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Original Article

Bridging potential of Taurine-loading PCL conduits transplanted with hEnSCs on resected sciatic nerves

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ABSTRACT

Reconstruction of nerve conduits is a promising method for functional improvement in peripheral nerve repair. Besides choosing of a suitable polymer for conduit construction, adding factors such as Taurine improve a more advantageous microenvironment for defect nerve regeneration. Showing several major biological properties of Taurine, for example, regulation of the osmotic pressure, modulation of neurogenesis, and calcium hemostasis, makes it an appropriate option for repairing of defected nerves. To this, we examined repairing effects of Taurine-loading PCL conduits cultured with human endothelial stem cells (hEnSCs) on resected sciatic nerves. PCL/Taurine/Cell conduits transplanted to a 10-mm sciatic nerve gap. Forty-two wistar rats were randomly divided to seven groups: (1) Normal group, (2) Negative control (NC), (3) Positive control (nerve Autograft group), (4) PCL conduits group (PCL), (5) Taurine loaded PCL conduits group (PCL/Taurine), (6) hEnSCs cultured on the PCL conduits (PCL/Cell), (7) hEnSCs cultured on the PCL/Taurine conduits (PCL/Taurine/Cell). Functional recovery of motor and sensory nerves, the action potential of exciting muscle and motor distal latency has seen in PCL/Taurine/Cell conduits. Histological studies showed also remarkable nerve regeneration and obvious bridging has seen in this group. In conclusion, PCL/Taurine/Cell conduits showing suitable mechanical properties and biocompatibility may improve sciatic nerve regeneration.

1. Introduction

Peripheral nerves develop a wide complex system that makes a connection between the brain and spinal cord and the other organs of the body [1,2]. Therefore, nerve trauma can obstruct the linkage between the brain and the controlled muscle, which affects the moving ability or normal perception [3]. Renovation of injured nerves depends on various factors and has been researched by

Abbreviations: hEnSCs, human endothelial stem cells; FDA, Food and Drug Administration; PD, Parkinson's disease; AD, Alzheimer's disease; DPN, peripheral neuropathy; MSCs, mesenchymal stem cells; PCL, polycaprolactone; PBS, phosphate-buffered saline; FBS, fetal bovine serum; MTT, dimethylthiazol diphenyl tetrazolium bromide; DAPI, diamidino phenylindole; SFI, sciatic functionl index; EMG, electromyography; EMAP, muscle action potential; HPL, hotplate latency; WRL, withdrawal reflex latency; HPF, high power fields; LFB, Luxol fast blue; ECM, extracellular matrix structure; TCP, tissue culture plate; PNS, peripheral nerve system; NGC, nerve guidance conduits.

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different techniques, such as grafting procedures, drug therapy, and designed degradable/non-degradable natural/synthetic scaffolds [4,5]. One of the usual graftings in surgery is autograft defined as utilizing a part of the nerve from elsewhere in the body. However, there are several limitations including multiple surgeries, loss of function and morbidity of donor nerves, lack of appropriate size and structure for nerve tissue [6–8], while allograft and xenograft transplantation are associated with immune system stimulation [9–11]. Hence, more investigators considered utilizing artificial neural guidance conduits to reconstruct neural pathway [12–15]. The main objective of constructing artificial neural guidance conduits is to best imitate the structure and constituents of autologous nerves. Within the last decades, fabricating methods could improve the structure of nerves in terms of appropriate permeable and biodegradable porous conduits, along with ideal tensile strength in resisting *in vivo* mechanical forces [16]. Electrospinning is an eminent technique to produce nanofibers with suitable porosity, degradability, mechanical properties, and extensive surface to volume for compatible cell attachment [17,18]. These special characteristics of nanofibers structures persuade wide investigation on its application for neural tissue engineering [12–15,19–21].

To success in neural tissue engineering, proper material selection plays an important role in achieving tailored material degradation rate and tensile strength. Polycaprolactone (PCL) is an absorbable aliphatic polyester, which is degradable due to susceptibility of aliphatic ester linkages. PCL products metabolized both through carbocyclic acid or kidney pathways. *In vitro* and *in vivo* suitable compatibility of PCL and efficacy studies lead to confirmation of US Food and Drug Administration (FDA) for medical drug delivery applications [22–24]. Recently, compatible products such as drug delivery systems, absorbable sutures, and cartilage and bone graft substitutions [25–27]. PCL synthetic biomaterials showed that they could be used as nerve regeneration conduits. Mohammadi et al. fabricated PCL/collagen/nanobioglass (NGB) conduits and exhibited that they have the potential to regenerate sciatic nerves in the rat animal model [28].

Taurine is a fundamental amino acid, which has cytoprotective properties in different kinds of tissues [29]. It is mostly produced by methionine and cysteine metabolism in the heart, brain, liver, and spinal cord [30]. There are multiple studies have considered the neuroprotective effect of Taurine in central and peripheral nervous system diseases, like Parkinson's disease (PD), Alzheimer's disease (AD), stroke, epilepsy, cognitive disorders, diabetic peripheral neuropathy (DPN), and etc. [31–35]. Taurine showed several major biological properties, such as membrane consistency, regulation of the osmotic pressure, modulation of neurogenesis, neuro-inflammation, anti-apoptotic, and calcium hemostasis [36].

Regeneration of endometrium is mediated by stem cells populations and signaling molecules [37]. The uterine endometrium is one of the richest tissues containing endometrial stem cells (EnSCs) and a possible source of multipotent mesenchymal stem cells (MSCs) [38]. Human endometrial stem cells (hEnSCs) are a new source of stem cells in the postmenopausal endometrium that may play an important role in a monthly regeneration and remodeling of the human endometrium [39–42]. Recently, hEnSCs have been widely used in regenerative medical studies, mainly in cell replacement therapy due to their easy access to culture, rapid development without any crucial ethical and technical problems, high ability to differentiate into different cell lineages such as adipocyte, dendrocyte, osteoblast, and neuron cells [43–46]. Accordingly, the main objective of this study was to evaluate the nerve regeneration of polycaprolactone/Taurine (PCL/Taurine) nanofibrous conduits cultured with hEnSCs in resected sciatic nerves in the rat animal model.

2. Material and methods

2.1. Materials

PCL (MW = 80,000 g/mol), phosphate-buffered saline (PBS), fetal bovine serum (FBS), trypsin–EDTA, Dimethylthiazol diphenyl tetrazolium bromide (MTT), Penicillin/Streptomycin, (Pen-Strep, 10,000 U/mL), Diamidino phenylindole (DAPI) and collagenase I were purchased from Sigma-Aldrich, UK. Dulbecco's Modified Eagle's Medium F12 (DMEM/F12) was obtained from Invitrogen (Carlsbad, CA, USA). Also, chloroform and methanol were prepared from Merck.

2.2. Production and characterizations of the nanofibrous conduits

Nanofibrous nerve conduits were synthesized by electrospinning technique with PCL polymer. PCL polymer (12% w/v) was solved in chloroform:methanol (7:3 v/v) and stirred for 24 h. For fabrication of PCL/Taurine, Taurine (20% w/w relative to PCL weight) was added to PCL solution and stirred for another 24 h. The nerve conduits were constructed using an electrospinning setup included a syringe driver, a positive high-voltage supply (18 kV), and a rotating template with 2 mm diameter. The solution was transferred to a 22-gauge blunt tip needle at a steady flow rate of 1 ml/h using a syringe driver. The distance between the needle tip and the rotating template was 13 cm, and the rotation speed of the rotating template was about 600 rpm. Eventually, the samples were prepared with gold coating using a Sputter (SCD 050, BAL-TEC USA) before morphology evaluation of the conduits under field emission scanning electron microscopy (Philips XL-30 SEM, Netherlands) at the acceleration voltage of 25 kV. The diameter of fibers and the cross-sectional external and internal diameter of conduits were measured by using Image J software (Image J, National Institute of Mental Health, Bethesda, Maryland, USA).

2.3. Mechanical properties

Tensile strength, elongation at break and Young's modulus of each conduit (30 mm length- 5 mm width) were measured using a universal testing machine (INSTRON, USA) with ASTM 638-5 standard) that had contained 10 N of load capacity and 1 mm/min of tensile rate. The fibers were cut into 30 × 5 mm sections (n = 5). The results were reported as mean ± SD.

2.4. Isolation of human endometrial stem cell (hEnSCs)

Human endometrial stem cells were isolated using our previously described protocol [28]. Briefly, human endometrial tissue was enzymatically digested with 1 mg/ml collagenase type I at 37 °C for 1 h. The digested suspensions were filtered through 70- and 40-μm cell strainers and centrifuged to separate the hEnSCs. Eventually, the isolated stem cells were suspended in DMEM/F12 containing 10% FBS and 1% pen-strep and incubated in 37 °C and 5% CO_2. The hEnSCs at passage 3 were characterized by flow cytometry and used for the following experiments.

2.5. Cell adhesion and morphology analysis

Cell adhesion and morphology analysis is an appropriate method to evaluate the cell-scaffold compatibility and cell attachment. To the cell seeding, conduits were cut into 24-well plate, sterilized by exposure to ultraviolet radiation for 30 min in each side and then alcohol 70% for 30 min after that, washed with PBS for three times and incubated with DMEM/F12 medium

containing 10% FBS for 24 h. Then, 25 × 10^3 hEnSCs cells/well were seeded on the nanofibrous conduits and incubated at 37 °C and 5% CO_2. To assess cell attachment, the cell-scaffold composite was stabilized in Karnovsky's Fixative (paraformaldehyde 2% (w/v) and glutaraldehyde2.5% (w/v) for 45 min, rinsed in PBS, and dehydrated in ascending alcohols concentrations at room temperature for 10 min. The morphology of cells onto conduits was determined by SEM (Philips XL-30) at an accelerating voltage of 25 kV on the fifth days of the cell seeding period.

2.6. Cell survival and proliferation study

To quantify the cell viability and proliferation of the hEnSCs, a colorimetric MTT assay was performed on day 1, 3, and 5 [47]. The hEnSCs were seeded at a density of 10 × 10^3 cell/well of 96-well plate onto the nanofibers conduits and incubated in DMEM/F12 at 37 °C and 5% CO_2 for 1, 3 and 5 days. The absorbance of each well was examined at 570 nm using a plate reader (Epoch, BioTeck, USA). The results were repeated for six times and reported as mean ± SD.

The cell attachment and proliferation was evaluated using DAPI staining. hEnSCs were seeded on the nanofiberous conduits and cultured in DMEM/F12 for fifth days. Morphological analyses of the stained hEnSCs were examined using a confocal fluorescent microscope (OPTIKA B-500TiFL, Italy).

2.7. In vivo studies

2.7.1. The groups of study

To evaluate the nerve regeneration, sums of 42 adult male wistar rats were applied in this study. The rats were randomly divided into 7 groups (n = 6 rats). (1) Rats without any manipulation for nerve surgery (Normal), (2) Nerve defect group with 10-mm gap without treatment (Negative control; NC), (3) Nerve Autograft group that 180-degree reversed autograft was transplanted into the 10-mm sciatic nerve gap (Positive control; Autograft), (4) PCL conduits group (PCL), (5) Taurine loaded PCL conduits group (PCL/Taurine), (6) hEnSCs cultured on the PCL conduits (PCL/Cell), (7) hEnSCs cultured on the PCL/Taurine conduits (PCL/Taurine/Cell).

The rats were anesthetized by intraperitoneal ketamine (60 mg/kg) and xylazine (10 mg/kg) injection. The surgery was performed on the right-side sciatic nerve in all groups. In fully anesthesia condition, the sciatic nerve was exposed with an incision in the posterior muscle of the right thigh. The Sciatic nerve was resected into proximal and distal parts (10 mm gap) in the center of the right thigh. Then a 12-mm conduit was utilized to bridge the gap between the proximal and distal stumps, where the ends of stumps were anchored into the conduits to a depth of 1 mm with 7–0 nylon.

In Autograft group, a 10-mm sciatic nerve was resected and then sutured again by a 180-degree rotation. In NC, no conduits were used to bridge the gap. All the rats were housed in a controlled temperature and humidity with a dark-light cycle and easy access to water and food. The rats were sacrificed after 12-week post-surgery and nerve regeneration was evaluated by sciatic functional index (SFI), hotplate study, Electromyographic examination as well as histological assessments.

2.7.2. Electromyographic evaluation

Electromyography (EMG) was carried out on the animals 12-week post-surgery to evaluate nerve regeneration. The electrophysiological response of gastrocnemius muscle was analyzed by measuring the evoked muscle action potential (EMAP) using an electromyographic sensor device (Negarandishegan, Tehran, Iran). The rats were anesthetized by intraperitoneal injection and surrounding adipose and fibrous tissues of sciatic nerve were entirely removed. To analysis the EMAP, the site of proximal sciatic nerve injury was provoked using needle electrodes (3–5 mA), while a ground electrode was located in surrounding muscle tissues to eliminate any potential interference. The cap and needle electrodes placed in the gastrocnemius muscle with a sweep speed of 1 ms/division, the sensitivity of 2 mV/division and filtering frequency of 10 Hz to 10 kHz.

2.7.3. Walking-foot-print analysis

As described previously, the sciatic functional index (SFI) was determined based on the rats' footprints of 1,4, 8 and 12 weeks' post-surgery [48]. The rats' posterior feet were impregnated with ink and located inside a walking passage (100 × 20 × 15 cm) covered with white millimeter papers. SFI was measured by the following equation; where N and E are the normal and experimental feet, respectively; PL is the interval between the heel and the uppermost point of the third toe; TS is the interval between the first and the fifth toe, and IT is the interval between the second to the fourth toe. This procedure was repeated at least 3 times in order to obtain obvious footmarks. If the result was calculated around zero, it showed normal function while the result of -100 implied the total impairment.

$$SFI = -38.3 \times \frac{EPL - NPL}{NPL} + 109.5 \times \frac{ETS - NTS}{NTS} + 13.3 \times \frac{EIT - NIT}{NIT} - 8.8$$

2.7.4. Hotplate latency test

The hotplate latency (HPL) was used to analyze the sensory function and sensitivity to thermal pain in 1, 4, 8, and 12 weeks' post-surgery. To this, the rats' injured limbs were located in the midpoint of an open-ended cylinder on a hotplate of 56 °C and the time recorded in seconds until they jumped their feet. From the onset of hotplate touch to drawing back of the limb, the time interval was recorded by a timer and considered as withdrawal reflex latency (WRL). The cut off time for their response was considered at 12 s. The HPL was repeated three times and the interval time was figured out about 10 min between the repetitions to avoid sensitization.

2.7.5. Histological examination

To analysis the histological sections, the animals were euthanized after 12 weeks' post-surgery, the distal part of the sciatic nerves was resected and immediately transferred to 10% buffered formalin for 2 days. The histological sections were prepared to stain with hematoxylin and eosin (H&E) and Luxol fast blue (LFB) for light microscopy evaluations (Olympus BX51; Olympus, Tokyo, Japan). Stained tissue sections were analyzed according to nerve growth from proximal and distal, vacuolation rate, and nerve necrosis and fibrosis. The number of axons was counted in 5 high power fields (HPF) and analyzed by an independent reviewer. Remyelination was evaluated by LFB staining which stains the myelin blue. The total number of myelinated axons in these 5 HPF (axon counts) was derived and analyzed, using computer software Image-Pro Plus® V.6 (Media Cybernetics, Inc., Silver Spring, USA).

2.8. Statistical analysis

The results were analyzed by GraphPad Prism 6 software and reported as mean ± standard deviation (SD). For nerve histomorphometry, unpaired one-way ANOVA and Tukey post hoc were used to compare the axon counts between experimental groups. The significance level was considered as $p<0.05$.

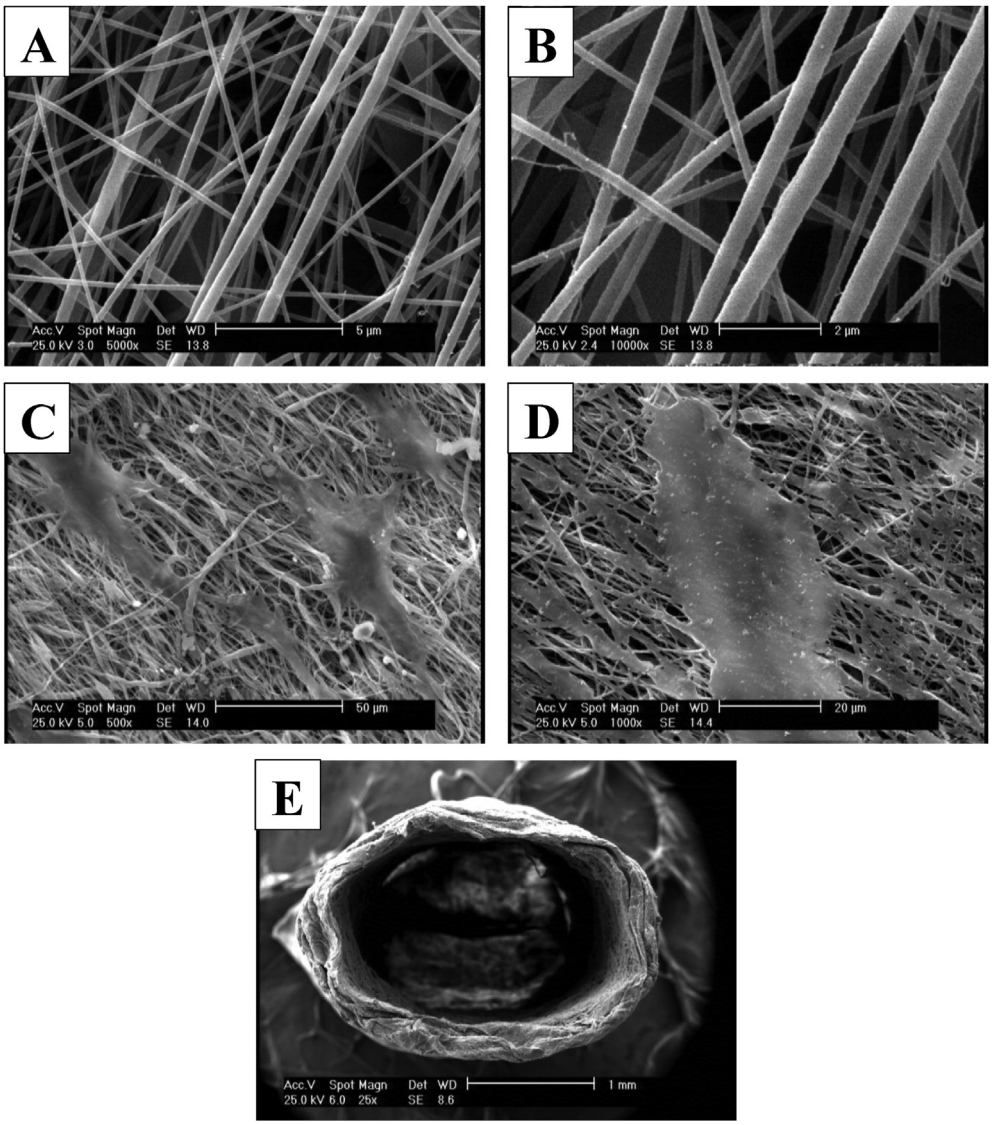

Fig. 1. The electrospun mat of PCL conduit without cells (A, B) and an electrospun mat of PCL conduit with cells grew on this conduit 5days after seeding (C, D), The photographs of composite nerve conduit fabricated (E).

3. Results

3.1. Evaluation of microstructures of nanofibrous conduits

Fig. 1 showed the SEM micrographs of PCL fabricated conduits containing Taurine 20% w/w. According to our observations, the nanofibers conduits are composed of non-beaded and uniformed fibers with an average diameter of 261 ± 84 nm. The template collector and the polymer solution volume could affect the internal diameter and thickness of the conduits. The electrospun neural conduits had an internal and external diameter, and a length of 2 mm, 2.2 mm, and 12 mm, respectively. Fig. 1(C, D) showed that PCL could mimic the extracellular matrix structure (ECM) and provide hEnSCs adhesion and proliferation.

3.2. Mechanical properties

The neural nanofibers conduits must have appropriate mechanical properties to endure the grafting surgery and forces during the regeneration stage. Fig. 2 showed the mechanical properties of PCL and PCL/Taurine nanofibers conduits. Incorporation of Taurine into PCL significantly increased the young's modulus ($p < 0.05$) and decreased the percentage of elongation at break ($p<0.001$). However, the ultimate tensile strength of the PCL and PCL/Taurine conduits has no significant difference.

3.3. Cytotoxicity and proliferation assay

To analyze cell viability, diamidino-2- phenylindole (DAPI) stained the hEnSCs nuclei cultured on the conduits. For this reason, human EnSCs were cultured on the nanofibrous conduits in DMEM/F12 medium for 5 days. Results showed that both PCL and PCL/Taurine conduits could provide a compatible scaffold to support adhesion and proliferation of the human EnSCs (Fig. 3(A)). MTT method was used to determine the viability of hEnSCs cultured on the PCL and PCL/Taurine/Cell conduits compared with the positive control (tissue culture plate (TCP)) at 1, 3 and 5 days (Fig. 3(B)). Until day 1, the cells cultured on TCP revealed higher viability than cells cultured on the PCL conduits. Thereafter, the viability of cells cultured on PCL conduit significantly increased relative to cells

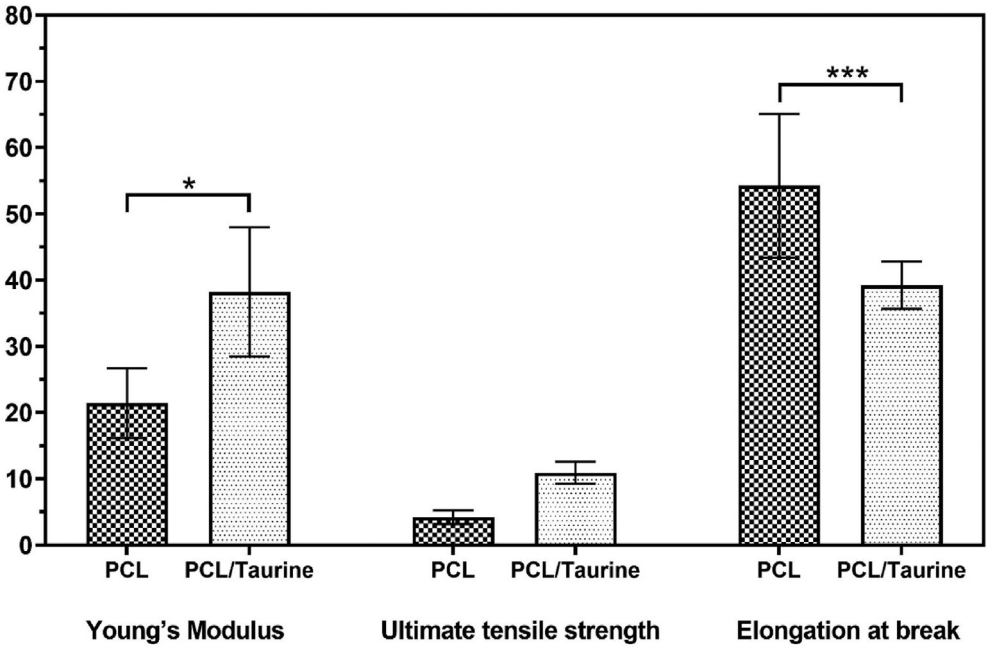

Fig. 2. Mechanical properties of the PCL and PCL/Taurine nanofiberous conduits.

cultured on the TCP group in days 3 and 5. However, the PCL/Taurine/Cell conduits showed significant viable cultured cells relative to TCP group in 1, 3, and 5 days. These results displayed that PCL and PCL/Taurine/Cell nanofibrous conduits had contained competent materials than TCP to provide proliferation and attachment of hEnSCs.

3.4. Electromyographic studies

The electrophysiological analysis was applied to evaluate the efficacy of nanofibrous fabricated conduits to determine functional neural regeneration. After 12-week post-surgery, the electrophysiological study showed that there were no significant differences between PCL/Cell and PCL/Taurine group in amplitude evaluations ($p > 0.05$) (Fig. 4). Results of amplitude measurement exhibited significant difference between Normal group and the other groups, and NC group and the other groups except PCL conduits. In the NC group, the weakest signals and muscle contractions were observed in relation to the other groups. Results showed that PCL/Taurine/Cell nanofibers conduits had almost a similar level of muscle regeneration as Autograft group, however still to reach full maturity in the normal group.

3.5. Walking-foot-print evaluation

Fig. 5 shows the average of the walking pattern of the injured foot as sciatic functional index (SFI) in all study groups at 1, 4, 8 and 12-week post-implantation. As a standard method of nerve bridging to compare with other experimental groups, Autograft group showed better practical recovery results. The Autograft the group revealed the improvement process from -90.27 ± 1.17 at the end of the 1st week to -54.43 ± 0.92 at the end of 12th week. At the 4th week, the Autograft group did not show significant difference with PCL/Taurine/Cell group and any significant difference with group PCL/Taurine/Cell and PCL/Taurine groups, at the 8th week. At 12-week post-surgery, PCL/Taurine/Cell group has the least significant difference compared to the other groups with the Autograft group. Therefore, generally speaking that PCL/Taurine/Cell group reached a similar level of functional recovery as the Autograft group. At the end of three months, SFI level had the best to the least value of Autograft group (-54.43 ± 0.92), PCL/Taurine/Cell group (-60.48 ± 1.86), PCL/Taurine group (-65.82 ± 2.37), PCL/Cell group (65.81 ± 3.69), PCL group (-74.33 ± 1.75), and NC group (-81.51 ± 2.59), respectively. There was an obvious significant difference between the PCL/Taurine, PCL/Cell, and PCL/Taurine/Cell groups with NC group ($p < 0.001$) at the 8th and 12th, suggesting the NC group make a little practical improvement compared to PCL nanofiberous conduit loaded with Taurine, Cell, and both together.

3.6. Hotplate latency analysis

Fig. 6 displays the results of the hotplate examination at the 1st, 4th, 8th, and 12th weeks' post-implantation. One week after neural surgery, the rats of all groups reply to the hotplate test by drawing back their hind feet in considered cut-off time of the WRL (12s). This delay is the time necessary to create bridging of nerve axons through a 10-mm gap, with an average regeneration rate of 0.2 mm per day [49]. Ending the 1st week, significant differences were observed between Autograft group ($p < 0.01$) and PCL/Taurine/Cell conduits ($p < 0.05$) compared to the NC group. As depicted in Fig. 6, the WRL continuingly improved during three months' recovery time. Three months' post-surgery, the WRL response showed better recovery results in Autograft ($p < 0.001$), PCL/Taurine/Cell ($p < 0.001$), PCL/Taurine ($p < 0.001$), PCL/Cell ($p < 0.001$), PCL ($p < 0.05$) conduits compared to NC group, respectively. However, no significant differences were noticed between PCL/Taurine, PCL/Cell, and PCL/Taurine/Cell conduits.

3.7. Histological studies

Fig. 7 shows the H&E and LFB staining of the regenerated nerves in different groups at three-month post-surgery. The normal structure of myelinated sciatic nerve fibers was arranged beside the other groups for comparison. The arrangement of sciatic nerve fibers in the NC group was disrupted and they had necrotic or missing axons. Also, multiple signs of nerve damage observed,

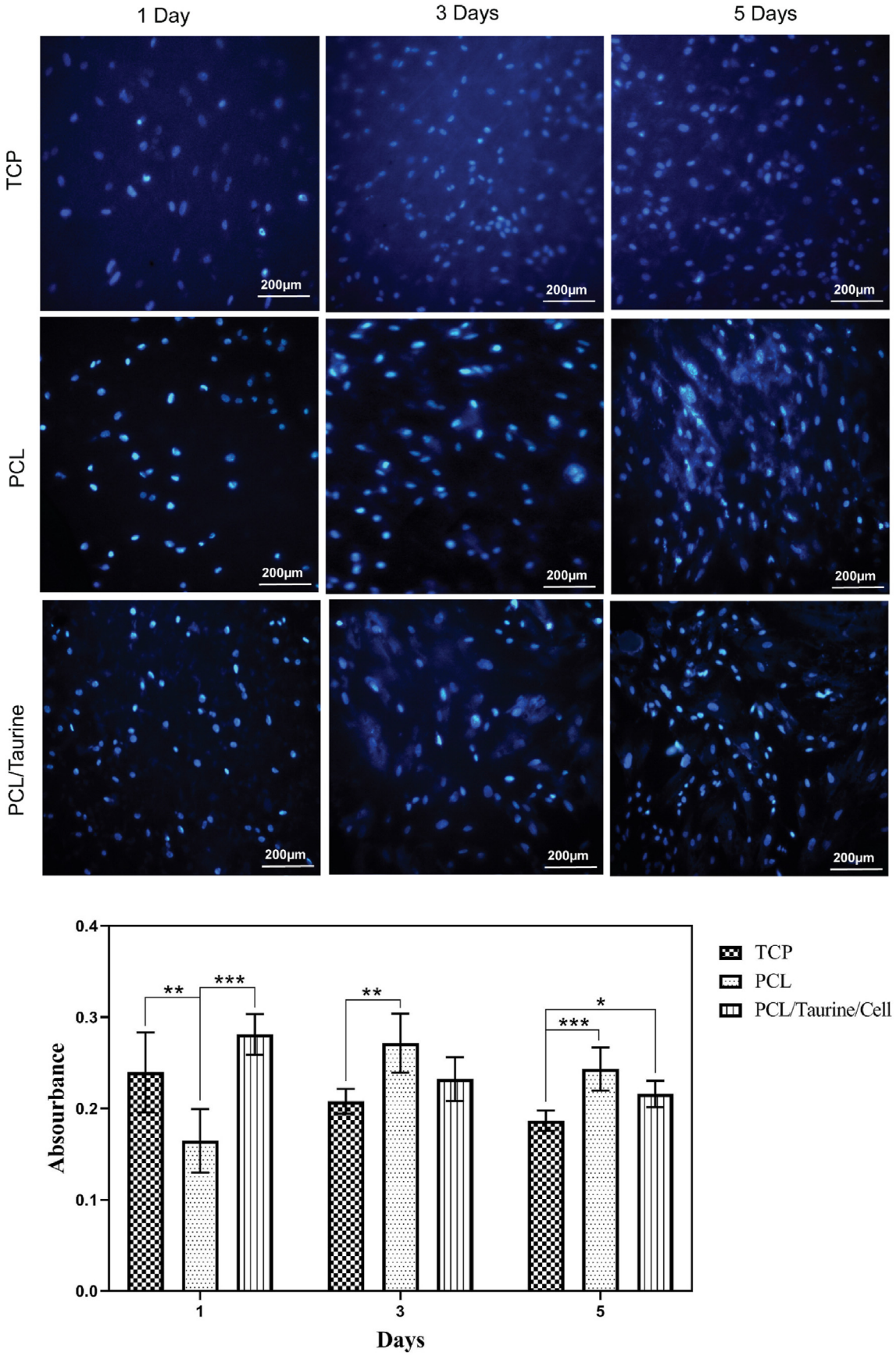

Fig. 3. Fluorescent microscopic results of hEnSCs density (Top), MTT analysis results (Below) on TCP, PCL, and PCL/Taurine conduits after 1, 3, and 5 days.

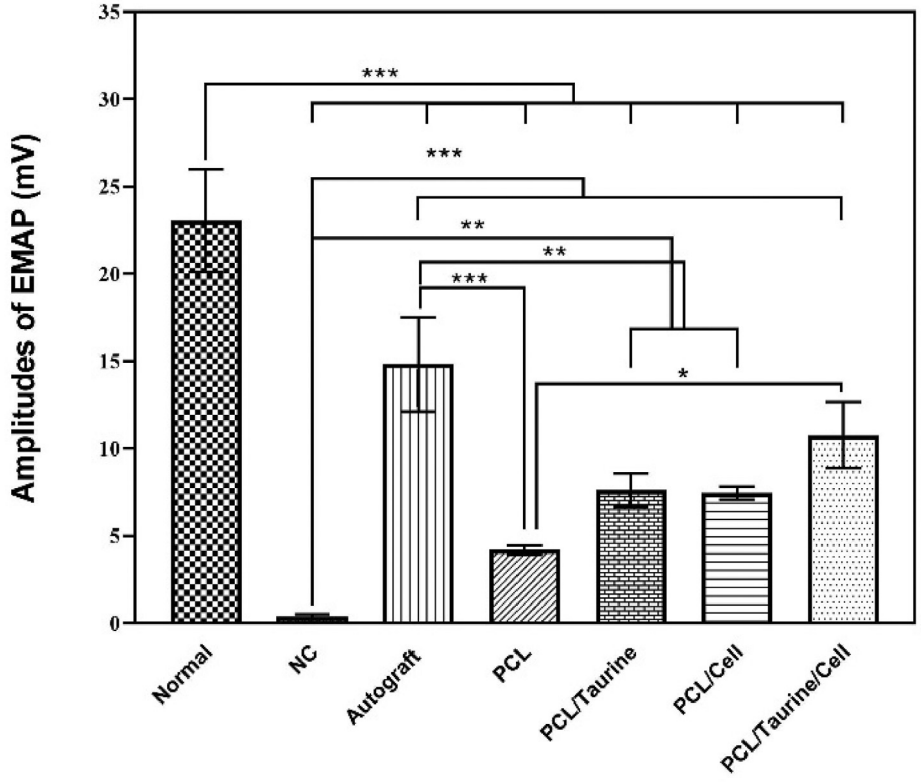

Fig. 4. Evoked muscle action potential (EMAP) of Normal, NC, Autograft, PCL/Taurine, PCL/Cell, and PCL/Taurine/Cell nanofiberous Conduit groups after 12 weeks' implantation in rats and compared with each other. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

including irregular distribution and variable thickness of myelin sheath, degeneration of fibers, axonal disintegration and notable edema of the nerve fibers. The histopathological examination of the Autograft group showed various degrees of vacuolation. However, the arrangement of the sciatic nerve fibers was acceptable, and the bridging regeneration was completed. In PCL and PCL/Taurine, fibrosis was observed in the area of the end-to-end junction and several multinucleated giant cells were seen in the grafting site. Fibrosis significantly decreases in PCL/Cell and PCL/Taurine treatment group when compared to those of NC and PCL treatment. In PCL/Taurine/Cell treatment, the nerve fibers had almost returned to the normal state, showing uniform myelin sheaths and normal axonal structure. The histopathological evaluation of this group was more similar to those in the normal group than others.

The results of the histomorphometrical study have been displayed in Fig. 8. The results demonstrated that the PCL/Taurine/Cell

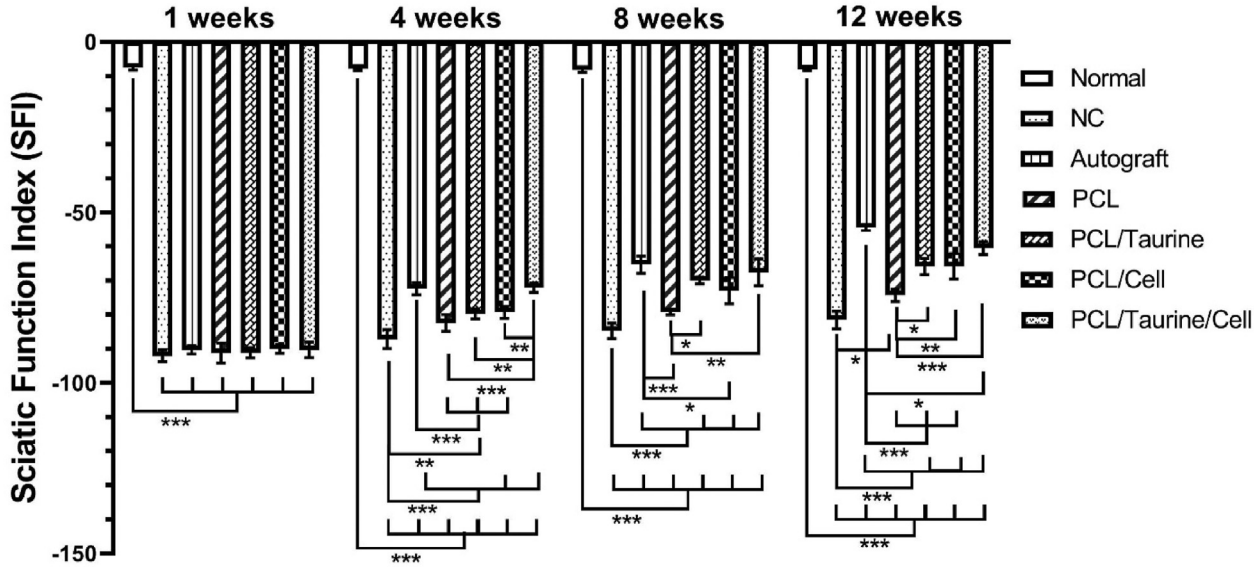

Fig. 5. The comparison of sciatic functional index (SFI) analysis at 1, 4, 8, and 12 weeks. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

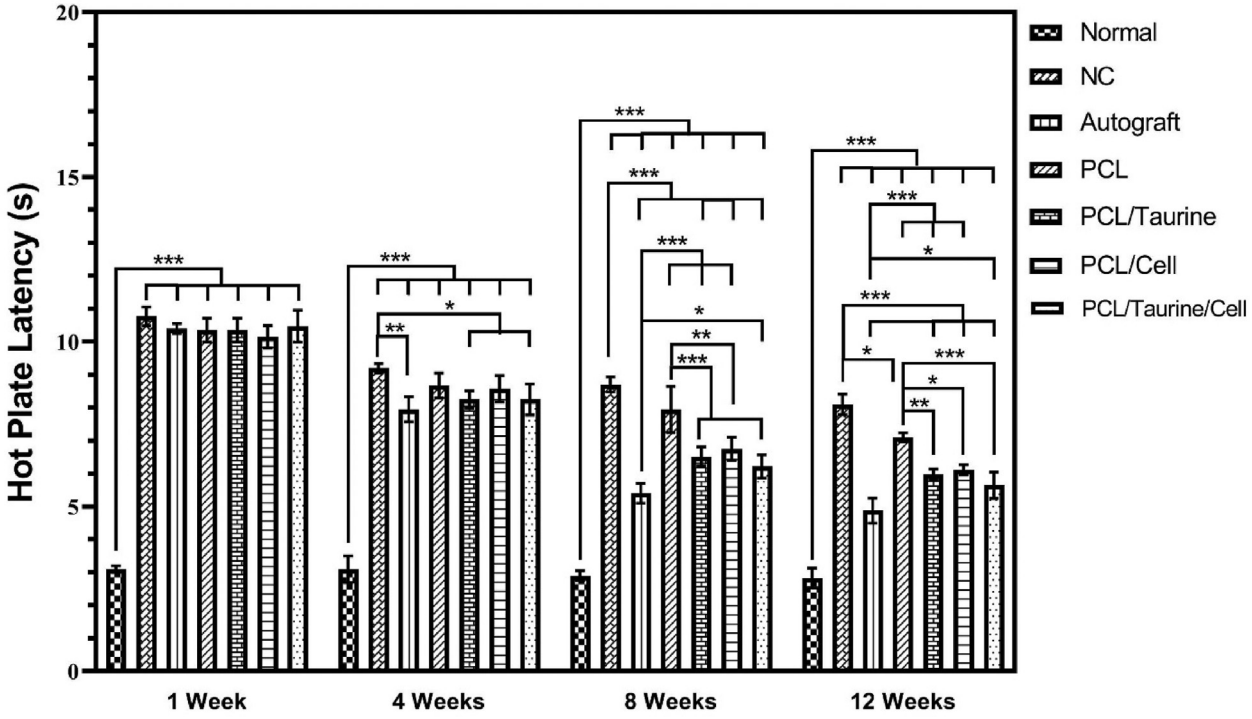

Fig. 6. Hotplate jumping response of male rats at 1, 4, 8 and 12-week post-surgery. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

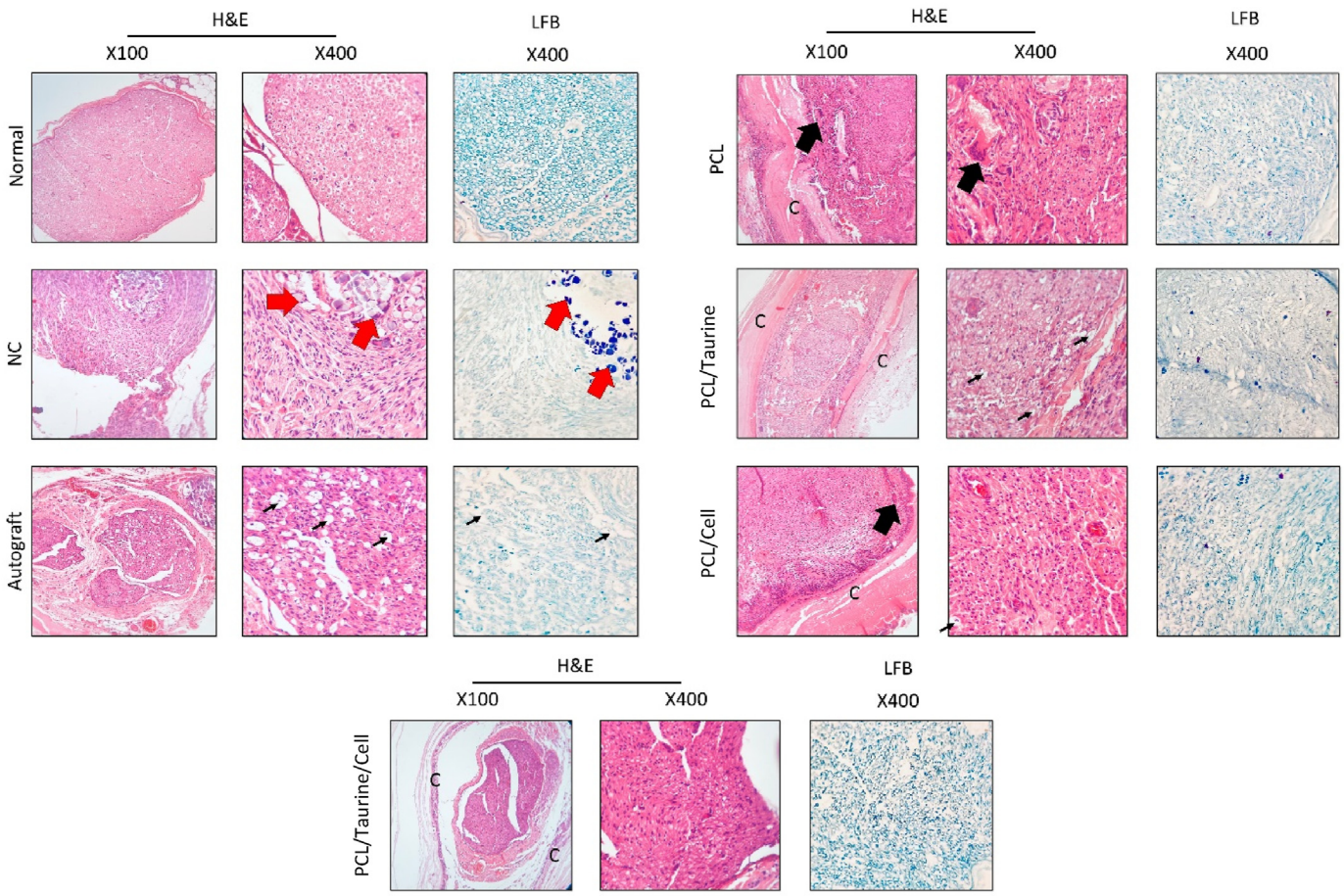

Fig. 7. Hematoxylin/Eosin and LFB staining of the sciatic nerve regeneration in study groups at 12-week post-surgery. Red arrows: necrosis, Thin black arrows: vacuolation, Thick black arrows: multinucleated giant cells.

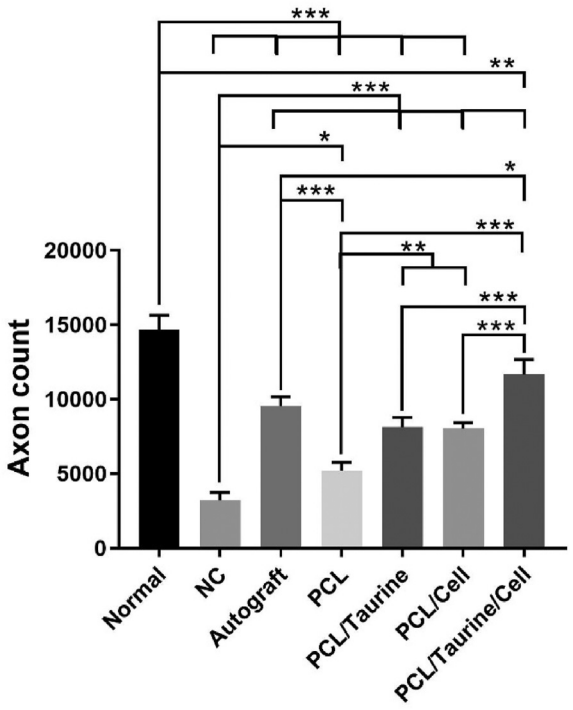

Fig. 8. Histomorphometric analysis of the axon count of sciatic nerves in study groups.

had higher mean axon counts per nerve than other treatments ($p < 0.01$). More importantly, there is a statistical difference between the PCL/Taurine/Cell and Autograft in terms of axon counts ($p < 0.05$).

4. Discussion

Since some potential for regeneration of peripheral nerve system (PNS), numerous investigations have been performed to develop procedures and improve PNS regeneration [50]. Until the last several decades, autologous nerve transplant clinically has been used in the bridging of PNS defects. As regards, minimal availability and complication of donor resection of autograft surgery remain as an inevitable drawback [51]. Therefore, several studies have been investigated on the engineered nerve guidance conduits (NGCs) as a potent procedure in the case of PNS regeneration [47]. NGCs provide a bridging guidance and an appropriate microenvironment to support nerve regeneration. Electrospinning method is a simple and functional technique to develop nanofiberous conduits promising high porosity and extensive surface area for better cell attachment and development [52]. Recent attention towards applying electrospinning for conduits production is substantially because of biological and tensile strength properties being simply controlled by changing polymer and device properties [53]. Considering the advantages of PCL polymer such as desirable biocompatibility, biodegradability, and mechanical properties, this synthetic polyester has been widely used in the field of tissue engineering [54]. On the other hand, recent investigations show that Taurine has the capability of cytoprotection in different kinds of tissues and exhibits multiple important roles including neuromodulator because of its antioxidant and anti-inflammatory properties [55,56]. Taurine also could protect against PNS damage because of its ability to controlling apoptosis [57].

In this study, we showed that PCL/Taurine nanofiberous conduits fabricated by electrospinning method and transplanted with human EnSCs are as a beneficial nerve conduit to increase nerve regeneration. In order to develop an ideal nanofiberous conduit, besides the conduit materials, incorporation of beneficial factors and stem cells providing nerve regeneration should also be evaluated. Several investigations showed that neuromodulator and anti-apoptotic factors and stem cell transplantation could play a critical role in functional nerve reconstruction after neural damage [28,31,57]. Taurine is one type of neuromodulator, which play significant roles as an anti-apoptotic, anti-oxidant, and anti-inflammatory as well as improving the velocity of nerve conduction and nerve blood flow during nerve regeneration [36]. Most investigations have depicted the important role and advantageous effects of Taurine in PNS regeneration of a nerve deficiency are in diabetes disease field [31,36,57–60]. In diabetic neuropathy, excessive accumulation of sorbitol and Taurine depletion was found to regenerate diabetic nerves [61]. Exposing the cells to high glucose reduces the expression of Taurine transporter, while treatment of aldose reductase inhibitor, converting enzyme of excessive glucose to sorbitol, increase the expression of Taurine transporter [62,63].

Previous studies have shown the useful effect of 1% Taurine dietary supplements against oxidative stress and deficiency in the peripheral nerves of diabetic rats and human Schwann cells. Obrosova et al. suggested that the antioxidant activity of Taurine was mediated in part by the antioxidant defense system of ascorbate [59]. Askwith et al. also investigated the role of Taurine in adjusting of glucose-induced nitrosative stress in Schwann cells and showed that Taurine supplementary diet restores the cell growth to normal by inhibiting the activity of glucose-induced inducible nitric oxide synthase and its regulatory signaling pathway [64].

Taurine depletion results in nerve conduction slowing, which may be associated with functional, vasculature, and metabolic deficits in diabetic neuropathy. Pop-Busui et al. showed that 1% Taurine dietary supplements for 6 weeks correct Taurine depletion in diabetic rats and prevents velocity slowing of motor nerve conduction and impaired endoneurial nutritious blood flow [58]. Taurine can also reduce diabetic neuropathic pain. Although the etiology of diabetic neuropathies is not yet fully understood, however, it may derive from neuronal hyperexcitability to changes of calcium signaling in sensory neurons. Since Taurine is known as an osmolyte and calcium modulator, thus its depletion in diabetes can lead to neuronal hyperexcitability and pain. Confirming the modulating ability of Taurine, Li et al. reported that the use of a 2% Taurine supplementary diet could affect hyperalgesia and calcium homeostasis in diabetic rats [60].

To understand the protective mechanism of Taurine, Shi et al. showed that Taurine affects the expression profile of microRNAs in peripheral nerve tissue in diabetic neuropathy. Following microRNAs tracking using target analysis software, they found that twelve microRNAs are involved in encoding genes of axon conduction, neuron production, nervous system development, and neurogenesis [31]. There is other evidence that Taurine plays an important role in the improvement of diabetic peripheral nerve neuropathy because of its ability to prevent apoptosis and the destruction of the myelin sheath of Schwann cells. According to the underlying mechanisms, taurine increased the levels of NGF expression and Akt and GSK3 phosphorylation, whereas preventing NGF activation and phosphorylation of Akt and GSK3 increased the death of high glucose-exposed RSC96 cells. These findings suggest that taurine may be a viable and effective therapy option for peripheral nerve damage since it reduced the myelin sheath degeneration of the sciatic nerve in rats via reducing SC apoptosis via NGF/Akt/GSK3 signaling pathways. In addition, other study showed that taurine might have a positive effect on axonal regrowth [57,65]. Li et al. showed that the use of

Taurine supplements improves myelin sheath destruction by preventing Schwann cell apoptosis in the sciatic nerves of diabetic rats. They found that Taurine inhibited apoptosis through the NGF/Akt/GSK3β signaling pathway [57]. PCL/Taurine conduits also significantly enhanced the mechanical properties compared with PCL conduits, which presented tensile strength improvement with taurine incorporation.

Endometrial stem cells are derived from the uterine endometrium, which is a new type of MSCs [38]. EnSCs have an easier collection, more proliferation, reduced rejection risk, and lower immunogenicity compared to other MSCs sources [39–44]. There are several studies showing that EnSCs cultured on the scaffold can improve transplantation and stimulate angiogenesis and endothelial cell branching [28,66]. There have been no reports of the use of EnSCs in PCL/Taurine nanofiberous conduits for neural tissue engineering.

Conduction velocities of motor and sensory nerves and evoked muscle action potential have been improved in PCL conduits containing hEnSCs and/or Taurine. In an electrophysiological study, EMAP measurement increases the function of test duration showing sufficient improvement of resected nerve. Extended walking results revealed that Autograft group showed higher SFI score compared to another six experimental groups in the first-week post-surgery, but after three months, PCL/Taurine/Cell conduit showed significant functional recovery as the same of Autograft group. Lack of Taurine transporter in taut−/− mice resulted to the reduced level of tissue Taurine more than 98% in heart and skeletal muscle compared to control group [67]. Reduced level of Taurine led to a decrease in action potential rate showing by electromyography in skeletal muscles of taut−/− mice [67]. Taurine is the most abundant amino acid in numerous mammalian tissues with an intracellular concentration of 20–70 mmol/kg in heart and skeletal muscles [68,69]. The most part of intracellular Taurine in mammalian skeletal muscles has an important role in excitation-contraction coupling mechanism [70]. Taurine is a contractile function modulator through regulation of ion channels, which has a crucial role in normal excitation-contraction coupling mechanism in skeletal muscles [70].

To evaluate sensory nerve recovery, a behavioral examination such as a hotplate test has been used. In 1st week, Autograft and PCL/Taurine/Cell groups showed better responses than the other groups, but three-month post-surgery, Autograft and conduits containing Taurine and hEnSCs showed better than blank conduit and NC. Heat response recovery of conduits containing Taurine and hEnSCs in 1st week shows suitable regeneration of sensory axons. Therefore, using of Taurine and cell improved nerve regeneration in the primary phase. MTT test results and DAPI staining also exhibited suitable cell survival, showing compatibility and non-toxicity of conduits for hEnSCs.

Histological evaluations showed regeneration of numerous sciatic nerves in PCL/Taurine/Cell group as the same of Autograft group and significantly better than the other groups. It could be due to the incorporation of Taurine and hEnSCs in the structure of the conduit. Histological assessment with H&E staining displayed an average repair in conduits containing Taurine or cell, while remarkable nerve regeneration and obvious bridging have seen in rats receiving PCL/Taurine/Cell conduits, confirming by images of LFB staining. Histological assay in line with other beneficial results of conduits containing Taurine and hEnSCs support bridging of the resected sciatic nerve and axon repair.

5. Conclusion

In this study, we fabricated a PCL conduit containing Taurine transplanted with hEnSCs using electrospinning method to evaluate the regeneration potential of the sciatic nerve. Functional and histological examinations showed that PCL/Taurine/Cell conduits increase the recovery rate of injured sciatic nerve compared with PCL conduit. The role of Taurine and hEnSCs have precise evaluations, however, our study showed that stem cell therapy and nerve-regeneration substances introduce new therapies in neuropathies.

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Author contributions

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Declaration of competing interest

The authors declare no competing interests.

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