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ORIGINAL ARTICLE

The prevention effect of poly (L-glutamic acid)/chitosan on spinal epidural fibrosis and peridural adhesion in the post-laminectomy rabbit model

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Abstract
Purpose Spinal epidural fibrosis and adhesion are implicated as one of the key factors of failed back surgery syndrome, which may cause dura mater compression or peridural tethering, resulting in persistent backache and leg pain. Various materials or drugs have been used to inhibit formation of epidural fibrosis and reduce the compressive effect on neural structures. Nevertheless, the effects are not satisfied. In this study, we investigated the prevention effect of poly (L-glutamic acid)/chitosan (PLGA/CS) barrier on epidural fibrosis developing post-laminectomy in a rabbit model.
Methods Sixteen rabbits were divided randomly into two equal groups: group A (experimental group, $n = 8$) and group B (non-treatment group, $n = 8$). In both groups, total L5–6 laminectomy was performed; further both ligamentum flavum and epidural fat were removed gently. In experimental group, the laminectomy sites were treated with PLGA/CS barriers, while no additional treatment was received in non-treatment group. At 1, 12 and 24 weeks post-surgery, the animals were subjected to magnetic resonance imaging (MRI) evaluation. Following last MRI examination, all rabbits were sacrificed and their spinal columns were totally removed for further macroscopic and histological evaluation.
Results MRI showed that rabbits treated with PLGA/CS barrier at 12 and 24 weeks post-surgery had less epidural fibrosis or scar tissue, peridural adhesion, foreign body reaction and low pressure of spinal cord in comparison with the non-treatment group. In consistence with the radiographic results, macroscopic analysis and histological examination showed that the amount of scar tissue and the extent of epidural adhesion decreased significantly in experimental groups. Concerning the fibroblast density evaluated, the scores were significantly lower in experimental group compared with those in non-treatment group.
Conclusion The results of our study demonstrate that PLGA/CS barrier is effective in inhibiting epidural fibrosis and peridural adhesions in post-laminectomy rabbit model.

Keywords Epidural fibrosis · Laminectomy · Poly (L-glutamic acid)/chitosan · Failed back surgery syndrome · MRI

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Introduction

Failed back surgery syndrome (FBSS), representing a cluster of symptoms following spine surgery, is characterized by persistent, chronic, disabling pain. Epidural fibrosis formation between dura mater and paravertebral tissue is now widely accepted as being a major contributing factor to chronic nerve radicular pain and low extremity weakness post-laminectomy, which could account for as much as 24 % of all cases of FBSS [1, 2]. It is acknowledged that a direct contact of the exposed dura mater and invading fibroblasts results in the generation of localized dense fibrotic tissue, tethering of the thecal sac or nerve foot [3]. The existence of epidural fibrosis makes re-operation more difficult. When repeated surgery is indicated, the secondary procedure is considerably hampered by the presence of fibrosis tissue and adhesions, which could limit visualization and identification of major blood vessels and nerve at the original surgery site. Although extensive epidural fibrosis tissue could be removed out and the tethered dura mater and nerve root could be released, the adhesion will potentially recur and some patients' syndrome will be worse than before the secondary operation in many cases. Therefor, it is necessary to develop a novel therapeutic method that reliably reduces the formation of epidural scar adhesion post-laminectomy.

Based on the above theory, numerous types of biological and non-biological materials, including autologous fat graft, polytetrafluoroethylene membrane, protein-based polymer, high-molecular weight hyaluronan, collagen, gelatin foam, ADCON-L and more recently, have been implanted to serve as barrier to reduce epidural adhesion by means of prophylactic intervention [3–9]. Although these materials have been widely evaluated to prevent epidural fibrosis formation, their efficacy is surrounded with contradictory. Some complications from use of autologous free-fat graft have been reported such as fat tissue necrosis, seroma formation and even compression on the spinal cord [10]. It is also reported that ADCON-L may result in cerebrospinal fluid leakage and infection [11].

Currently, the usage of a physical barrier to reduce epidural fibrosis adhesion has been shown to be one of the most effective methods in spine surgery [12]. PLGA/CS, which is a porous scaffold based on electrostatic interaction between carboxyl groups of water-soluble PLGA and amido group of CS, exhibits good biological and physicochemical properties. Due to its non-toxic, biodegradable and hydrophilic characteristics, PLGA/CS has been widely used in tissue engineering [13]. Studies have demonstrated that scar tissue is not normally stable and mature until around 80 days post-surgery in rodents, which is match to the reabsorption time of PLGA/CS barrier [14]. Xu et al. have documented that PLGA could prevent fibroblast migration and reduce the formation of epidural fibrosis in a laminectomy model [15]. However, due to its outstanding water solubility, PLGA alone cannot maintain a stable construction [16]. Chitosan, which could reduce scar formation by inhibiting fibroblast proliferation, procollagen production as well as regulation of TGF-β, bFGF and IL-8 production, can form an amide bond with PLGA to enhance stability of PLGA/CS barrier and increase the capacity of inhibiting fibrosis formation [14, 17]. Cross-linking of PLGA and CS is an effective way to retard hydrolytic degradation and stability property, which could rapidly occur in biological environments [18].

In this study, we examined the effect of PLGA/CS as a barrier to confine fibroblast migration and reduce epidural fibrosis adhesion in the laminectomy rabbit model.

Materials and methods

Animal

All the procedures were performed with the permission of the animal ethics committee of Fudan University. Sixteen normal male, New Zealand white rabbits (Animal Center, Shanghai Medical College, Fudan University, Shanghai), weighing 2–2.5 kg were randomly divided into two groups of eight animals each, non-treatment and experimental group. In both groups, total L5–L6 laminectomy was performed; further both ligamentum flaum and epidural fat were removed gently. In experimental group, the surgery site was treated with PLGA/CS barrier, while no additional treatment was received in non-treatment group.

Preparation of PLGA/CS barrier

The material based on PLGA and CS was fabricated by a chemical cross-linking method [19]. First, PLGA was dissolved in a low concentration of solidum hydroxide solution (PH = 9) to firm a homogeneous solution. Then, CS was added for activation of the carboxyl groups of PLGA and the suspension was stirred at 800 rad min^{-1} to make sure that the powder was uniformly distributed in the PLGA solution. After dialysis, freezing and lyophilization, pore scaffolds with a cavernous structure were obtained. The mole ratio of PLGA to CS was set at 1:1 to achieve the strongest electrostatic interaction and the solid content was 2 wt%. The scaffold was sheared in a size of 1.5 cm in length, 0.8 cm in width and 0.2 cm in thickness. Before seeding, the scaffold was sterilized with 75 % ethanol for 1 h and washed three times by sterile saline.

Surgical procedure

The procedure was carried out under general anesthesia by intraperitoneal injection of ketamine (35 mg/kg). All rabbits were fixed in the prone position and the surgery field was sterilized with povidone–iodine soap and solution. A posterior midline skin incision was performed from L5 to L7 vertebrae and the lumbar fascia was opened bilaterally. With blunt dissection, the paravertebral muscles were subperiosteally dissected and the lumbar vertebral segments were exposed. After removing the spinous process and vertebral plate with a rongeur for total laminectomy in each rabbit, the ligamentum flavum and epidural fat were removed and the dura mater of L6 level was exposed. After the laminectomy site was well prepared and hemostasis was obtained, the PLGA/CS barrier was implanted over the exposed dura site in experiment group. In no-treatment group, only laminectomy was carried out. Following that, the dense dorsal spinous fascia was reapposed using absorbable suture, whereas the skin was closed with 3–0 nylon sutures. Postoperatively, the animals were then housed in individual cages, received for free food and water consumption and allowed for normal activity.

Magnetic resonance image examination

Magnetic resonance image (MRI) examination was carried out immediately at 1, 12 and 24 weeks post-surgery. After general anesthesia, the MR images were acquired with the use of a 1.5-T whole-body MRI scanner (Magnetom Vision, version VB-33D, Siemens Medical Systems, Germany) with quadrate extremity receiving coil according to previous study [20]. Three sets of MR images were performed; they were spin echo T1-weighted images in sagittal (TR_500 ms, TE_12 ms) and transverse (TR_500 ms, TE_12 ms) planes of the animals and gradient recalled echo T2-weighted images in the same locations with spin echo images (TR_3000 ms, TE_100 ms) for sagittal images, and (TR_3600 ms, TE_115 ms) for transverse images.

The scoring system was developed using a version of a previous score system [21]. Briefly, the spinal canal in each level was further subdivided into four quadrants by drawing perpendicular lines from the central aspect of the dura sac. For each quadrant at each imaging slice encompassing the operative level, the amount of epidural fibrosis was graded on the following scale of 0–4: Grade 0, no scar/trace of a scar; grade 1, >0 to ≤25 % of the quadrant filled with scar; grade 2, >25 to ≤50 % of the quadrant filled with scar; grade 3, >50 to ≤75 % of the quadrant filled with scar; and grade 4, >75 to ≤100 % of the quadrant being filled with scar. Two radiologists analyzed MR image of epidural fibrosis independently on three occasions, with an interval of 3 weeks in three readings.

Table 1 Pathological analysis scoring system

Grade	Pathological features
Grade 0	The dura is free of scar tissue
Grade 1	Presence of only thin fibrous bands between dura and scar tissue
Grade 2	Continuous adherence between dura and scar tissue involving <2/3 of laminectomy defect
Grade 3	Scar tissue adherence ≥2/3 of laminectomy defect and/or extend to nerve roots

Table 2 Fibroblast cells scoring system

Grade	Cell density
Grade 1	Less than 100 cells in each area at 400×
Grade 2	100–150 cells in each area at 400×
Grade 3	More than 150 cells in each area at 400×

Macroscopic observation and pathological analysis

After MRI examination at 24 weeks, all specimens were harvested to assess the whole anatomy of fibrosis formation on a space between the dura mater and surrounding soft tissues. Subsequently, the vertebral column segments from L5 to L7 level were resected, fixed in 10 % buffered formaldehyde solution for 48 h, and decalcified in 10 % neutral ethylenediaminetetraacetic acid (EDTA) for approximately 4 weeks. Following that, vertebral columns were sectioned in 6-mm-thick slices horizontally for optimal visualization of the laminectomy site. All the sections were embedded in paraffin blocks separately, stained with Hematoxylin–Eosin and photographed by a microscope. Peridural fibrosis and adhesions were evaluated according to the following classification that was previously described by He et al. [21, 22], which were summarized in Table 1. Fibroblast cells density was evaluated using the criteria previously described by He et al. [22, 23] in Table 2. All analyses were assessed by two similar histopathologists who were blinded independent to the treatment.

Statistical analysis

Data were presented as mean standard error of the mean (SEM) and analyzed using the SPSS19.0 statistical package. The differences between groups were evaluated with a non-parametrical test (Mann–Whitney U test). P values less than 0.05 were considered statistically significant.

Results

Magnetic resonance image

MRI was an effective tool to evaluate post-operative peridural scar adhesion. Sagittal MRI obtained at the laminectomy level of non-treatment animals exhibited noticeable fibrotic scarring compression in the spine cord at 12 and 24 weeks post-operatively (Fig. 2). However, the experiment group showed a discrete hyposignal space between the dura mater and the surrounding fibrosis tissue nearby skin. There was apparent change between the extent of scar (Fig. 1) at 12 and 24 weeks both in experimental and non-treatment groups. Axial MR images showed extensive scar formation with scar tissues abutting the spinal canal in non-treatment group, and there was little fibrosis tissue in experimental group at 12 and 24 weeks post-surgery. The signal intensity in no-treatment group was higher than that of experiment group at 1 week post-surgery (Figs. 1, 2).

MRI score demonstrated that the score was lower in experimental group when compared with that in non-treatment group ($n = 8$) at 12 ($P < 0.05$) and 24 ($P < 0.05$) weeks post-surgery. The epidural fibrosis score at 12 week was increased in comparison with that at 24 weeks post-surgery both in experimental and non-treatment rabbits. However, there was no significant difference between experimental and non-treatment groups (Fig. 3).

Macroscopic observation and histological analysis

Macroscopic observation showed soft or weak fibrosis adhesion around the laminectomy sites in experimental group, which could be dissected by manual traction. However, there was severe epidural scar tissue disrupted dura mater around the laminectomy sites, which was difficult to dissect the scar in non-treatment group.

At 24 weeks post-operative, abundant epidural fibrosis and excessive proliferation of collagenous tissue were observed between the paraspinal tissue and dura mater in non-treatment group, which adhered to dura mater and compressed the spine cord (Fig. 4a). In addition, a large amount of fibroblasts mingled with fibrocytes was present in the laminectomy site (Fig. 4b). However, the rabbits treated with PLGA/CS barrier, there was only loosely arranged epidural fibrosis tissue with little or without dura adhesion to dura mater in the laminectomy site (Fig. 4c). The proliferation of collagenous tissue was significantly decreased compared with non-treatment group (Fig. 5). As far as the fibrosis severity of post-operative epidural was concerned, there was a significant difference between experimental group and non-treatment group. The fibroblasts density evaluation showed statistical difference between experimental group and non-treatment group ($P < 0.05$) (Fig. 6).

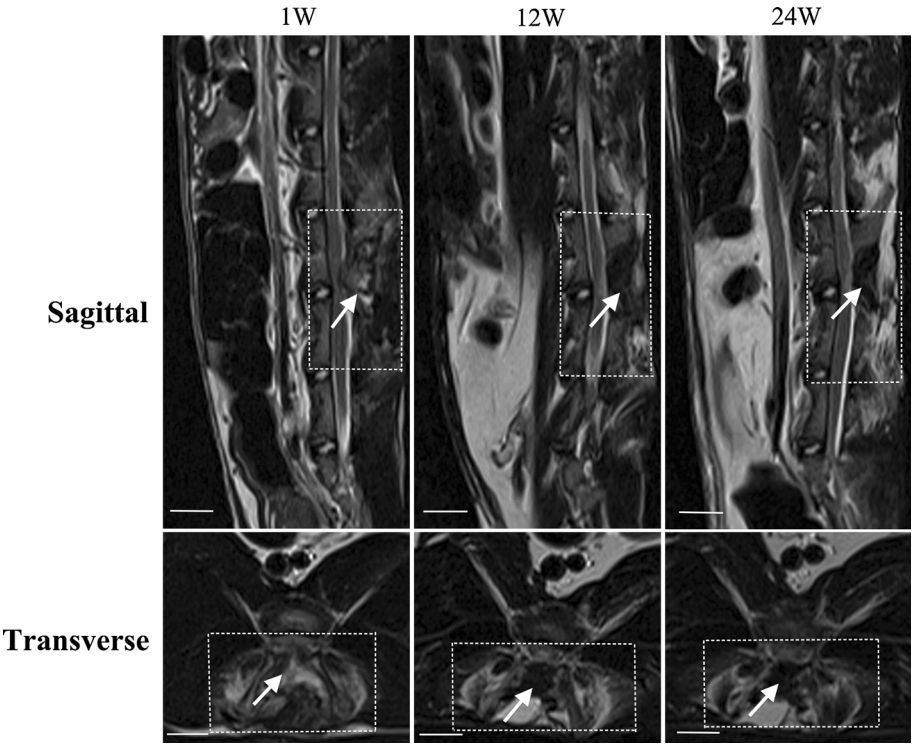

Fig. 1 Sagittal and transverse MR images of laminectomy site in experimental group at 1, 12 and 24 weeks post-surgery. MRI exhibits mild-signal intensity at laminectomy site at 1 week post-surgery. There is a low-signal space between the dura mater and surrounding scar tissue shown in MRI observation at 12 and 24 weeks after surgery. *White arrow* PLGA/CS barrier, *scale bar* 10 mm

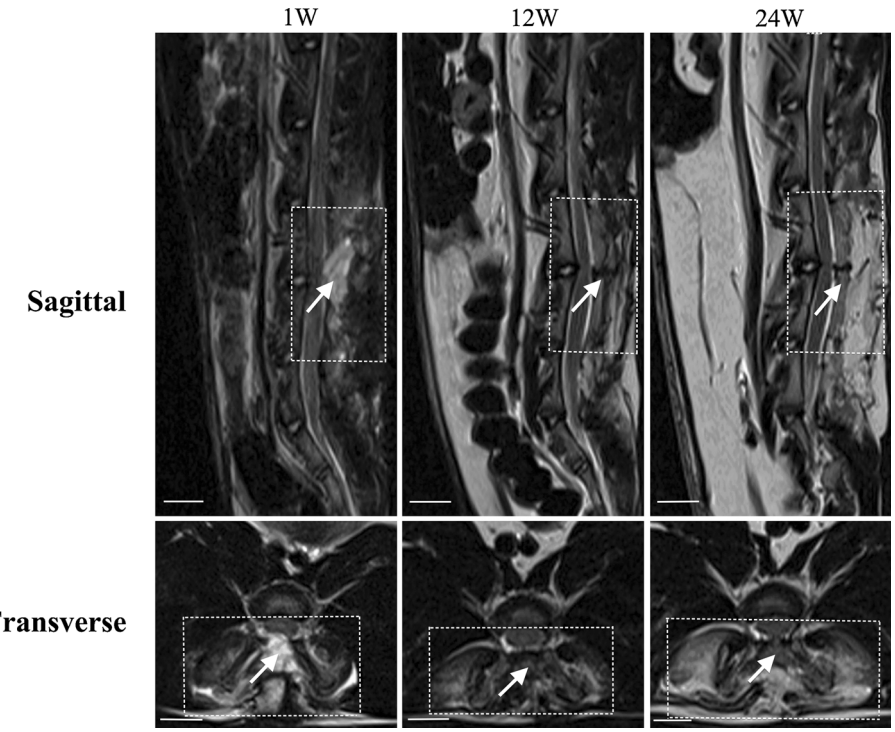

Fig. 2 Sagittal and transverse MR images of laminectomy site in non-treatment group at 1, 12 and 24 weeks post-surgery. MRI shows high-signal intensity at laminectomy site at 1 week after surgery, which is different from experimental group. There is a mount of dense scar tissue showed in MRI observation at 12 and 24 weeks after surgery, which adheres to the dura mater. *White arrow* epidural hematoma or fibrosis tissue, *scale bar* 10 mm

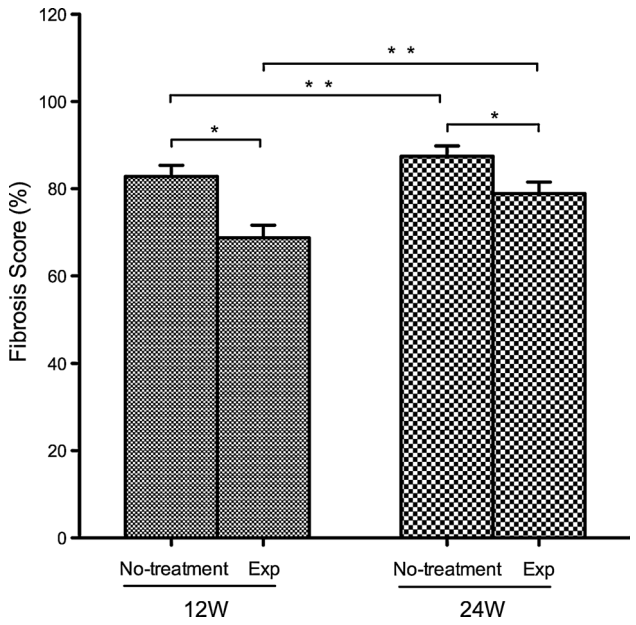

Fig. 3 Comparison of the epidural fibrosis score calculated from MR images by a modified version of a pervious score fibrosis. The epidural fibrosis score was significantly decreased in experimental (Exp) rabbits compared with that in non-treatment rabbits at 12 and 24 weeks post-surgery. $*P < 0.05$, $**P > 0.05$, $n = 8$

Discussion

The present study demonstrated that a PLGA/CS barrier could be implanted as a physical barrier to reduce effectively epidural fibrosis formation and scar adhesion without affecting wound healing. The results provided sufficient evidence that PLGA/CS barrier was a potential biomaterial to reduce epidural fibrosis for future clinical application. The formation of epidural fibrosis post-surgery is an inevitable sequel of spine laminectomy. Excessive epidural scar adhesion was likely contributed to one's chance of developing to FBSS, which is characterized as continued recurrent pain or neurological dysfunction [2]. One of the possible mechanisms of epidural fibrosis formation is that fibroblasts originated from paravertebral tissue are carried by blood into the surgery area, which contributes to the progression of peridural fibrosis. [24, 25]. Therefore, blocking or restricting the migration of fibroblast and reducing the degree of hemostasis in laminectomy area may be one of the most important strategies to decrease epidural fibrosis formation. The application of biophysical barriers to restrict cell migration and avoid the contacting between peridural fibrosis and dura mater are considered an effective approach to improve the recovery [3, 5]. It has been reported some complications from the use of physical barriers including cerebrospinal fluid leakage, infection, seroma formation, dimpling of the scar and cauda equine syndromes [11, 26–28], even though various biological and synthetic barriers have been proposed to prevent or minimize scar formation [3–9, 12, 29].

In the present study, we investigated the effect of PLGA/CS barrier on reducing epidural adhesion post-laminectomy in a rabbit model. PLGA, one part of this barrier, is a polypeptide with outstanding biological and physicochemical properties, which is supposed to be an effective material to mimic the natural ECM and has been

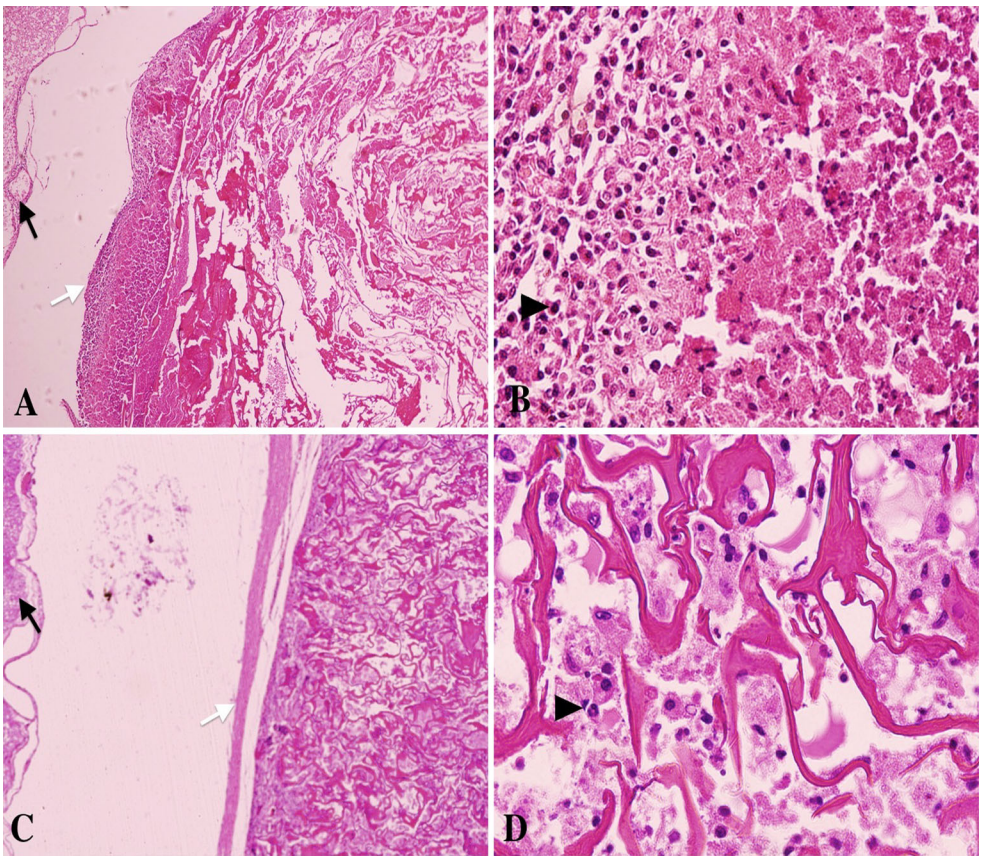

Fig. 4 Photomicrographs of the epidural adhesion issues in the laminectomy sites in no-treatment and experimental groups. There was dense scar tissue adhered to dura maters in non-treatment group (**a**). Loose scar tissues without adherence to dura mater were present in experimental group (**c**). The number of fibroblasts in experimental rabbits (**d**) was less than that in non-treatment rabbits (**b**). The section was stained with hematoxylin–eosin and the magnification is ×40 (**a**, **c**) and ×400 (**b**, **d**). *White arrow* spinal cord, *black arrow* dura mater, *black triangle* fibroblasts

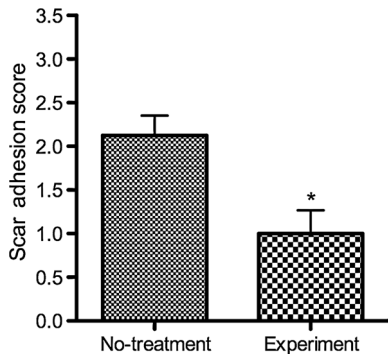

Fig. 5 The results of epidural scar adhesion scoring from histological evaluation in each group. *$P < 0.05$, compared with non-treatment group

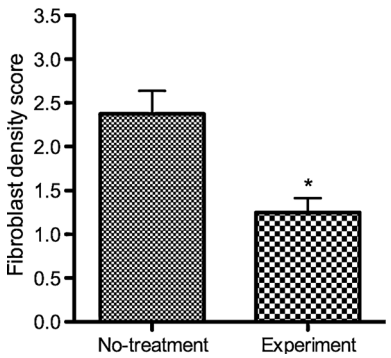

Fig. 6 The effect of PLGA/CS barrier on fibroblast counts in epidural scar tissue based on histological analysis. *$P < 0.05$, compared with non-treatment group

proved to be a promising polymer material for biomedical applications [12, 30–32]. Xu et al. [15] reported that PLGA also could reduce epidural fibrosis formation. We supplemented the Chitosan with PLGA to enhance the antifibrosis effects of PLGA. Chitosan (CS), a functional, renewable, non-toxic and biodegradable biopolymer, has been widely used in tissue engineering and transdermal drug delivery [33]. It was reported that Chitosan with triple compression

bandage could promote recovery of venous leg ulcers [34]. Chen et al. [18] reported that chitosan could reduce the ratio of type I/III collagen in keloid fibroblast by inhibiting the secretion of collagen type I. On the contrary, it had no apparent effect on the secretion of types I and III collagen in the normal skin fibroblast. We fabricated PLGA/CS barrier by electrostatic interaction between carboxyl groups of water-soluble PLGA and amido groups of CS. Chitosan can form an amide bond with PLGA to improve the instability PLGA/CS barrier [19, 35]. When the PLGA/CS barrier was implanted in the laminectomy site, it could form a barrier with some similarities to normal soft tissue lamina to limit the fibroblast migration and reduce hemorrhage or hematoma.

MRI has been proved to be an effective diagnostic tool to identify and assess epidural fibrosis non-invasively, which could monitor the forming process of fibrosis tissue dynamically [36]. In this investigation, MR image showed scar tissue, which was consistent with the scar tissue formation in every surgery area. T2-weighted transverse and sagittal MR images were good at identifying the scar tissue [37]. The PLGA/CS barrier was low-signal intensity, while the fibrosis tissue was high-signal intensity in T2-weighted MR image, which could assess the change of PLGA/CS barrier and the formation of fibrosis tissue. There was little scar tissue formation in MR image and lower MRI score in experimental rabbits when compared with non-treat group at 12 and 24 weeks post-surgery. In addition, MRI score was higher at post-operative 24 weeks than that post-surgery 12 weeks in all rabbits. In addition, the extent of epidural scar in experimental animals was significantly less than non-treatment group at 12 and 24 weeks post-surgery. Moreover, it was noteworthy that the laminectomy site was high-signal intensity in non-treatment group compared with the mild-signal intensity in experimental group at 1 week post-surgery. One possible reason for this phenomenon was that the PLGA/CS barrier had good absorbency similar with sponge, which may play a hemostatic role and contribute to a decrease of the post-surgery hematoma in early phase of fibrosis formation.

Macroscopic and histopathological analysis showed abundant fibrosis and strong tissue adhesion in non-treatment group. However, there was less amount of epidural fibrosis tissue and lower fibroblast score observed after implanting PLGA/CS barrier. These results evidenced that the usage of PLGA/CS barrier could significantly reduce the formation of epidural fibrosis. Furthermore, we also noticed that a mount of epidural fibrosis and dura mater had complete adhesion, which may increase the risk of nerve root injury and dura tears in re-operation. The experimental group manifested no adherence or incomplete adhesions. Thus, we supposed that the PLGA/CS barrier implantation confined fibroblast infiltration into laminectomy area and reduced the risk of fibrosis formation during granulation.

In summary, three possible reasons might be responsible for the reduced scar. First of all, prior animal studies demonstrated that bio-absorbable polylactide barrier, which showed good biocompatibility with spinal cord, was effective in reducing hemorrhage or hematoma, and also diminishing the volume and tenacity of epidural fibrotic tissue. Second, the porous structure of PLGA/CS scaffold used in this study might act as a barrier for limiting fibroblasts migration to the exposed dura mater. In addition, some major advantages of PLGA/CS barrier found in this study were that the usage of PLGA/CS barrier gave perfect adaptation to the defect, and the barrier function of PLGA/CS scaffold reduced the risk of leakage into soft and neural elements. More importantly, PLGA/CS with a suitable degradability was resorbed within 2–5 months by the organism [38, 39], which was match to the time of mature fibrosis tissue formation, preventing the compression of spine cord and nerve root during fibrosis tissue formation. We also found that the fibrosis tissue adhered to dura mater in non-treatment rabbits, which might oppress the spine cord and increase the risk of damaging the dura mater in re-operation. However, there was some space shown between dura mater and paravertebral tissue in experimental rabbits. Ross et al. [40] demonstrated that there was no apparent linear correlation between the radicular pain scores and MRI scar scores in a prospective, controlled, randomized, blinded multicenter clinical trial. They thought that scar tissue did not induce pain directly and other factors also contributed to pain. More importantly, increasing scar tissue significantly increased likelihood of experiencing recurrent radicular pain. Although, we demonstrated the effect of PLGA/CS barrier on preventing epidural fibrosis adhesion by morphology, unfortunately, we did not test the rabbits' behavior, which was essential to understand the physiological function of it. It is deserved for further investigation to evaluate the effect of PLGA/CS barrier application on improving the neurological function.

Conclusion

The present study suggests that PLGA/CS barrier is an effective material to decrease epidural scar adhesion after spinal laminectomy in the rabbit model. Our findings indicate that applying PLGACS barrier in clinical practice may potentially minimize post-operative complications.

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Conflict of interest None.

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