Novel biodegradable α -TCP/poly(amino acid) composite artificial lamina following spinal surgery for prevention of intraspinal scar adhesion

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Abstract Biodegradable copolymer α -TCP/poly(amino acid) composite artificial lamina was prepared and used in goat cervical vertebra resection repair. Cervical 4 was removed by laminectomy, and a vertebra defect of 27×9 mm was made. α -TCP/poly(amino acid) composite artificial lamina was inserted in the test group. The efficiency of the copolymer during repair and reconstruction of the goats' vertebra was tested by using X-ray, CT scanning, and histological and biomechanical measurements. In the 24 weeks following the operation, the artificial lamina refrained from shifting, and no dural adhesion pressure was observed. In contrast, the control group suffered from infiltration of soft tissue in the spinal canal, dural pressure and α -TCP/poly(amino acid) degradation. In conclusion, α -TCP/poly(amino acid) composite artificial lamina can significantly prevent scar tissue from infiltrating the spinal canal.

Keywords Adhesion · Artificial lamina · Biodegradable materials · Cervical

Introduction

Laminectomy is an effective surgical method for spinal decompression. However, laminectomy often leads to

postoperative epidural adhesions that cause well-recognized complications in the lumbar spine failed back surgery syndrome (FBSS), the ratio of which is around 6–15% [1]. In some patients, the difficulty and risk may increase due to FBSS post-laminectomy. As a result, the need for a more difficult and dangerous second surgery may increase. Therefore, it is of great importance to enhance the efficacy of lumbar spine surgery to prevent and reduce the formation of epidural scars. Many researchers have conducted extensive clinical and experimental studies, but the results have not been satisfactory. Lamina defects following laminectomy include fibrous connective tissue proliferation, inflammation, granulation tissue development, scar tissue formation and other pathological processes [2]. Trauma, hematoma, fibroblast proliferation and scar formation result from a series of interactions between inflammatory cells and repair cells. As a main component of repair cells, fibroblasts usually appear in the lamina defect area 2–3 days following an injury under the chemotactic stimulation of inflammatory mediators and growth factors. The proliferation of fibroblasts also results in the synthesis of collagen and the formation of collagen fibers. Fibroblasts change to static fiber cells, and granulation tissue gradually converts into scar tissue with the proliferation and maturation of collagen fibers. A laminal membrane is formed that exerts pressure on the nerve root, causing barriers to both nutrition and nerve conduction in the back of the spinal cord. Regional scar adhesion was caused by an injury on the rough surface of the sacral spine and surgical stripping of the lamina fiber layer. In 1990, Songer [3] first proposed his three-dimensional fibrosis formation theory. Songer detailed how epidural scar adhesion is induced not only by sacral spine muscle injury, but also by damage to the fiber layer and longitudinal ligaments. Furthermore, the nerve root is enveloped by

This study was conducted according to the guidelines laid down in the Declaration of Helsinki.

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fibrous tissue, which only exacerbates the problem. Thus, intraspinal scar adhesion and compression are the primary causes of FBSS.

The establishment of a barrier between scar tissue and dura is an effective way to prevent scar adhesions. Both biodegradable and non-biodegradable materials have been used to fabricate the barrier. The predominant biological materials used are hyaluronic acid and chitosan [4, 5]. Hou and colleagues [4] evaluated sodium hyaluronate's anti-adhesion, tissue reparation and intraspinal hematoma prevention capabilities. Ni and coworkers [5] confirmed that chitosan can promote physical rehabilitation, inhibit the formation of scar tissue, enhance the hemostatic effect and act as a barrier. However, there is much work to be done to overcome the poor mechanical properties of biodegradable materials [6]. Of the many non-biodegradable materials used for barrier construction, poly(tetrafluoroethylene) (PTFE) is currently predominant. PTFE is an excellent biocompatible material that allows fibrous tissue ingrowth and maintains a stable body position. However, as a foreign body, PTFE increases the body's natural inflammatory response, potentially leading to scar formation and adhesion [7, 8].

The ideal biomedical material should have the following characteristics: (a) the initial size must be maintained to avoid blood mass formation and pressure; (b) during medium-term, the material must act as a semi-fluid that may be filled with a large but movable hematoma absorption area to cover dura or parcels to live nerve; and (c) the material must be absorbed upon completion of recovery [9, 10].

We developed a new biodegradable α -TCP/poly(amino acid) copolymer composite artificial lamina at the Sichuan University that satisfied the aforementioned characteristics. In the present study, biodegradable copolymer, α -TCP/poly(amino acid) composite artificial lamina was fabricated. However, because symptoms from epidural fibrosis are more likely to be seen in cervical vertebral laminoplasty in clinical settings, the efficacy of the material was tested through the prevention of cervical vertebra scar formation after laminectomy in animals. The novel material, α -TCP/poly(amino acid), has significant advantages including biodegradability, compatibility, plasticity and the abilities to induce and guide bone regenerations. Thus, α -TCP/poly(amino acid) can be potentially used as implantations to replace natural lamina in patients with resected lamina.

Materials and methods

Composite materials

The biodegradable α -TCP/poly(amino acid) copolymer composites used were from the National Nano-Biomedical

Materials Industry Incubation Center in Sichuan University. The composite material was combined with polyamide structure and α -calcium phosphate composition to procure a compression strength of 173 mPa, modulus of 1.3 GPa and viscosity of 186. The composite material was made of a smooth rectangular plate of size $28 \times 16 \times 2.5$ mm. There was a flat panel on the copolymer surface with two small circle holes of diameter 3.5 mm. The distance from the center of the hole to the edge of the plate was 17 mm. The composites were sterilized using ^{60}Co irradiation.

Animals

The animal experiments were reported to the Ethic Committee of Sichuan University, which permitted performing the investigations for the study. Seventeen 2-year-old goats with weights of 30 ± 2 kg were obtained from the Sichuan University Animal Center. The goats were randomly divided into three groups: an experimental group consisting of nine goats that underwent cervical 4 laminectomies, followed by α -TCP/poly(amino acid) copolymer composite artificial lamina implantations; a control group of six goats whose cervical 4 vertebra plates were removed; and a normal group of two goats that did not receive any operations or treatments.

Surgery procedures

The goats received anesthesia via intramuscular injection of Aetna (0.1 ml/kg). In addition, tracheal intubation and balloon-assisted breathing were used. The goats' limbs were fixed in a prone position and skin disinfection was applied. A sterile neck incision was made and subcutaneous tissue was separated. Cervicals 3–5 were exposed before cervical 4 was removed using laminectomy. A vertebra defect of 27×9 mm was made without damaging the small facet joints. Furthermore, the dura was kept intact under surgery. In the test group, biodegradable α -TCP/poly(amino acid) copolymer composite artificial laminas were used to cover the dura; they were fixed on the pedicle cervical 4 via two screws. The incision was washed with saline solution before the surrounding skin and soft tissue were sutured. In the control group, the incision was closed directly without a polymer artificial lamina implantation. The goats then received penicillin shots via intramuscular injection 5 days after the operation to prevent infection.

On weeks 4, 12 and 24, three goats in the test group and two in the control group were selected using X-ray and subsequently killed for histological examinations. CT scans were acquired on week 24 to measure the spinal canal area of cervical vertebrae 3–5 and sagittal diameter of the spinal canal in cervical 4. The adhesion and repression of scar tissue on the dura and nerve root were also investigated.

The adhesion level of scar tissue was examined according to Rydell's degree of adhesion criteria [1].

Biomechanical measurements

To investigate the impact of α -TCP/poly(amino acid) composite artificial lamina on the repair and reconstruction of the goats' vertebrae, the biomechanical properties of the goats' cervical spine were analyzed using the bending test and the vertical compression test (Instron 8874, UK). The samples were $118 \times 48 \times 42$ mm for the bending test and $45 \times 35 \times 33$ mm for the compression test of 60 N.

Statistical analysis

Data were presented as the mean of six individual observations with standard deviation. Statistical analysis was performed using a one-way analysis of variance (ANOVA), followed by the Bonferroni *t* test for comparison with the control group. Statistical significance was determined at $P < 0.05$.

Results

The animal numbers of the study groups are not from the same size due to financial support. The mean values of the parameters among the three groups were compared and there was no significant effect on the results and conclusions.

Gross observation

After 4 weeks, soft-tissue membranes on the surface of artificial laminas in the test group remained undamaged,

and they were easily separated from the materials. There were no traces of broken or shifted lamina in the spinal canal. Moreover, there were no dural adhesions observed. As for the control group, granulation tissue had formed, and it had entered into the spinal canal and dura. Adhesion of bone tissue was apparent. After 12 weeks, the test group's laminas were surrounded by fibrous tissue. However, in the control group, the dura of the defective lamina was pressed inward by fibrous scars. After 24 weeks, the test group showed fibrous tissues surrounding the artificial materials (Fig. 1a). Some parts of the copolymer were broken. In the control group, adhesion of the scar tissue to the dura was clearly observable (Fig. 1b). Moreover, the dura was pressed by fibrous tissue in the control group.

Histological analysis

After 4 weeks, connective tissue had formed in the test group, and it had separated the fibrous/polymer materials comprising the inner artificial lamina. In addition, small amounts of osteoblast bone cells and osteoid formations were seen (Fig. 2a). In contrast, soft tissue grew in the lamina defects of the control group, and inter-organizational vascular proliferation was not obvious. Also, there was a small amount of osteoid bone stump and osteoblasts on the bone side (Fig. 2b). After 12 weeks, the trabecular bone in the test group could be observed between the inner artificial lamina's organization and materials. In addition, the defect was almost closed off with new bone tissue, and there was no adhesion or pressure on the dura. However, in the control group, the defect was filled with dense connective tissue. Furthermore, striated muscle and fibrous tissue had broken into the spinal canal, and pressure exerted by the scar tissue on the dura was evident. After 24 weeks, natural trabecular and lamellar bone had formed

Fig. 1 Photographs of the goat cervicals from the test group (a) and the control group (b) after week 24. In the test group, the laminar surface was covered by a fibrous connective tissue and the artificial lamina has partly degraded. In the control group, adhesion between dura mater and scar tissue in the defect can be found, resulting in the compression of the dura mater

Fig. 2 Histological images of the goat cervicals from the test group (a, c) and from the control group (b, d).

a Appearance of osteoblasts and formation of a small amount of osteoid after 4 weeks (HE $\times$ 200). **b** Both osteoblasts and scar tissue can be seen after 4 weeks (HE $\times$ 200). **c** After 24 weeks, trabecular bone and lamellar bone appeared. Fragments of degraded artificial lamina were wrapped by fibrous tissue, and there was no observed compression on the subdural. **d** After 24 weeks, defects were filled with fibrous scar tissue that exerted pressure on the dura mater

on the artificial lamina/polymer interface, and degraded polymer fragments appeared wrapped by fibrous tissue. Again, no dural adhesion or pressure was found (Fig. 2c). In the control group, fibrous scar tissue filled the defect and the scar tissue continued to exert pressure on the dura (Fig. 2d). Table 1 summarizes these results. We observed that the experimental group was significantly better than the control group ($P < 0.01$).

X-ray images

After 4 weeks, the artificial lamina shape was still maintained in the test group without displacement of the copolymer. Also, a longitudinal translucent shadow was observed at the defect (Fig. 3a). Figure 3b illuminates the defects of cervical lamina 4 and the spinous process. After

12 weeks, the artificial lamina was still maintained without any displacement or shift. However, the edge of the artificial lamina was slightly blurred. Additionally, the density of the lamina increased and new bone formation was observed. After 4 weeks, there was no change in the control group. At 24 weeks after the operation, no artificial laminar location shift was seen in the test group, but fragmentation and melting polymers were observed. The laminar defect density had increased and new goat cervical autologous bone was reconstructed in the defect. As for the control group, the X-ray image showed a very small amount of new bone formation, a longitudinal translucent shadow at cervical lamina 4 and the spinous process.

CT examination results

After 24 weeks, a CT scan of the test group detected no shift of artificial lamina, many degraded blocks of debris and no subdural adhesion pressure. Furthermore, a 2-mm thick bone tissue shadow was found beside the spinal canal (Fig. 3c). In the control group, the CT scan revealed lamina defects, spinal canal containing fragments of soft tissue shadow and pressure on the dura (Fig. 3d). Spinal area measurements of cervicals 3–5 for both the test and control groups are illustrated in Table 2. Significant differences can be seen in cervical 4, while no significant differences

Table 1 Adhesion levels of surgical parts in the test and control groups

Grade	0	1	2	3	Total
Test group (case)*	8	1	0	0	9
Control group (case)	0	0	2	4	6

Compared with the control group ($P < 0.01$)

* $P < 0.05$

Fig. 3 X-ray (a, b) and CT scanning (c, d) images of the goat cervicals from the test group (a, c) and the control group (b, d). **a** Defect was covered by C4 artificial lamina 4 weeks postoperation. **b** There was no regeneration or repair of the C4 spinous process lamina segments after 4 weeks. **c** Some parts of the artificial lamina degraded after 24 weeks. New bone formation in the defect and no compression of the dural sac. **d** A small amount of new bone formed and a large amount of fibrous scar tissue in the C4 defect that correspond to the adhesion and dural sac compression at 24 weeks postoperation

Table 2 Cervical spinal canal area of the test, control and normal groups ($X \pm S$) (mm^2)

Groups	Samples	Cervical spinal canal area (mm^2)		
		C3	C4	C5
Test group*	3	103.5 ± 8.5	128.6 ± 10.6	137.3 ± 12.9
Control group	2	$100.8 \pm 9.1^{**}$	$81.7 \pm 6.5^{***}$	$140.9 \pm 11.7^{**}$
Normal group	1	102.6 ± 9.3	130.3 ± 11.7	139.1 ± 12.6

In the control group, the four cervical spinal canal areas were significantly smaller than the experimental and normal group

* Compared with the normal group in the cervical spinal area of cervicals 3, 4 and 5 ($P > 0.05$)

** Compared with the normal group in the cervical spinal area of cervicals 3 and 5 ($P > 0.05$)

*** Compared with the normal group in the cervical spinal area of cervical 4 ($P < 0.05$)

are observed in cervicals 3 and 5 ($P > 0.05$). CT scanning also showed significant difference in the sagittal diameter of cervical 4 between the experimental group and control group with $P < 0.05$ (Table 3).

Biomechanical tests

As shown in Table 4, a 60-N load displacement of the vertical compression among the three groups caused no significant difference in the spinal specimens ($P > 0.05$). Similarly, there was no statistical difference in anti-bending strength of spinal specimens in the three groups ($P > 0.05$). As such, we can see that absorbable α -TCP/poly(amino acid) composite artificial lamina is similar to human cortical bone in compressive strength and bending resistance, and it had no adverse effects on spine biomechanical function. Therefore, for maintaining biomechanical property, it is not necessary to implant artificial lamina.

Discussions

α -TCP/poly(amino acid) copolymer composite is a novel biodegradable material that can be applied to prevent intraspinal scar tissue adhesion. Biodegradation can be divided into bulk degradation and surface corrosion degradation [10]. Surface corrosion degradation is characterized by the degradation that occurs on the material surface

Table 3 Sagittal diameters of spinal canal of cervical 3 to cervical 5 ($X \pm S$) (mm)

Groups	Samples	Sagittal diameters of spinal canal (mm)		
		C3	C4	C5
Test group*	3	11.5 $\pm$ 1.5	12.8 $\pm$ 1.3	13.8 $\pm$ 1.2
Control group	2	11.4 $\pm$ 1.2**	10.2 $\pm$ 1.1***	13.9 $\pm$ 1.5**
Normal group	1	11.6 $\pm$ 1.2	13.1 $\pm$ 1.2	13.8 $\pm$ 1.3

In the control group, the four cervical spinal canal areas were significantly smaller than the experimental and normal groups

* Compared with the normal group in the cervical spinal area of cervicals 3, 4 and 5 ($P > 0.05$)

** Compared with the normal group in the cervical spinal area of cervicals 3 and 5 ($P > 0.05$)

*** Compared with the normal group in the cervical spinal area of cervical 4 ($P < 0.05$)

Table 4 Biomechanical properties: compression and bending of cervical 4 ($X \pm S$)

Groups	Samples	Bending strength (mPa)	Compressed displacement (mm)	Compressive strength (mPa)
Test group*	3	1.80 $\pm$ 0.03	2.13 $\pm$ 0.05	4.95 $\pm$ 0.06
Control group	2	1.68 $\pm$ 0.08	2.07 $\pm$ 0.03	4.77 $\pm$ 0.09
Normal group	1	1.88 $\pm$ 0.05	2.11 $\pm$ 0.07	4.89 $\pm$ 0.06

* Compared with the control and normal groups ($P > 0.05$)

while the material's internal structure remains unchanged. Surface corrosion degradation materials have obvious advantages when used as implant material. Degradation rate is only related to the material's contact with surrounding tissue fluid. Thus, the degradation rate is easily controlled. Moreover, the material maintains its high mechanical properties during the process because the internal structure does not change.

The α -TCP/poly(amino acid) copolymer composite is a surface corrosion degradation material that has bending strength and compressive elastic modulus similar to that of the human cortical bone. During a simulated immersion in artificial body fluid, the composite material lost 20.1% per week in weight. Also, there was no significant change in the material or release of a large amount of acidic by-product during the material's degradation. As a result, the composite material will not increase the chance of severe inflammatory reactions or scar tissue formation. Thus, α -TCP/poly(amino acid) copolymer is an ideal anti-adhesion material for lamina repair.

In our experiment, slight inflammatory cell infiltration was observed. During the recovery period, there was no observed toxicity, rejection, material exposure or tissue necrosis. Test results on the material's compatibility revealed physical properties similar to that of human cortical bone in bending strength and compressive elastic modulus. The implantation of artificial lamina in a fixed location can significantly prevent scarring of the muscle behind the spinal canal, as shown by the intact spinal morphology seen in the experimental group's CT scans. There was also no compression observed on the spinal canal epidural. However, the control group's epidural was compressed as a result of scar formation, muscle penetration and dural adhesions. With regard to the sagittal diameter of the spinal canal, there was no significant difference from the normal group. α -TCP/poly(amino acid) copolymer composite artificial lamina was partially degraded at week 24. The lamina was wrapped by fibrous tissue and osteoblasts were detected. The formation of new bone and repair of the defect could be seen. In the control group, only a small amount of new bone was formed. The defect was filled with a large amount of fibrous scar tissue that exerted compressive pressure on the dura. The results indicate that a composite artificial vertebra plate as a mechanical barrier prevents the growth of fibroblasts and connective tissue surrounding the defect while supporting the formation of new bone plates.

Conclusions

Biodegradable α -TCP/poly(amino acid) composite artificial lamina has similar mechanical properties to the cortical bone. Its biodegradability and biocompatibility, consistent with the ideal characteristics of material degradation, afford it immunity from an immune response. The material effectively inhibits the compression on and adhesion to the dura mater and nerve root that is caused by scarring behind the spinal canal. The composite is an effective mechanical barrier that prevents the growth of the connective tissue surrounding the defect while promoting effective bone tissue repair and new bone formation and reconstruction during degradation.

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