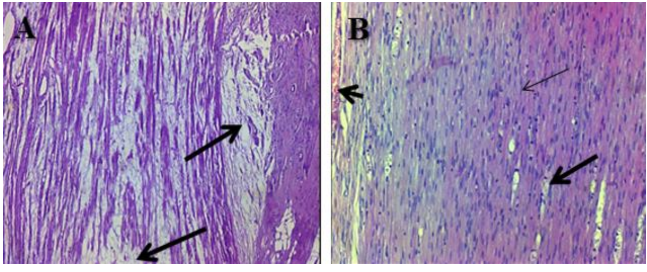


Figure 5: A photomicrograph of the proximal segment of 112 days post sciatic nerve defect. A. Chitosan nanoparticles group, Poor healing process of the sciatic nerve was observed, where low density of neural axons with large spaces (arrow) in the affected area was noted. B. Stem cells group, Schwann cells(thine arrow)at the epineurium and perineurium, degenerated nerve fibers (thike arrow), and the angiogenesis activity (head arrow) were noted. H&E, X100.

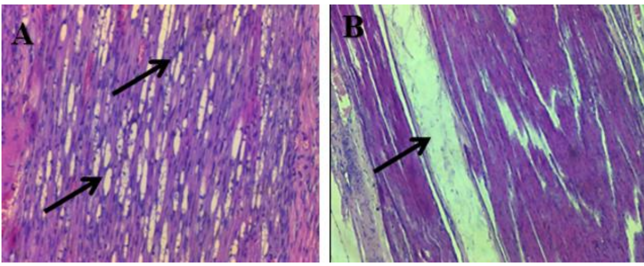


Figure 6: A photomicrograph of the middle segment of 112 days post sciatic nerve defect. A. Chitosan nanoparticles group. Note the vacuoles (arrow) in new growth nerve observed in neural fibers of the proximal segment. B. Stem cells group, a complete healing process of the sciatic nerve, was observed. However, a large space (arrow) separated neural fibers. H&E, X100.

Histopathological study of the distal segment of the sciatic nerve in the CS NPs group on 112 days postoperatively showed an incomplete healing process of the sciatic nerve was observed, where the neural fibers were interrupted by collagen fibers surrounding the neural fibers (Figure 7). The stem cells group showed good remyelination regeneration of the nerve fibers. Also, angiogenesis activity was noted, where new blood vessels were observed between neural fibers (Figure 7).

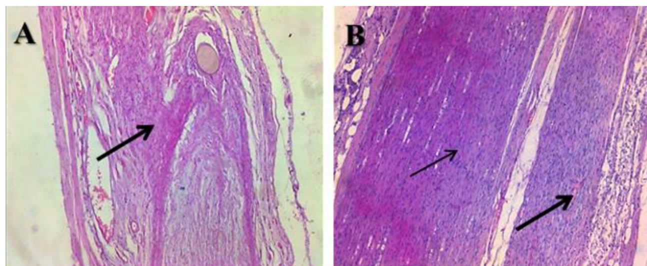


Figure 7: A photomicrograph of distal segment of 112 days post sciatic nerve.defect A. Chitosan nanoparticles group, an incomplete healing process of the sciatic nerve, was observed, where the neural fibers were interrupted by collagen fibers (arrow) that surrounded the neural fibers. B. Stem cells group shows good remyelination and regeneration of the nerve fibers; also, angiogenesis activity was noted (thick arrows), where new blood vessels were observed between neural fibers with increased Schwann cells (thin arrows). H&E, X100.

Discussion

This investigation showed that the start of limb movement and walking in MSC animals was faster than in the CS NPs group. All the animals returned to normal gait; however, the MSCs group (53 days) regained normal gait faster than the CS NPs group (57 days). The disappearance of knuckling in the MSCs (62 days) groups differed substantially (p 0.05) from that of the CS NPs (74 days). Muscle contraction forces were significant in both groups; however, contraction started earlier in the MSCs (62 days) group than in the CS NPs (64 days). The ability of the animal to walk on its operated left hind foot was assessed based on the intensity of the discomfort, which was categorized as neuropathic or inflammatory. According to the outcomes of this study, all animals in the MSCs group progressed to normal gait, which was faster than those in the CS NPs group. This finding implies that MSCs have therapeutic potential and may play a role in neuropathic pain and faulty sciatic nerve regeneration. Previous research found that implantation of adipose-derived stem cells reduced neuropathic pain, perhaps due to the production of neurotrophic, angiogenic, and anti-apoptotic substances (24,25).

Another study found that injecting stem cells around the sciatic nerve lesion reduced the development of mechanical and thermal allodynia (26). Muscle contraction was related to muscle denervation and disuse, and greater Muscle contraction force was connected with improved sciatic nerve motor function. Due to the facilitation of reinnervation, the MSC animals regained muscular force contraction more quickly than the CS NPs group because a sciatic nerve lesion hindered muscle force contraction. (27) discovered that muscular innervation-maintained muscle contraction force. Sensory reactivity, particularly toe spreading, improved with time, and peroneal nerve function returned (28). As a result, rabbit sciatic nerve regeneration was considered an indication of functional recovery. Implantation of fibroblast-like MSCs improved active healing of the damaged sciatic nerve in the MSCs group. MSCs have been found to express trophic factors and supporting substances such as nerve growth factor, brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, collagen, fibronectin, and extracellular matrix components, all of which influence endogenous neural cell activity (29,30).

In this study, the histological obtained at 56 days post-operation revealed variations in the rate of nerve regeneration among the treatment groups, likely attributed to differences in the quantity and quality of cells and the release of various neurotrophic factors supporting myelin sheath formation, neovascularization, and reduced inflammatory reaction, all contributing to the regeneration process. Particularly noteworthy was the CS NPs group, which showed an increased number of Schwann cells at 56 days post-operation, similar to the Stem cells group, due to a higher concentration of stem cells in the conduit. It is worth mentioning that the nerve conduit employed in this investigation had a high concentration of stromal stem cells (31). This conduit delivered factors directly to the transected peripheral nerve, resulting in increased angiogenesis and the release of growth factors such as VEGF and FGF, creating a favorable environment for stem cell differentiation into Schwann cells (32-34). Previous research has established the crucial role of Schwann cells in removing myelin and axon debris, with a codependent interaction with macrophages, where macrophages stimulate Schwann cell growth and collaborate in providing essential trophic and tropic factors for regenerating axons (35).

These findings align with a study conducted which evaluated the use of a decellularized bovine urinary bladder matrix conduit as a nerve guidance conduit, revealing an abundance of nerve fibers and enhanced radial function, elongation, and bridging of a 10 mm nerve gap in dogs. Similarly, another investigation involved the implantation of a xenogeneic acellular nerve matrix (ANM) into the sciatic nerve of rats, with the removal of a 1 cm nerve segment. The results demonstrated significant nerve regeneration properties of the constructed nerve graft, indicating its potential as a promising alternative to autologous grafts for repairing peripheral nerve defects at PN repair sites (36,37).

Various stem cell-based tissue engineering techniques have been used, training the environment to offer elements that encourage nerve regeneration (38,39). The findings of this study are consistent with a previous study (40) involving the implantation of mesenchymal stem cells into the gap of the sciatic nerve, bridged with a conduit in rats, resulting in enhanced motor function recovery, improved nerve regeneration, and reduced muscle atrophy.

The mechanisms behind neovascularization and axon regeneration after peripheral nerve injury are unclear (41). Despite this, several studies show a close link between angiogenesis and neurogenesis, which is mediated by the innate immune response to peripheral nerve injury (42). Macrophages are assumed to play a key role in responding to and guiding endothelial cell migration, leading to the formation of new blood vessels in hypoxic tissues. These newly created blood vessels are thought to direct Schwann cells and enable axon regeneration over peripheral nerve damage (43).

Surprisingly, after 112 days, the portions of the proximal segment in the stem cell group showed no evidence of degeneration or vacuolation. Instead, there was an increase in Schwann cells, proper nerve fiber alignment, and little scar tissue in the epineurium. Significant angiogenesis, nerve fiber alignment, and myelination were seen in the proximal, middle, and distal parts. These findings indicate that combining UBM conduits with MSCs promotes regeneration and increases functional recovery in sciatic nerve damage. This impact is linked to the production of various neurotrophic factors, which aided in developing more myelin sheaths in the middle segment, enhanced neovascularization, and decreased inflammatory responses. When combined with MSCs, a functional UBM conduit can support regenerating nerve fibers and control their growth in the ideal direction to bridge the nerve gap. As expected, employing both UBM conduits with MSCs resulted in better functional recovery than UBM conduits with chitosan conduits alone, exhibiting greater regeneration capacity. This discovery is consistent with a recent work (44) that used chitosan conduits with bone marrow mesenchymal stem cells (BMSCs) to bridge a fundamental gap in adult rat sciatic nerves in addition, combining MSCs with chitosan conduits expedited sciatic nerve regeneration and enhanced functional recovery compared to employing chitosan alone.

Furthermore, a study explored the effectiveness of nanofibrous nerve conduits supplemented with nerve growth factors and Bone Marrow stem cells (45). However, due to the limited accessibility of Schwann cells (SCs) and inadequate coverage of seeded cells on the nerve guidance conduits (NGCs), nerve regeneration across long gaps and achieving full functional recovery can be challenging. Nonetheless, the study indicated that mixing diverse parts improves peripheral nerve regeneration by boosting

neurotrophic factor production, especially in situations with extensive nerve damage intervals. Research on the other hand, found that using nerve guidance conduits (NGCs) in conjunction with adipose-tissue-derived multipotent mesenchymal stromal cells (ADMSCs) improves nerve regeneration in a Wistar rat model with a 12-mm sciatic nerve defect gap. After 112 days of therapy, regeneration was detected (46).

Furthermore, a research discovered that the presence of Schwann-like cells was increased in the sciatic nerve treated with BMSCs (47). BMSCs, like Schwann cells, have been postulated to prevent neuronal cell death and promote directed axonal growth. They form longitudinal columns known as Bungner bands as they multiply to take up home in the endoneuria sheaths (48,49). Within a few days of the damage, Schwann cells start division and form dedifferentiated daughter cells without axon interaction. They downregulate the expression of their normal proteins, such as peripheral myelin protein-22 (PMP-22), myelin basic protein (MBP), myelin-associated glycoprotein (MAG), and connexin-32 (50), to change into a premyelinating cell phenotype.

The Schwann cells undergo a process of dedifferentiation, proliferation, and redifferentiation. Their crucial role in guiding axon regeneration and the influence of neurotrophins, which communicate through paracrine receptor-mediated signals (51), Nerve growth factor (NGF), neurotrophic factors, cytokines, and other substances are examples. This increase stimulates Schwann cell development and proliferation, critical in avoiding neuronal death following damage. Furthermore, it promotes Schwann cell migration, adherence to axonal outgrowth, and the major augmentation of neovasculogenic withdrawal in any inflammatory process and subsequent promotion of regeneration time (52-54).

Conclusions

The study evaluated the impact of CS NP implantation within an acellular urinary bladder matrix conduit (AUBM) on repairing peripheral nerve regeneration defects. Although the implanted CS NPs in AUBM showed improvement in regeneration, the recovery rate was relatively lower. On the other hand, the implantation of ASCs within an acellular urinary bladder matrix conduit (AUBM) demonstrated a more significant effect in repairing a 10mm peripheral nerve gap in rabbits, particularly for sciatic nerve defects.

Acknowledgments

The College of Veterinary Medicine, University of Baghdad provided the study materials.

Conflicts of interest

According to the authors, there is no conflict of interest.

References

1. Li Y, Ma Z, Ren Y, Lu D, Li T, Li W, Wang J, Ma H, Zhao J. Tissue engineering strategies for peripheral nerve regeneration. Front Neurol. 2021;12:768267. DOI: 10.3389/fneur.2021.768267
2. Altaee MM, Qassim AH. The histological effect of the injection of nonsteroidal anti-inflammatory drugs on sciatic nerve of rats. Iraqi J Vet Sci. 2022;36(3):699-707. DOI: 10.33899/ijvs.2021.131529.1961
3. Essa HH, Jasim HS, Kadhim HA. Immunological and hematological response to local transplantation of stem cells in injured radial nerve of dogs. Iraqi J Vet Med. 2020;44(2):45-55. DOI: 10.30539/ijvm.v44i2.976
4. Yi S, Zhang Y, Gu X, Huang L, Zhang K, Qian T, Gu X. Application of stem cells in peripheral nerve regeneration. Burns Trauma. 2020;8:002. [available at]
5. Al-Timmemi HA, Ibrahim R, Zuki AZ, Azmi TI, Al-Jashamy K. Bone marrow stromal cells implantation and suture repair of peripheral nerve: A comparative study of functional, histopathological, morphometric and relative gastrocnemius muscle weight in rabbits. Afr J Biotechnol. 2011;10(44):8976-83. DOI: 10.5897/AJB11.1279
6. Kabu S, Gao Y, Kwon BK, Labhasetwar V. Drug delivery, cell-based therapies, and tissue engineering approaches for spinal cord injury. J Control Release. 2015;219:141-54. DOI: 10.1016/j.jconrel.2015.08.060
7. Salih SI, E'atelaf A, Mahdi AK, RA S. Role of chitosan application in postoperative abdominal adhesions in rabbits. Iraqi J Vet Med. 2015;39(1):105-11. DOI: 10.30539/iraqijvm.v39i1.206
8. Gao S, Zhao P, Lin C, Sun Y, Wang Y, Zhou Z, Yang D, Wang X, Xu H, Zhou F, Cao L. Differentiation of human adipose-derived stem cells into neuron-like cells which are compatible with photocurable three-dimensional scaffolds. Tissue Eng A. 2014;20(7-8):1271-84. DOI: 10.1089/ten.tea.2012.0773
9. Elmasry DM, Fadel MA, Mohamed FH, Badawy AM, Elsamadony HH. Copper chitosan nanocomposite as antiviral and immune-modulating effect in broiler experimentally infected with chicken anemia virus. Iraqi J Vet Sci. 2022;36(4):999-1009. DOI: 10.33899/ijvs.2022.132826.2135
10. Meyer C, Stenberg L, Gonzalez-Perez F, Wrobel S, Ronchi G, Udina E, Suganuma S, Geuna S, Navarro X, Dahlin LB, Grothe C. Chitosan-film enhanced chitosan nerve guides for long-distance regeneration of peripheral nerves. Biomater. 2016;76:33-51. DOI: 10.1016/j.biomaterials.2015.10.040
11. Zhao Y, Wang Y, Gong J, Yang L, Niu C, Ni X, Wang Y, Peng S, Gu X, Sun C, Yang Y. Chitosan degradation products facilitate peripheral nerve regeneration by improving macrophage-constructed microenvironments. Biomater. 2017;134:64-77. DOI: 10.1016/j.biomaterials.2017.02.026
12. AL-Zaidi AA, Shabeeb ZA, Al-Timmemi HA. Efficacy of acellular-lyophilized human umbilical cord powder seeded with human umbilical cord mesenchymal stem cells in peripheral nerve regeneration in dogs models. Med leg Upd. 2021;21(2). DOI: 10.37506/mlu.v21i2.2674
13. Mahmood MB. A Comparison between ketamine-xylazine and ketamine-midazolam or all of them to induce balance anesthesia in rabbits. Iraqi J Vet Sci. 2022;36(2):499-506. DOI: 10.33899/ijvs.2021.130618.1852
14. Flecknell P, Lofgren JL, Dyson MC, Marini RR, Swindle MM, Wilson RP. Pre-anesthesia, anesthesia, analgesia, and euthanasia. In: Fox JG, Anderson LC, Otto GM, Pritchett-Corning KR, Whary MT, editors. Laboratory animal medicine. USA: Academic Press; 2015. 1135-1200 p. DOI: 10.1016/B978-0-12-409527-4.00024-9
15. Ghadi A, Mahjoub S, Tabandeh F, Talebnia F. Synthesis and optimization of chitosan nanoparticles: Potential applications in nanomedicine and biomedical engineering. Caspian J Intern Med. 2014;5(3):156. [available at]
16. Lyles VD, Serem WK, Yu JJ, Garno JC. Surface characterization using atomic force microscopy (afm) in liquid environments. In: Bracco G, Holst B, editors. Surface Science Techniques. Germany: Springer; 2013. 599-620 p. DOI: 10.1007/978-3-642-34243-1_20

17. Jasim NA. Efficacy of paromomycin-loaded chitosan nanoparticles as a therapeutic agent against *Giardia lamblia* [master's thesis]. Baghdad: University of Baghdad; 2021.73 p.
18. Araña M, Mazo M, Aranda P, Pelacho B, Prosper F. Adipose tissue-derived mesenchymal stem cells: Isolation, expansion, and characterization. In: Race LK, editor. Cellular Cardiomyoplasty: Methods and Protocols. USA: Humana Totowa; 2013. 47-61 p. DOI: 10.1007/978-1-62703-511-8_4
19. Wall ME, Bernacki SH, Loboa EG. Effects of serial passaging on the adipogenic and osteogenic differentiation potential of adipose-derived human mesenchymal stem cells. Tissue Eng. 2007;13(6):1291-8. DOI: 10.1089/ten.2006.0275
20. Eberli D, Atala A, Yoo JJ. One and four-layer acellular bladder matrix for fascial tissue reconstruction. J Mater Sci Mater Med. 2011;22:741-51. DOI: 10.1007/s10856-011-4242-6
21. Al-Timmemi H, Hussein H, Ammal B. Acellular Xenograft bovine urinary bladder submucosa a novel conduit for regeneration of sciatic nerve defect in rabbit model. Adv Anim Vet Sci. 2017:5(1):1–10. DOI: 10.14737/journal.aavs/2017/5.1.14.22
22. Xu K, Liu Y, Bu S, Wu T, Chang Q, Singh G, Cao X, Deng C, Li B, Luo G, Xing M. Egg albumen as a fast and strong medical adhesive glue. Adv Healthc Mater. 2017;6(19):1700132. DOI: 10.1002/adhm.201700132
23. Schmidhammer R, Zandieh S, Hopf R, Mizner I, Pelinka LE, Kroepfl A, Redl H. Alleviated tension at the repair site enhances functional regeneration: The effect of full range of motion mobilization on the regeneration of peripheral nerves histologic, electrophysiologic, and functional results in a rat model. J Trauma. 2004;56(3):571-84. DOI: 10.1097/01.TA.0000114082.19295.E6
24. Gadelkarim M, Abushouk AI, Ghanem E, Hamaad AM, Saad AM, Abdel-Daim MM. Adipose-derived stem cells: effectiveness and advances in delivery in diabetic wound healing. Biomed Pharmacother. 2018;107:625-33. DOI: 10.1016/j.biopha.2018.08.013
25. Siniscalco D, Rossi F, Maione S. Stem cell therapy for neuropathic pain treatment. J Stem Cells Regen Med. 2007;3(1):2. DOI: 10.46582/jsrm.0301002
26. Forouzanfar F, Amin B, Ghorbani A, Ghazavi H, Ghasemi F, Sadri K, Mehri S, Sadeghnia HR, Hosseinzadeh H. New approach for the treatment of neuropathic pain: Fibroblast growth factor 1 gene transfected adipose-derived mesenchymal stem cells. Eur J Pain. 2018;22(2):295-310. DOI: 10.1002/ejp.1119
27. Passipieri JA, Dienes J, Frank J, Glazier J, Portell A, Venkatesh KP, Bliley JM, Grybowski D, Schilling BK, Marra KG, Christ GJ. Adipose stem cells enhance nerve regeneration and muscle function in a peroneal nerve ablation model. Tissue Eng A. 2021;27(5-6):297-310. DOI: 10.1089/ten.tea.2018.0244
28. Alsmadi NZ, Bendale GS, Kanneganti A, Shihabeddin T, Nguyen AH, Hor E, Dash S, Johnston B, Granja-Vazquez R, Romero-Ortega MI. Glial-derived growth factor and pleiotrophin synergistically promote axonal regeneration in critical nerve injuries. Acta Biomater. 2018;78:165-77. DOI: 10.1016/j.actbio.2018.07.048
29. Przyborski SA, Hardy SA, Maltman DJ. Mesenchymal stem cells as mediators of neural differentiation. Curr Stem Cell Res Ther. 2008;3(1):43-52. DOI: 10.2174/157488808783489471
30. Maltman D, Hardy S, Przyborski S. Role of mesenchymal stem cells in neurogenesis and nervous system repair. Neurochem Int. 2011;59(3):347-56. DOI: 10.1016/j.neuint.2011.06.008
31. Mohamadi F, Ebrahimi S, Nourani M, Mansoori K, Salehi M, Alizadeh A, Ai J. Enhanced sciatic nerve regeneration by human endometrial stem cells in an electrospun poly (ε-caprolactone)/collagen/NBG nerve conduit in rat. Artif Cells Nanomed Biotechnol. 2018;46(8):1731-1743. DOI: 10.1080/21691401.2017.1391823
32. Idrisova KF, Zeinalova AK, Masgutova GA, Bogov Jr AA, Allegrucci C, Syromiatnikova VY, Salafutdinov II, Garanina EE, Andreeva DI, Kadyrov AA, Rizvanov AA. Application of neurotrophic and proangiogenic factors as therapy after peripheral nervous system injury. Neural Regen Res. 2022;17(6):1240. DOI: 10.4103/1673-5374.327329
33. Ahmed H, Hameed A, Nada H. Role of acellular bovine urinary bladder submucosa on skin wound healing in Iraqi goats. Iraqi J Vet Med. 2016;40(1):53-60. DOI: 10.30539/iraqijvm.v40i1.138
34. Zedan ZK, Hassan SA. In vivo study of impact transplantation hematopoietic progenitor cells on induced cutaneous wound healing in rabbit's model. Iraqi J Vet Sci. 2022;36(3):579-89. DOI: 10.33899/ijvs.2021.130949.1899
35. Camara J, Griessenauer C. Stem cells and peripheral nerve. In: Tubbs RS, Rizk E, Shoja MM, Loukas M, Barbaro N, Spinner RJ, editors. Nerves and nerve injuries. USA: Academic Press; 2015. 947-969 p. DOI: 10.1016/B978-0-12-802653-3.00108-1
36. AL-Zaidi A. Evaluation the role of human umbilical cord mesenchymal stem cells and acellular human umbilical cord tissue on healing of radial nerve in dogs [Ph.D. Dissertation]. Baghdad: University of Baghdad; 2021. 176 p.
37. Zhang Y, Luo H, Zhang Z, Lu Y, Huang X, Yang L, Xu J, Yang W, Fan X, Du B, Gao P. A nerve graft constructed with xenogeneic acellular nerve matrix and autologous adipose-derived mesenchymal stem cells. Biomater. 2010;31(20):5312-24. DOI: 10.1016/j.biomaterials.2010.03.029
38. Hong IS. Enhancing stem cell-based therapeutic potential by combining various bioengineering technologies. Front Cell Dev Biol. 2022;10:901661. DOI: 10.3389/fcell.2022.901661
39. Mahmood SK, Abdul Razak IB, Yaseen MT, Yusof LM, Jeber ZK, Gimba F, Zakaria MB. 3-D nanocomposite scaffolds: Tissue engineering for bone reconstruction. Iraqi J Vet Sci. 2022;36(2):433-444. DOI: 10.33899/IJVS.2021.130481.1831
40. Raisi A, Azizi S, Delirezh N, Heshmatian B, Farshid AA, Amini K. The mesenchymal stem cell–derived microvesicles enhance sciatic nerve regeneration in rat: A novel approach in peripheral nerve cell therapy. J Trauma Acute Care Surg. 2014;76(4):991-7. DOI: 10.1097/TA.0000000000000186
41. Scheib J, Höke A. Advances in peripheral nerve regeneration. Nat Rev Neurol. 2013;9(12):668-76. DOI: 10.1038/nrneurol.2013.227
42. Lavorato A, Raimondo S, Boido M, Muratori L, Durante G, Cofano F, Vincitorio F, Petrone S, Titolo P, Tartara F, Vercelli A. Mesenchymal stem cell treatment perspectives in peripheral nerve regeneration: Systematic review. Int J Mol Sci. 2021;22(2):572. DOI: 10.3390/ijms22020572
43. Ren T, Faust A, van der Merwe Y, Xiao B, Johnson S, Kandakatla A, Gorantla VS, Badylak SF, Washington KM, Steketee MB. Fetal extracellular matrix nerve wraps locally improve peripheral nerve remodeling after complete transection and direct repair in rat. Sci Rep. 2018;8(1):4474. DOI: 10.1038/s41598-018-22628-8
44. Zheng L, Cui HF. Enhancement of nerve regeneration along a chitosan conduit combined with bone marrow mesenchymal stem cells. J Mater Sci Mater Med. 2012;23:2291-302. DOI: 10.1007/s10856-012-4694-3
45. Zhou G, Chang W, Zhou X, Chen Y, Dai F, Anwar A, Yu X. Nanofibrous nerve conduits with nerve growth factors and bone marrow stromal cells pre-cultured in bioreactors for peripheral nerve regeneration. ACS Appl Mater Interfaces. 2020;12(14):16168-77. DOI: 10.1021/acsami.0c04191
46. Rodríguez D, Pinto G, Cartarozzi L, Alexander O, Ana B, Carvalho M, Silva J, Dernowsek J, Golim M, Barraviera B, Ferreira R. 3D-printed nerve guidance conduits multi-functionalized with canine multipotent mesenchymal stromal cells promote neuroregeneration after sciatic nerve injury in rats. Stem Cell Res Ther. 2021;12(1):303. DOI: 10.1186/s13287-021-02315-8
47. Hameed AT, Ibrahim R, Karim AJ, Abz Z, Ti A. Clinical and histopathological observations of autologous bone marrow stromal cells implantation on the regeneration of sciatic nerve neurotmesis in rabbit. Afr J Biotechnol. 2011;10(33):6310-8. DOI: 10.5897/AJB11.1279
48. Stoll G, Müller HW. Nerve injury, axonal degeneration, and neural regeneration: Basic insights. Brain Pathol. 1999;9(2):313-25. DOI: 10.1111/j.1750-3639.1999.tb00229.x
49. Arthur-Farraj PJ, Latouche M, Wilton DK, Quintes S, Chabrol E, Banerjee A, Woodhoo A, Jenkins B, Rahman M, Turmaine M, Wicher GK. C-Jun reprograms Schwann cells of injured nerves to generate a

repair cell essential for regeneration. Neuron. 2012;75(4):633-47. DOI: 10.1016/j.neuron.2012.06.021
50. Kidd GJ, Ohno N, Trapp BD. Biology of Schwann cells. In: Said G, Krarup C, editors. Handbook of clinical neurology. Netherlands: Elsevier B.V.; 2013. 55-79 p.
51. Schuh CM, Sandoval-Castellanos AM, De Gregorio C, Contreras-Kallens P, Haycock JW. The role of Schwann cells in peripheral nerve function, injury, and repair. Cell Eng Reg. 2020:215-36. DOI: 10.1159/000370324
52. Ismail HK, Al-Sabawy RA, Jumaa HJ. Protective effect of placental mesenchymal stem cells on histological changes of pancreas experimentally induced by alloxane in mice. Iraqi J Vet Sci. 2020;34(1):1-8. DOI: 10.33899/ijvs.2020.163563
53. Correale J, Fiol M, Villa A. Neuroprotective effects of inflammation in the nervous system. NeuroImmune Biol. 2008;6:403-31. DOI: 10.1016/S1567-7443(07)10020-X
54. El-Shaer AJ, Badr HA, AlSadek DM. The combined effect of the human umbilical cord blood with chitosan scaffold on the full thickness wound healing process in rats. Iraqi J Vet Sci. 2023;37(2):391-403. DOI: 10.33899/ijvs.2022.134782.2404

فعالية جسيمات الكيتوسان النانوية والخلايا الجذعية لتجديد العصب الوركي في نموذج الأرنب

ضرغام حميد الحيدري[١] و حميد علي كاظم التميمي[٢]

[١]فرع السريريات، كلية الطب البيطري، جامعة الكوفة، النجف، [٢]فرع الجراحة والتوليد البيطري، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

الخلاصة

تعد إصابة الأعصاب المحيطية من أكثر الإصابات حدوثا في الحيوانات والتي يسببها عدد من العوامل، ومنها الصدمات والعمليات العلاجية، والخيارات العلاجية تكون محدودة للمساعدة في التعافي الوظيفي لهذه الحالات. كان الهدف من هذه الدراسة هو مقارنة الخلايا الجذعية المشتقة من الدهون وجزيئات الكيتوسان النانوية في تجدد العصب الوركي المقطوع للأرانب. حيث تم تقسيم عشرين أرنبًا من السلالات المحلية إلى مجموعتين بشكل عشوائي حيث تم سد القطع المحدث بقناة لا خلوية من المثانة البولية للأبقار بطول أربعة عشر ملم ومملوءة بمادة الكيتوسان النانوي شبه الهلام ١ ملغم/مل في المجموعة الأولى، وتم سد القطع المحدث بقناة لا خلوية من المثانة البولية للأبقار مملوءة بالخلايا الجذعية ٥*١٠[٦] في المجموعة الثانية. وفي نهاية اليوم السادس والخمسون واليوم المئة وأثني عشر، تم فحص الأعراض السريرية والوظيفة الحركية والحسية والتقدم النسيجي للعصب الوركي. وفقًا للنتائج، زادت القدرات الحركية والحسية للعصب الوركي بشكل أسرع في مجموعة الخلايا الجذعية. في مجموعة الخلايا الجذعية لإصلاح القطع المحدث في العصب الوركي بقياس عشرة ملم، وكشف الفحص المرضي النسيجي عن نوى قاعدية لخلايا شوان المتكاثرة مع غمد المايلين سميك والألياف العصبية جيدة التوجه ومجموعة محورية كثيفة وقطر محوري كبير ونخاع ألياف عصبية وأنسجة ليفية منخفضة. وخلص إلى أن تأثير الخلايا الجذعية يعزز تجديد العصب الوركي المقطوع مقارنة بجزيئات الكيتوسان النانوية.