

Evaluation of regular treadmill exercise plan combined with electrospun scaffolds loaded with green tea extract and bone marrow-derived mesenchymal stem cells for spinal cord injury repair in a rat model

Research Article

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Abstract: Spinal cord injuries (SCIs) continue to pose a significant challenge in regenerative medicine and neuroscience, inflicting severe consequences on affected individuals. This study aims to evaluate the therapeutic efficacy of sports medicine along with gelatin electrospun scaffolds loaded with green tea extract (GTE) and seeded with bone marrow-derived mesenchymal stem cells (BM-MSCs) for SCI repair in a rat model. The electrospun scaffolds were fabricated from GTE-loaded polycaprolactone/gelatin polymeric solution. BM-MSCs were then seeded onto the scaffolds, which were implanted into rat models of SCI. The animals then underwent daily treadmill exercise. Histological analysis and functional assessments were conducted to evaluate the extent of spinal cord repair and functional recovery. The study showed that the hybrid treatment strategy augmented SCI "repair" and restored tissue function. Enzyme-linked immunosorbent assay results showed that our method upregulated tissue expression levels of brain-derived neurotrophic factor and nerve growth factor and decreased the tissue expression levels of tumor necrosis factor-alpha and interleukin-1 beta.

Keywords: *Spinal cord injury • Sport medicine • Drug delivery*

1. Introduction

Spinal cord injuries (SCIs) continue to pose a significant challenge in regenerative medicine and neuroscience, inflicting severe consequences on affected individuals. Despite advances in medical technology and treatment methods, there is a pressing need for innovative therapeutic approaches to facilitate functional recovery and neural tissue regeneration following SCI [1]. In recent years, tissue engineering strategies have received

increasing interest as promising options for spinal cord repair [2].

This study proposes an original and comprehensive therapeutic approach that combines regular treadmill exercise with the utilization of electrospun scaffolds containing green tea extract (GTE) and bone marrow-derived mesenchymal stem cells (BM-MSCs) for SCI repair. This multifaceted strategy aims to capitalize on the synergistic potential of each component to address the intricate challenges associated with spinal cord regeneration. Regular treadmill exercise has demonstrated considerable promise as an effective rehabilitation method for SCI patients. Exercise-based therapies are known to enhance functional

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recovery, stimulate neuroplasticity, and improve locomotor outcomes [3]. Engaging in regular treadmill exercise may lead to enhanced motor function, increased sensory feedback, and decreased muscle atrophy, ultimately contributing to an improved quality of life [4].

Incorporating electrospun scaffolds into the treatment plan represents a significant advancement in tissue engineering. Electrospinning is a versatile technique that enables the creation of nanofibrous structures resembling the native extracellular matrix. These electrospun scaffolds offer a conducive microenvironment for cell adhesion, migration, and proliferation, thereby promoting tissue regeneration at the injury site [5]. Augmenting the scaffolds with GTE further enhances their regenerative potential, as green tea polyphenols possess neuroprotective, anti-inflammatory, and antioxidant properties [6]. Furthermore, the use of BM-MSCs holds great promise for SCI repair due to their immunomodulatory characteristics and potential to differentiate into neural lineages. These multipotent cells can interact with the host tissue, attenuating inflammatory responses and releasing various trophic factors that facilitate neural tissue repair and axonal regrowth [7]. Integrating these elements into a comprehensive therapeutic strategy presents a multifaceted approach to addressing the complex pathophysiology of SCI. This study aims to explore the combined impact of regular treadmill exercise, electrospun scaffolds with GTE, and BM-MSCs in promoting functional recovery, axonal regeneration, and neural tissue repair using a rat model of SCI.

2. Methods and materials

2.1 Preparation of polycaprolactone (PCL)/gelatin scaffolds

First, PCL (Mn80000, Sigma Aldrich, USA) and gelatin (Type A, Sigma Aldrich, USA) were dissolved in acetic acid at 14 w/w% for 6 h. The weight ratio of PCL to gelatin was 80:20. Then, GTE (ethanolic extract, Iran Pharma Co., Ltd.) was added to PCL/gelatin solution at 3 v/v% and mixed for 6 h. Finally, the polymeric solution was loaded into a disposable syringe connected to a fine-gauged metal needle. A positive high voltage (18 kV) was applied to the needle tip, leading to the formation of polymeric jets. The polymer feeding rate was set at 0.5 ml/h, and the distance between the collector and the needle was set at 15–16 cm. The turning rate of the mandrel ranged between 60 and 700 rpm. The scaffolds loaded with CTE and those

without it were named GTEPCLGEL and PCLGEL scaffolds, respectively.

2.2 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The viability of PC-12 cells cultured on GTEPCLGEL and PCLGEL scaffolds was assessed using the MTT assay. PC-12 cells were cultured in a growth medium consisting of RPMI (Roswell Park Memorial Institute) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin (Sigma Aldrich, USA). The cells were maintained in a humidified incubator at 37°C with 5% CO₂ to facilitate optimal cell growth. To assess cell viability on the scaffolds, the PC-12 cells were trypsinized and counted using a Coulter counter. After reaching the desired confluence, the cells were detached from the culture dish and seeded onto the GTEPCLGEL and PCLGEL scaffolds at 10,000 cells per scaffold in 96-well plates. Then, the cells were cultured on the scaffolds for 5 days. The culture medium was changed every 48 h. On days 1, 3, and 5, the MTT assay was performed to assess cell viability. MTT solution (0.5 mg/ml) was added to each well, and the plates were incubated for an additional 4 h to allow the MTT to be converted into formazan crystals by viable cells. Next, MTT solution was carefully removed, and 200 µl dimethyl sulfoxide was added to dissolve the formazan crystals. The plates were gently agitated to ensure complete dissolution. Finally, the absorbance of the samples was measured at 570 nm.

2.3 Cell viability assay under oxidative stress

The viability of PC-12 cells cultured on GTEPCLGEL and PCLGEL scaffolds under oxidative stress was assessed using the MTT assay. Briefly, cells were seeded onto the scaffolds at 7,000 cells/scaffold density and cultured for 48 h. Then, culture media was supplemented with 1% H₂O₂, and cells were further incubated for 1 h. Finally, cell viability was assessed using the MTT assay as described in Sections 2.2.

2.4 DPPH assay

The radical scavenging potential of GTEPCLGEL and PCLGEL scaffolds was assessed using the 2,2-diphenyl-1-

picrylhydrazyl (DPPH) assay. For the DPPH assay, stock solutions of the GTEPCLGEL and PCLGEL scaffolds were prepared by grinding the scaffolds into fine powders and preparing stock solutions of each sample with a concentration ranging from 50 to 400 µg/ml. DPPH reagent was prepared by dissolving DPPH in absolute ethanol to achieve a concentration of 0.1 mM. In parallel, a standard curve was prepared using ascorbic acid as a positive control, with concentrations ranging from 50 to 400 µg/ml. Next, 180 µl of the diluted scaffold solutions and ascorbic acid standard solutions were mixed with 20 µl of the DPPH reagent in a 96-well microplate. The microplate was then incubated in the dark at room temperature for 30 min. After incubation, the absorbance of each well was measured at 517 nm using a microplate reader. The percentage of DPPH radical scavenging was calculated using the following formula:

Percentage of DPPH Radical Scavenging

$$= \frac{[(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}] \times 100.}{}$$

The data obtained were analyzed using appropriate statistical software to determine the radical scavenging potential of the GTEPCLGEL and PCLGEL scaffolds.

2.5 Biodegradation rate measurement

The degradation rate of GTEPCLGEL and PCLGEL scaffolds was evaluated through an *in vitro* degradation study. To initiate the degradation study, both types of scaffolds were cut into uniform disc-shaped samples (150–170 mg) with predetermined dimensions. The samples were then immersed in a solution of phosphate-buffered saline (PBS) to mimic physiological conditions, with a constant temperature of 37°C simulating the body's internal environment. At specific time points (1 week, 2 weeks, 4 weeks, and 8 weeks), the samples were taken out of the PBS solution, gently washed with deionized water, and carefully dried to eliminate any excess liquid. The initial weight (W_0) of each sample was recorded before being placed in the PBS solution. To determine the extent of degradation, the samples were subsequently dried at 60°C until reaching a constant weight (W_t). The percentage of weight loss was then calculated using the formula:

$$\text{Percentage of weight loss} = [(W_0 - W_t) / W_0] \times 100.$$

2.6 Ultimate tensile strength measurement

To conduct the tensile strength assessment, rectangular strip-shaped specimens were prepared from both GTEPCLGEL and PCLGEL scaffolds, ensuring a standardized size ($5 \times 2 \text{ cm}^2$). Precise cutting tools were employed to maintain uniformity in the size and shape of the samples. Subsequently, the specimens were securely affixed to the grips of a universal testing machine, which was equipped with a load cell capable of precisely measuring the applied force. The gauge length, representing the distance between the grips, was set to an appropriate value to ensure an accurate measurement of tensile strength. The tensile testing was carried out at a constant crosshead speed (1 mm/min), subjecting the samples to gradual uniaxial tension until failure occurred. During the testing process, the load cell recorded the applied force, and the corresponding elongation of the samples was measured using an extensometer. The ultimate tensile strength was determined by dividing the maximum force observed at the point of failure by the original cross-sectional area of the samples. The original cross-sectional area was calculated by measuring the width and thickness (200–250 µm) of the specimens using a digital caliper prior to initiating the testing procedure.

2.7 Hemocompatibility assay

The hemocompatibility of GTEPCLGEL and PCLGEL scaffolds was assessed using rat-derived whole blood, which was anticoagulated and diluted with normal saline. The samples were incubated with 200 µl of the blood mixture at 37°C for 60 min, followed by centrifugation at 1,500 rpm for 10 min. The absorbance of the resulting supernatant was then measured at 545 nm using a Multi-Mode Microplate Reader (BioTek Synergy 2). For controls, whole blood diluted in normal saline served as the negative control, while whole blood loaded with triton x-100 was used as the positive control.

2.8 Anti-inflammatory assay

In this research, we examined the anti-inflammatory effects of GTEPCLGEL and PCLGEL scaffolds using RAW 264.7 macrophage cells, a commonly utilized mouse macrophage cell line. RAW 264.7 macrophage cells were cultured in complete Dulbecco's modified eagle medium

supplemented with 10% FBS and 1% penicillin–streptomycin at a temperature of 37°C with 5% CO₂. To trigger an inflammatory response, the RAW cells were exposed to lipopolysaccharide at a concentration of 1 µg/ml, while the non-stimulated cells were utilized as the control group. Subsequently, the activated RAW 264.7 macrophage cells were seeded separately onto the GTEPCLGEL and PCLGEL scaffolds at a density of 1×10^5 cells per scaffold. The scaffolds were then placed in 24-well plates and allowed to incubate for 24 h. Following the incubation period, the culture supernatants were collected for analysis, and the levels of pro-inflammatory cytokines, including interleukin-6, interleukin-1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α), were quantified using murine-specific enzyme-linked immunosorbent assay (ELISA) kits (Abcam, USA).

2.9 Scanning electron microscopy (SEM) imaging

The microstructure of GTEPCLGEL and PCLGEL scaffolds was investigated using SEM imaging. Briefly, the scaffolds were cut and coated with gold for 252 s followed by imaging under 26 kV accelerating high voltage.

2.10 *In vivo* study

Study was approved by Guangzhou Sport University and conducted according to the university Guidelines. The animal studies were performed on male Wistar rats according to the university guidelines, and the study was approved by the ethics committee. The surgical procedure was initiated by intraperitoneal injection of ketamine and xylazine (100 and 10 mg/kg, respectively). After inducing deep anesthesia, the skin on the surgery site was shaved and disinfected with betadine solution. Then, a laminectomy was surgically established at the T10 vertebra, involving the removal of the vertebral bone. Subsequently, an incision was made in the protective dura mater covering the spinal cord. Following this, a controlled compression of the dorsal spinal column was executed. This compression procedure was meticulously carried out using precision jeweler's forceps. The forceps' tips were introduced to a depth of 0.5 mm, with the tips spaced apart by 0.5 mm, and then firmly closed for a duration of 10 s. This precise technique ensured a consistent and controlled spinal column compression, simulating the injury process under controlled conditions. The animals were then randomly divided into

five groups as follows: (1) GTEPCLGELBM-MSCsPH group, in which the scaffolds were sterilized and seeded with 50,000 BM-MSCs per cm² and cultured for 48 h before implantation. The cell-seeded scaffolds were placed on the injury site, and the animals received 15 min daily treadmill exercise after week 2. (2) GTEPCLGELBM-MSCs group in which the animals received the same treatment as group 1, but without the treadmill exercise. (3) PCLG-ELPH group in which the PCLGEL scaffolds were sterilized and implanted at the site of injury. From week 2 onward, the animals received 15 min daily treadmill exercise until the end of the study. (4) PCLGEL group in which the animals received the same treatment as group 3, but without the treadmill exercise. (5) Negative control group in which the animals received no treatment after the injury. The animals were kept for 8 weeks and then sacrificed using ketamine overdose. Finally, the spinal cord tissues were harvested and used for histological evaluations. Briefly, histological evaluations were performed using the widely accepted hematoxylin and eosin (H&E) staining method. Briefly, the paraffin-embedded tissues were meticulously sectioned into 5 µm-thick slices using a microtome. These sections were then carefully mounted onto glass slides, each slide containing multiple tissue sections for comprehensive analysis. The H&E staining process involved immersing the tissue sections in hematoxylin solution to selectively stain cell nuclei shades of blue, followed by a counterstaining step using eosin solution to highlight cytoplasmic structures in shades of pink. The stained tissue sections were then meticulously dehydrated, cleared, and cover-slipped for microscopy. Under a high-powered microscope, the stained tissue sections were meticulously examined by experienced histopathologists who were blinded to the study. Cellular morphology, tissue architecture, and any potential pathological changes were meticulously assessed and documented.

2.11 Functional assessment results

Motor function recovery was systematically assessed within each experimental group at specific time intervals: weeks 2, 4, and 8 subsequent to the initial intervention. This intentional selection of assessment time points aimed to encompass both immediate and extended effects, contributing to a thorough comprehension of the plausible treatment outcomes. The method of motor function assessment involved the meticulous utilization of the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale, recognized for its credibility and sensitivity. This well-

established technique entailed placing the rats within an open field setting, enabling their unhindered movement and demonstration of locomotor capabilities. During the designated assessment periods, proficient evaluators meticulously observed and documented the hind limb motions and coordination of the rats. The BBB scale, which spanned from 0 (indicating total paralysis) to 21 (representing typical locomotion), facilitated the precise quantification of the levels of motor function recovery. For each assessment session, the rats from every group were individually introduced to the open field, and their locomotion behaviors were carefully recorded. This systematic methodology aimed to eliminate potential bias and ensure uniformity in the evaluation procedure. The detailed observation was extended across a specific time-frame, capturing an extensive overview of the rats' motor behavior throughout diverse locomotion attempts.

For the thorough evaluation of sensory function recuperation, a specialized assessment method known as the hot plate latency test was meticulously put into practice. This test was deliberately chosen owing to its specialized ability to precisely gauge sensory reactivity and thermal sensitivity in the rats that were exposed to the experimental conditions on week 8 after the surgery. In the carefully orchestrated execution of this test, each individual rat was gently positioned on a heated surface (56°C) of the designated hot plate equipment. The temperature of this surface was meticulously regulated to maintain a uniform and safe thermal stimulus. The rats' responses to this thermal stimulus were observed with painstaking attention, specifically focusing on their behaviors indicative of discomfort or reaction to the thermal sensation. Within this test, a crucial parameter involved recording the time interval spanning from the instant the rat was placed on the heated surface of the hot plate to the distinct manifestation of a clear behavioral response. These responses often encompassed actions such as paw licking, shaking, or lifting. This temporal duration, referred to as the

latency period, was methodically measured to quantitatively assess the degree of sensory function recovery and the extent of responsiveness to the thermal stimulus. In order to ensure precise and consistent outcomes, each rat underwent the hot plate latency test on multiple occasions at predetermined assessment junctures.

2.12 ELISA assay

At the culmination of the 8-week period, ELISA was performed in order to measure the tissue concentrations of brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), TNF- α , and IL-6. These biomolecules, renowned for their significance in neurological responses, were chosen as key markers to unravel the effects of the experimental interventions on a molecular level. Within this analytical process, specialized ELISA kits (Abcam, USA) specifically designed for each target biomolecule were utilized according to the instructions provided by the manufacturer.

2.13 Statistical analysis

Data were analyzed using GraphPad Prism version 5 by employing Student's *t*-test and one-way ANOVA methods.

3. Results and discussion

3.1 MTT assay results

The outcomes illustrated in Figure 1 depict a dynamic pattern of cell viability across the experimental conditions. Initially, on day 1, PC-12 cells cultivated on the conventional tissue culture plate exhibited notably higher

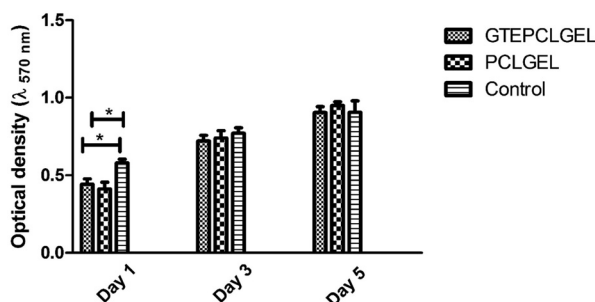


Figure 1. MTT assay with PC-12 cultured on GTEPCLGEL and PCLGEL scaffolds. * shows *p*-value < 0.05.

viability compared to cells cultured on both GTEPCLGEL and PCLGEL scaffolds. This discrepancy was statistically significant, with a p -value of less than 0.05, signifying a robust contrast in viability between these groups. As the study progressed to days 3 and 5, a noteworthy shift in the results emerged. At these later time points, statistical analysis indicated no substantial variance in cell viability among the GTEPCLGEL, PCLGEL, and control groups. The calculated p -values exceeded 0.05, indicating a lack of statistically significant distinctions among these groups. These trends underscore the dynamic interplay between substrate type and cellular behavior, emphasizing the significance of longitudinal observations in viability assessment. The convergence of cell viabilities suggests the potential adaptability of PC-12 cells to both GTEPCLGEL and PCLGEL scaffolds, indicating the scaffolds' ability to support cell growth and proliferation over the experimental timeframe [8].

3.2 MTT assay under oxidative stress results

Results (Figure 2) showed that under oxidative stress, GTE-PCLGEL scaffolds provided significantly higher protection against oxidative stress among experimental groups, p -value <0.05. Statistically, no significant difference was found between PCLGEL and control groups, p -value >0.05. Indeed, GTE's polyphenols possess anti-inflammatory properties that play a pivotal role in mitigating inflammatory responses, thereby reducing the generation of reactive oxygen species (ROS). Additionally, the polyphenols found in GTE have the ability to directly scavenge ROS and safeguard DNA from oxidative damage [9]. This protection of genetic material is crucial in maintaining its integrity and preventing mutations that can arise due to oxidative stress. Moreover, green tea polyphenols exhibit an impact on cellular signaling pathways implicated in oxidative stress responses, notably the nuclear factor

erythroid 2-related factor 2 (Nrf2) pathway [10]. Activation of this pathway instigates heightened expression of antioxidant enzymes and other protective proteins. Furthermore, green tea's polyphenols counteract lipid peroxidation, the oxidative disintegration of lipids that leads to harm in cellular membranes. This inhibition of lipid peroxidation plays a vital role in upholding the integrity of cell membranes, further contributing to the overall protective effects of GTE against oxidative stress [11].

3.3 DPPH assay results

Results (Figure 3) showed that at all concentrations, GTEPCLGEL scaffolds had significantly higher radical scavenging activity than PCLGEL scaffolds, p -value <0.05. Ascorbic acid had a significantly higher percentage of radical scavenging activity than both scaffolds, p -value <0.05. The capacity of GTE to counteract DPPH free radicals rests on its robust antioxidant attributes, primarily stemming from its rich content of polyphenolic compounds, notably catechins. DPPH free radicals serve as stable indicators for gauging the antioxidant prowess of substances or extracts. The process can be elucidated as follows: The polyphenols, particularly epigallocatechin gallate (EGCG), harbored within GTE harbor unpaired electrons within their molecular structure. This electron excess renders them apt for donation to the DPPH free radicals, effectively nullifying their reactivity. The neutralization of DPPH radicals is effectuated through a radical scavenging process. As these radicals accept electrons from green tea's polyphenols, they undergo a transformation into stabilized molecules bereft of unpaired electrons [12]. This results in their loss of radical attributes and subsequent diminished reactivity. This process is observable in the fading of the initial purple color associated with DPPH radicals. The progressive reduction in color intensity serves as a visual indicator of the radical quenching effect. The hydrogen atom transfer mechanism

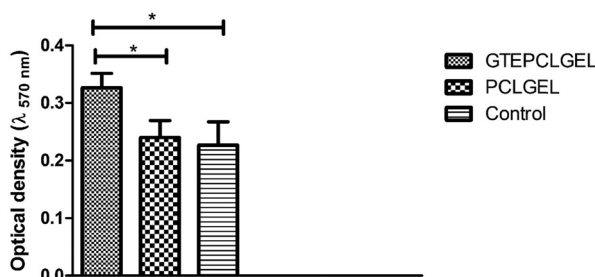


Figure 2. MTT assay under 1% H₂O₂ with PC-12 cells cultured with GTEPCLGEL and PCLGEL scaffolds. * shows p -value <0.05.

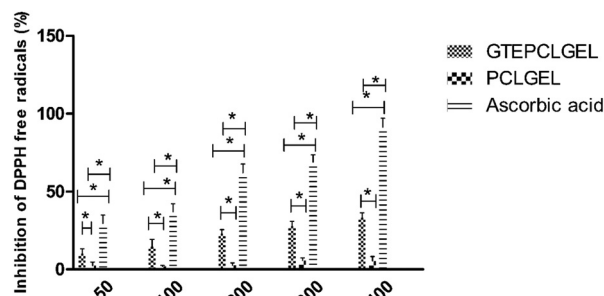


Figure 3. DPPH assay with GTEPCLGEL and PCLGEL scaffolds compared with ascorbic acid as the control group. * shows p -value < 0.05 .

serves as a principal modality in the neutralization of DPPH radicals. The polyphenolic constituents within GTE function as donors of hydrogen atoms to the radicals, thereby conferring stability and impeding the propagation of oxidative reactions [13].

3.4 Ultimate tensile strength measurement results

Results (Figure 4) showed that GTEPCLGEL and PCLGEL scaffolds had 4.19 ± 0.15 MPa and 4.59 ± 1.063 MPa of ultimate tensile strength, respectively. This high tensile strength ensures the easy applicability of the developed delivery system. The high tensile properties of GTE-loaded and GTE-free scaffolds could be attributed to the presence of PCL in the scaffolds. The elevated tensile strength observed in electrospun PCL/gelatin scaffolds can be attributed to a convergence of factors that encompass the inherent properties of these materials and the intricacies of the electrospinning technique itself. First, the mechanical attributes of both PCL and gelatin play a pivotal role. PCL, being a synthetic biodegradable polymer, boasts impressive tensile strength due to the elongated and pliable nature of its polymer chains [14]. Conversely, gelatin, a natural protein derived from collagen, contributes to the

scaffold's strength owing to its fibrous structure. The amalgamation of these two materials results in a composite that harnesses the strengths of both, fostering durability through PCL and biocompatibility via gelatin [15]. The orientation of electrospun fibers is paramount. The electrostatic forces inherent in the electrospinning process led to a pronounced alignment of fibers. This alignment significantly bolsters the scaffold's ability to bear loads, culminating in heightened tensile strength [8].

3.5 Hemocompatibility assay results

Results (Figure 5) showed that GTEPCLGEL and PCLGEL scaffolds had significantly higher hemolysis than normal saline solution, p -value < 0.05 . Triton x-100 had significantly higher hemolysis than other groups, p -value < 0.05 . Hemolysis is the rupture of red blood cells, and in the context of PCL/gelatin scaffolds, their interaction with blood components can trigger this response. The hemolysis mechanism involves the adsorption of blood proteins on the scaffold's surface, triggering complement activation and the formation of the membrane attack complex [8]. However, there are little data available in the literature to substantiate this theory.

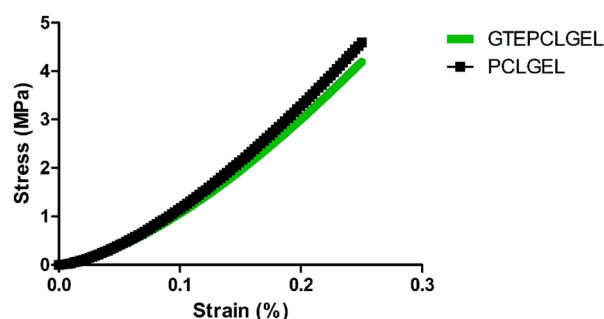


Figure 4. Stress-strain curve for GTEPCLGEL and PCLGEL scaffolds.

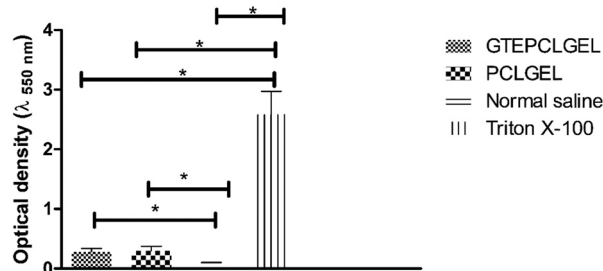


Figure 5. Hemolysis activity of GTEPCLGEL and PCLGEL scaffolds compared with normal saline and triton x-100 as the negative and positive control groups, respectively. * shows p -value <0.05 .

3.6 Anti-inflammatory assay results

Results (Figure 6) showed that the GTEPCLGEL group had significantly lower concentrations of TNF- α , IL-6, and IL-1 β than PCLGEL and control groups, p -value <0.05 . Statistically, no significant difference was found between PCLGEL and control groups, p -value >0.05 . Therefore, we can assume that GTE has imparted immunomodulatory properties to the scaffolds. GTE's anti-inflammatory activity is attributed to its rich content of polyphenolic compounds, primarily catechins such as EGCG. These polyphenols possess potent antioxidant properties that counteract ROS and reactive nitrogen species, thereby reducing oxidative stress-induced inflammation. GTE also modulates signaling pathways involved in inflammation, including nuclear factor-kappa B (NF- κ B) and mitogen-activated protein kinases. EGCG inhibits the activation of NF- κ B, a key regulator of pro-inflammatory gene expression, by blocking its translocation into the nucleus [16]. Additionally, GTE promotes the production of anti-inflammatory cytokines while suppressing pro-inflammatory cytokines. Moreover, it attenuates the activity of enzymes like cyclooxygenase and lipoxygenase, which are involved in prostaglandin and leukotriene synthesis, respectively [6].

3.7 SEM imaging results

The results presented in Figure 7 reveal the fibrous nature of both GTEPCLGEL and PCLGEL scaffolds, characterized by their intricate web-like structure. The fibers exhibited a uniform and smooth surface, preserving their structural integrity without signs of disintegration. Notably, an analysis of fiber size indicated a noteworthy effect on the incorporation of GTE into the PCLGEL scaffold matrix. Specifically, the mean fiber size experienced a significant increase, growing from 753.79 ± 353.92 to 890.73 ± 217.73 nm as a consequence of GTE integration. The electrospinning process facilitates the creation of nanofibers with high surface area, enabling enhanced cell-material interactions. These fibers can be tailored to mimic the size scale of native neural fibers, aiding in guiding axonal growth and promoting neural network formation [17]. Furthermore, the alignment of fibers can be controlled, mimicking the longitudinally oriented neural pathways within the spinal cord. Electrospun scaffolds can also incorporate bioactive molecules, such as growth factors or nerve guidance cues, fostering neural differentiation and axonal outgrowth. The modularity of electrospinning allows for the incorporation of multiple components,

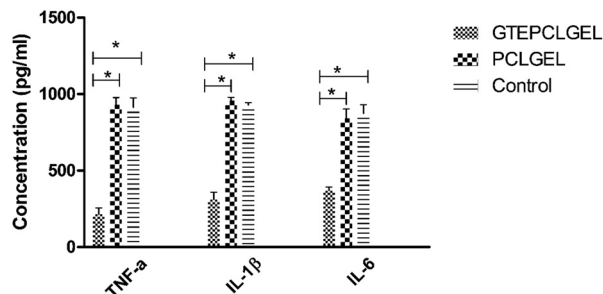


Figure 6. Anti-inflammatory assay with GTEPCLGEL and PCLGEL scaffolds compared with the macrophage cells cultured on the tissue culture plate as the control group. * shows p -value <0.05 .

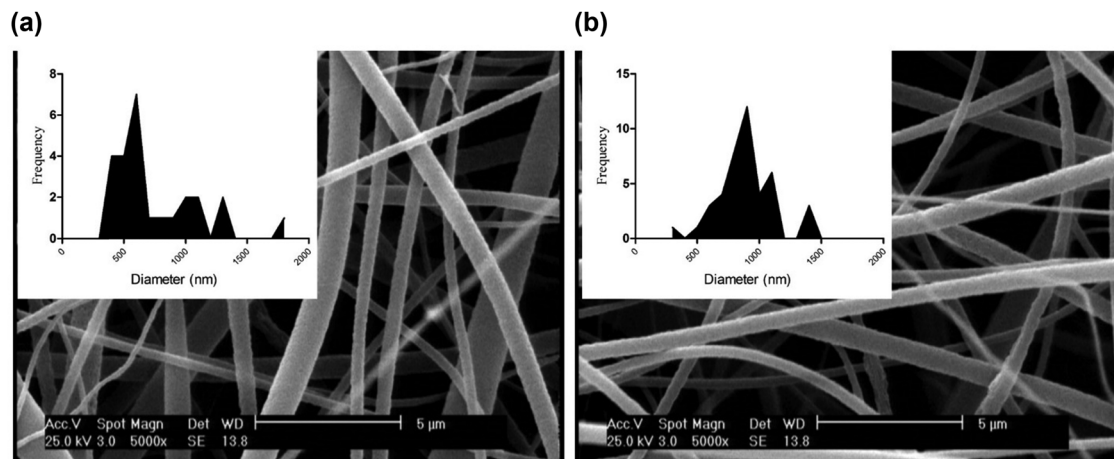


Figure 7. SEM images and size distribution of (a) GTEPCLGEL and (b) PCLGEL scaffolds.

optimizing mechanical support, cellular response, and bioactivity in spinal cord tissue engineering applications [18].

3.8 *In vivo* study

Histological assessments (Figure 8) provided significant insights into the regenerative effects of various treatments on spinal cord tissues. In the GTEPCLGELBM-MSCsPH group, an extraordinary and notable restorative impact was evident. Within this group, there was a remarkable improvement in the neural tissue architecture. This was discerned through the presence of well-defined gray and white matter regions, indicating a profound cellular and structural regeneration. The heightened neuronal density further supported the notion of cellular proliferation and maturation, signifying the potential for functional recovery. This amalgamation of bioactive components contributed to a synergistic healing trajectory, which positively influenced the overall recovery process. Similarly, the GTEPCLGELBM-MSCs group exhibited encouraging outcomes in terms of neural tissue regeneration. Histological cross-sections of spinal cord tissues demonstrated a notable surge in cellular density, accompanied by evident axonal growth. However, concurrent with these favorable aspects, subtle indications of edema, vacuolation, and axonal disintegration were still discernible. In contrast, the histological evaluation of the PCLGEL and PCLGELPHS groups revealed perceivable but modest advancements in tissue healing. Cellular repopulation was evident, although the extent of the regenerative response remained relatively restricted. Tissue disruption and structural anomalies, including signs of edema, vacuolation, and infiltration

of pro-inflammatory cells, were still noticeable. These observations underscored the complexity of spinal cord tissue regeneration and the challenges posed by the intricate microenvironment of the spinal cord. The negative control group, serving as a baseline, displayed minimal signs of tissue healing. Cellular repopulation was limited, and the preservation of tissue architecture was modest at best. The distinct lack of cellular organization and minimal axonal regeneration accentuated the challenges associated with spinal cord tissue recovery in the absence of therapeutic interventions. In conclusion, the histological evaluations unveiled a spectrum of tissue responses across the treatment groups. The GTEPCLGELBM-MSCsPH and GTEPCLGELBM-MSC treatments demonstrated commendable restorative effects with promising indications of cellular proliferation, neuronal maturation, and axonal growth. However, histopathological irregularities like edema and axonal disintegration in the GTEPCLGELBM-MSC group emphasized the higher healing activity of the GTEPCLGELBM-MSCsPH group. The PCLGEL and PCLGELPHS groups displayed negligible healing, while the negative control group underscored the low regeneration of natural healing processes in the spinal cord.

BBB assay results (Figure 9) showed that on weeks 2, 4, and 8, the GTEPCLGELBM-MSCsPH group had significantly higher BBB scores than PCLGELPH, PCLGEL, and negative control groups, p -value < 0.05 . On weeks 4 and 8, the GTEPCLGELBM-MSCsPH group had significantly higher BBB scores than the GTEPCLGELBM-MSCs group, p -value < 0.05 .

Sensory function assessment results (Figure 10) showed that GTEPCLGELBM-MSC and GTEPCLGELBM-MSCsPH groups had significantly lower hot plate latency time than other

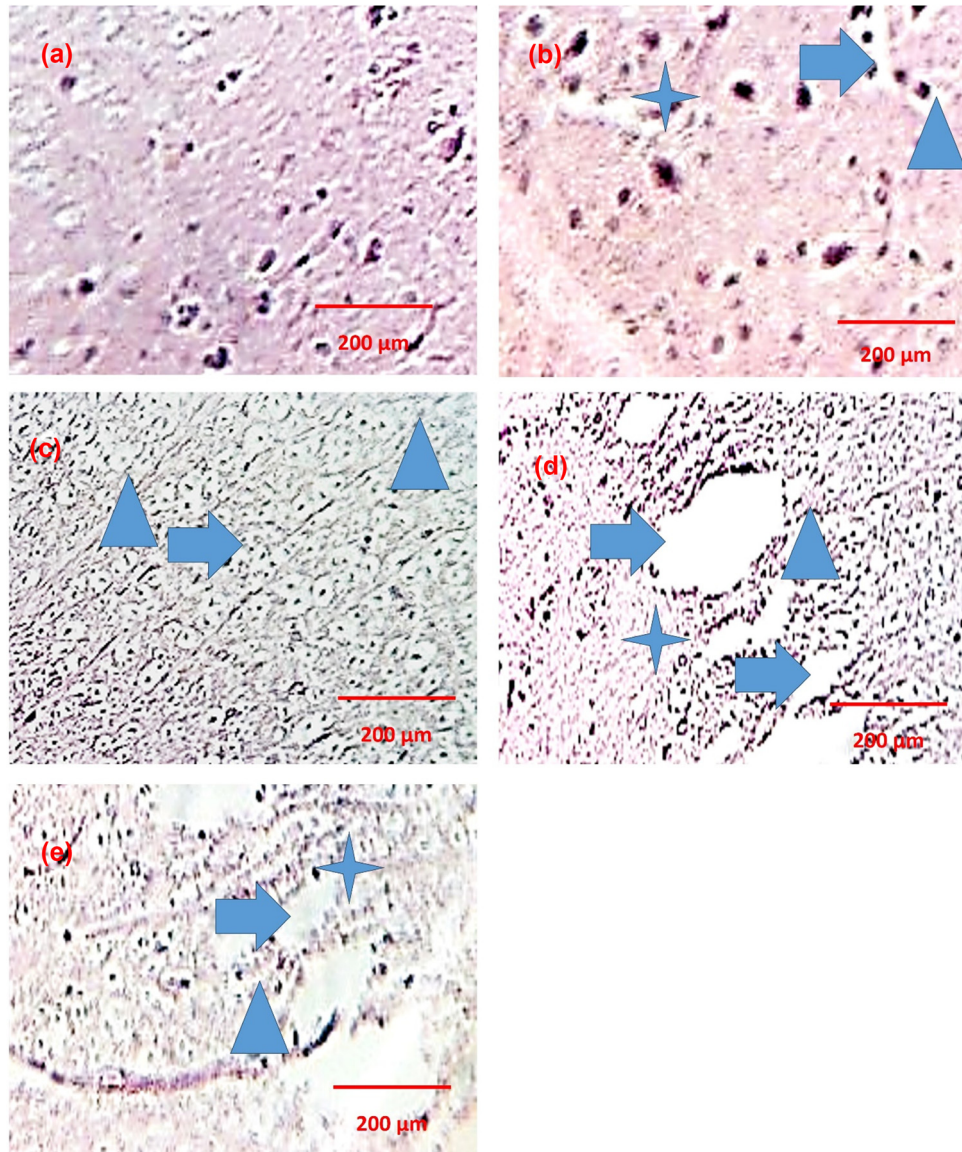


Figure 8. H&E staining images of spinal cord tissues in animals treated with (a) GTEPCLGELBM-MSCsPH, (b) GTEPCLGELBM-MSCs, (c) PCLGELPH, (d) PCLGEL, and (e) negative control. Arrows indicate vacuolation, asterisks denote edema, and axonal disintegration is indicated by arrowheads.

groups, p -value <0.05 . The GTEPCLGELBM-MSCsPH group had significantly lower hot plate latency time than the GTEPCLGELBM-MSCs group, p -value <0.05 . Therefore, we can assume that the physical activity plan has augmented sensory function recovery.

Based on these results, we can assume that the healing potential of the GTEPCLGELBM-MSCsPH group was the highest among experimental groups. This result could be due to the following reasons. The potential enhancement of SCI recovery through the synergy of GTE, BM-MSCs,

and treadmill exercise involves intricate mechanisms and signaling pathways. First, GTE's anti-inflammatory properties, enriched with compounds like EGCG, could suppress pro-inflammatory cytokines and pathways like NF- κ B, curbing inflammation at the injury site. This theory is in accordance with the results of *in vitro* anti-inflammatory assay [19]. Concurrently, BM-MSCs possess immunomodulatory capabilities, releasing interleukin-10 (IL-10) and transforming growth factor-beta (TGF- β), which effectively dampen inflammatory responses [20,21].

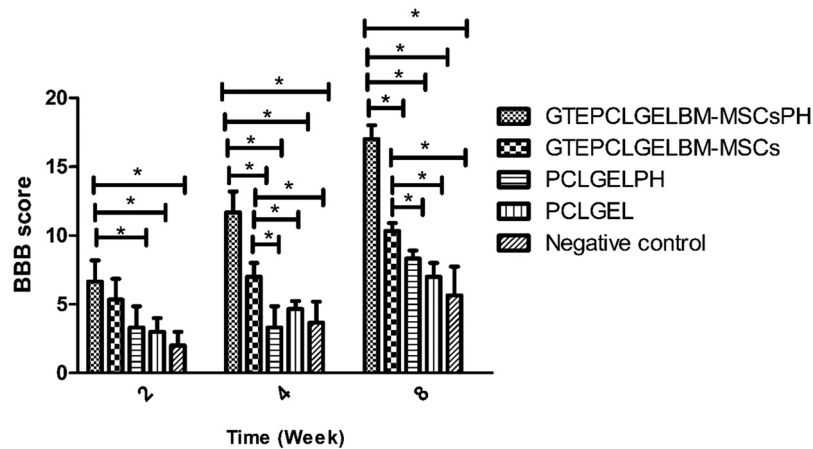


Figure 9. BBB scores in rats treated with different strategies on weeks 2, 4, and 8 after surgery. * shows p -value < 0.05.

Collaboratively, GTE and BM-MSCs contribute to neuroprotection and axonal regeneration. EGCG from GTE can stimulate neurite outgrowth and axonal regeneration, potentially through pathways such as ERK and PI3K. BM-MSCs release essential factors like NGF, BDNF, and glial cell-derived neurotrophic factor, fostering neuronal survival and axonal growth. Moreover, both GTE and BM-MSCs discharge exosomes, conveying microRNAs and proteins to recipient cells, thereby influencing gene expression and facilitating tissue regeneration [22]. Central to the strategy is treadmill exercise, which triggers the release of neurotrophic factors like BDNF and insulin-like growth factor-1, promoting neuronal resilience and function. This exercise also activates pivotal pathways such as PI3K/Akt and ERK, recognized for fostering cell viability, growth, and differentiation [23,24]. The intricate interplay between GTE, BM-MSCs, and treadmill exercise potentially creates a more

conductive microenvironment for the survival and integration of transplanted BM-MSCs, aiding their successful assimilation. However, it is imperative to recognize the intricacy of these interactions, and further research is required to assess the underlying mechanisms involved.

3.9 ELISA assay results

Results (Figure 11) showed that the tissue concentrations of BDNF and NGF in the spinal cord tissues treated with the GTEPCLGELBM-MSCsPH group were significantly higher than those other groups, p -value < 0.05. In addition, tissue concentrations of TNF- α and IL-6 in the GTEPCLGELBM-MSCsPH and GTEPCLGELBM-MSC groups were significantly lower than other groups, p -value < 0.05. Tissue concentrations of these cytokines were not statistically

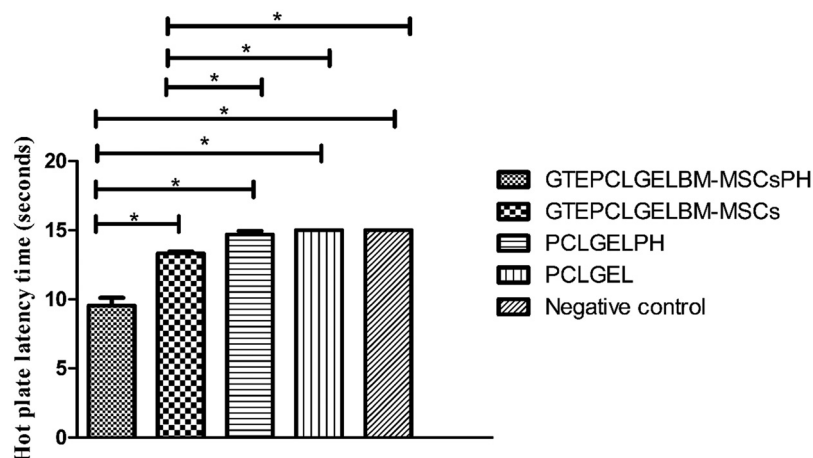


Figure 10. Hotplate latency test results in animals treated with different strategies, * shows p -value < 0.05.

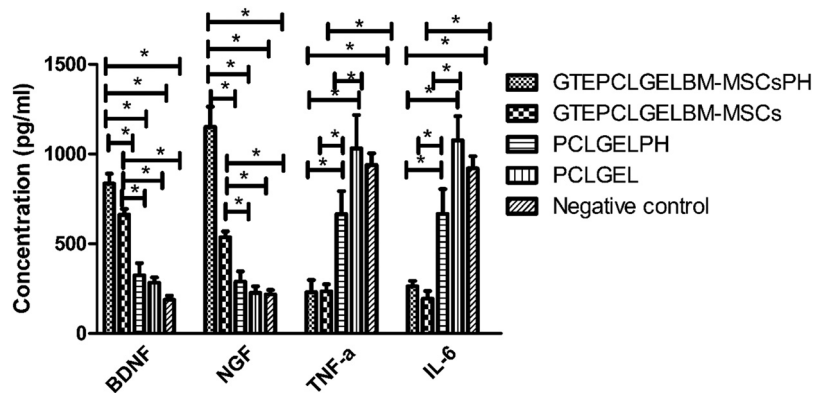


Figure 11. Tissue concentrations of BDNF, NGF, TNF- α , and IL-6 in spinal cord tissues treated with different strategies. * shows p -value < 0.05.

significant between GTEPCLGELBM-MSCsPH and GTEPCLGELBM-MSC groups, p -value > 0.05.

It could be that GTE, treadmill exercise, and BM-MSCs have worked in an orchestrated manner and augmented tissue expression levels of BDNF and NGF. Simultaneously, the combination of GTE and BM-MSCs may collectively decrease the expression levels of pro-inflammatory cytokines, including TNF- α and IL-6. GTE's anti-inflammatory properties, along with the immunomodulatory effects of BM-MSCs, can synergistically attenuate the production of TNF- α and IL-6 [25]. By integrating these elements, a dynamic interplay emerges: GTE's bioactive components and BM-MSCs' secreted factors collaboratively elevate BDNF and NGF, fostering nerve growth and survival. Concurrently, the multifaceted anti-inflammatory mechanisms, including those mediated by GTE, act to quell the production of TNF- α and IL-6, thereby ameliorating neuro-inflammation.

4. Conclusion

In the current research, we aimed to augment SCI repair by combining treadmill exercise with a co-delivery system composed of GTE and BM-MSCs. Our system was not toxic against PC-12 cells and showed strong immunomodulatory and antioxidative activities. *In vivo* study showed that the hybrid treatment augmented motor and sensory functions recovery following injury. The repair process was accompanied by the upregulation of BDNF and NGF and the downregulation of TNF- α and IL-6.

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Author contributions

Jianjun Wang and Tao Zhang were responsible for conceptualizing and designing the study. Yingqi Jiang and Wei Han conducted the experiments and collected the data. Shimeng Huang performed data analysis and interpretation. Jihong Zhao and Xiru Du contributed to the manuscript preparation, provided critical revisions, and supervised the overall project. Xiru Du served as the corresponding author and oversaw the submission process. All authors reviewed and approved the final manuscript.

Conflict of interest statement

The authors state no conflict of interest.

Data availability statement

Data will be shared upon request.

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