A Heterologous Fibrin Glue Enhances the Closure Effect of Surgical Suture on the Repair of Annulus Fibrous Defect in a Sheep Model*

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Summary: Improving the closure effect of surgical suture for repair of annulus fibrosus defects remains an unsolved problem. A new type of porcine fibrin glue was reported for the repair of annulus fibrous defects in sheep models in this study. Continuous axial loading test showed that this glue could effectively improve the closure effect of surgical suture for annulus fibrous defect. Magnetic resonance imaging (MRI) of the lumbar spine confirmed that, compared with non-fibrin glue treated intervertebral discs, it contributed to preservation of the nucleus pulposus and maintained the physiological hydration of the intervertebral discs. Moreover, histomorphology evaluation showed that the porcine fibrin glue could partially reverse degeneration of the injured intervertebral discs. Taken together, porcine fibrin glue can effectively enhance the closure effect of surgical suture on annulus fibrosus, improve the repair effect and slow down the degeneration of the intervertebral disc, and provide a potential therapeutic strategy for degenerative intervertebral disc disease.
Key words: annulus repair; fibrin glue; disc degeneration; closure effect

According to the statistics in recent years, the number of patients with lumbar disc herniation is on a rise year by year[1, 2]. Lumbar disc herniation has become a prevalent disease, which significantly increases the risk of disability and negatively affects the quality of daily life in the middle-age and elderly[3, 4]. Minimally invasive surgery for lumbar disc herniation is the most common surgical procedure performed to reduce the suffering of these patients. It reduces the surgical trauma to the patients and effectively relieves the pressure of nerve roots, so that the patients benefit a lot[5]. However, once the nerve nucleus is removed, the annulus tissue around the nucleus will also be destroyed. As a result, lumbar disc herniation may recur, and the nucleus will project from the damaged annulus and press on or chemically irritate adjacent nerve roots, which contributes to discogenic low back pain and muscle weakness. In order to prevent the recurrence, the easiest solution is to repair the annulus injury, however, as the heavy load on the spine and high pressure in the disc[6, 7], the fixation and sealing technology is challenged in the repair process, so an appropriate method is needed to ensure the success of the fixation and sealing[8].

The potential of biomaterials for annulus repair has been widely investigated, which has shown a good prospect for preventing intervertebral disc degeneration and reherniation[9–11]. The application of heterologous fibrin glue in the repair of intervertebral disc defect is an ideal method to close the intervertebral disc defect immediately and maintain the biomechanical properties of the intervertebral disc[12]. Medical fibrin has excellent sealing property after water initiated polymerization. It is usually used to repair wounds and lesions[13–15], which can reduce hemorrhage and protect the site against bacterial infection. It is also widely used in arterial vascular wall repair in cardiovascular surgery[16], clear corneal incision adhesion in cataract surgery[17], bone tissue engineering of maxillofacial surgery[18], nervous system repair[19], mesh fixation in hernia repair[20], reducing blood loss in primary total hip arthroplasty[21], and liver resection[22]. In recent annulus fibrosus repair studies, it has been proved that the sealing effectiveness of surgical sutures with fibrin glue is relatively great. Inspired from these previous studies, medical fibrin glue has been applied to repair annulus fibrosus defects of the intervertebral disc. In

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this study, we applied a commercial medical porcine fibrin sealant kit, along with surgical sutures, to repair the defect of the annulus fibrosus in a slit incision sheep model. The repair effect of the fibrin glue was tested by *in vitro* mechanical tests. The response of sheep lumbar intervertebral disc to fibrin glue was also studied by magnetic resonance imaging and histomorphology *in vivo*.

1 MATERIALS AND METHODS

1.1 Application of Medical Fibrin Glue in Repair of Annulus Fibrous Defect *In Vitro*

The experiment was performed in lumbar specimens *in vitro*, which were collected from 35 to 40 kg of adult experimental sheep. A circular fascia with a diameter of 5 mm was separated from the paraspinal muscle near the transverse process for repair. Based on previous studies[23], a 4-mm long incision was made with a scalpel on the anterolateral part of the annulus fibrosus; secondly, the defects were sutured with 4-0 polypropylene (Surgi-pro TM), but not tightened immediately; thirdly, 20 μL of fibrin sealant (Hangzhou Puji Biotechnology. Ltd., China) (fig. 1) was applied on the entrance of the defect according to the manufacturer's instruction, and immediately covered with a fascia, which can provide more sealing strength and protect the adjacent tissue from direct contact with heterogeneous fibrin glue, such as nerve; finally, to secure, we tightened the suture and made an extra simple suture through the fascial cover. The control group and operation group were established with the similar procedure, except no fibrin glue application in the control group.

1.2 Mechanical Test of Annulus Fibrous Defect after Fibrin Glue Repair *In Vitro*

Twenty motion segments of fresh sheep lumbar spine were used. All soft tissues and facet joint were carefully removed. Then specimens were randomized into two groups, 10 segments in each. The first group (group 1): 4-0 non-absorbable polypropylene suture and heterologous fibrin glue were used to repair the defect of annulus fibrosus. The second group (group 2): the defect of annulus fibrous was sutured without fibrin glue, as the control group. The cranial and caudal vertebral ends were embedded in mixed epoxy for fixation into load cell in an MTS 858 Materials Testing System (MTS Systems Corp, Minneapolis, USA). An axial compression loading test was then carried out. The axial compression force is set at a loading rate of 1 mm/min until failure occurs (the load suddenly drops and nucleus leaks from the repair site).

Fig. 1 A porcine fibrin sealant kit

1.3 Response of Annulus Tissue to Fibrin Glue *In Vivo*

Thirty-six healthy adult sheep (aged 30–36 months and weighing 35–40 kg) were divided into three groups: experimental group, control group and healthy control group (12 in each group). All of them were fasted for 12 h before surgery and placed in the prone position after anesthesia.

In experimental group, a dorsal paravertebral incision was made to expose the annulus fibrosus, and the integrity of annulus fibrosus was maintained. A slit incision (4 mm in length) was made through the entire annulus fibrosus and sutured as described above. During performing the repair, the fibrin glue compound was deposited strictly over the simulated defect with the help of a syringe and a hypodermic needle according to the instruction of the manufacturer.

The defects sutured without fibrin glue application served as control group. Following surgery, the antibiotics were administered for 5 days continuously. The rest of the healthy sheep served as healthy control group. The sheep were then euthanized under general anesthesia with pentobarbital overdose (120 mg/kg, I.V.) at 3, 6, 12 weeks post-surgery respectively. The lumbar spine specimens were harvested for magnetic resonance imaging (MRI) and histological evaluation. This study was approved by the Animal Ethics Committee of the Inner Mongolia Medical University (China).

1.4 MRI Analysis

Qualitative MRI was used to examine the morphological changes of the intervertebral disc after annulus fibrosus injury and to identify the degenerative changes. Imaging was obtained using a sagittal TurboRare sequence (TR=2192 ms, TE=128.2 ms, thickness=3 mm, matrix size=320×320). Degeneration was evaluated on a modified Pfirrmann scale (table 1), which was quantified according to nucleus pulposus signal intensity and homogeneity, as well as the intervertebral disc height loss.

1.5 Histological Analysis

The structural changes of the intervertebral disc were described by histomorphology, indicating degenerative changes. Samples were fixed with 4% paraformaldehyde, decalcified with EDTA, dehydrated with ethanol, embedded in paraffin and cut sagittally.

Table 1 Degenerative changes of intervertebral disc quantified by modified Pfirrmann grading system

Grade	Structural changes within nucleus pulposus	Signal intensity	Intervertebral disc height
Ⅰ	Homogeneous and bright	Hyperintense	Normal
Ⅱ	Heterogeneous	Intermediate	Normal
Ⅲ	Heterogeneous and gray	Intermediate	Decreased
Ⅳ	Heterogeneous and black	Hyperintense	Decreased or collapsed

After dewaxing and hydration, sections were stained by Masson staining according to manufacturer's instructions (Nanjing Jiangcheng Biotechnology Institute, China).

1.6 Statistical Analysis

The data were expressed as mean±standard deviation. The failure load data were compared by the Student's *t* test. T2-relaxation time data were evaluated by repeated measurement variance analysis (ANOVA). Significance level was defined as $P<0.05$.

2 RESULTS

2.1 Mechanical Test of Annulus Fibrous Defect after Fibrin Glue Repair *In Vitro*

The failure load in the experimental group with fibrin glue (2921.36±897.74 N) was significantly higher than that in the control group without fibrin glue (795.42±277.63 N) ($P<0.001$) (fig. 2). After failure, all specimens showed that the sutures remained unbroken. Nucleus pulposus tissue was forced to leak out through the conjunction of the suture.

2.2 Analysis of MRI

Similar to healthy discs, fibrin glue treated intervertebral discs still remained a relative hyperintensity of nucleus pulposus 12 weeks after operation compared to control group, which indicated a higher water content and no obvious degeneration. Modified Pfirrmann grades ranged from Ⅰ to Ⅱ. In contrast, the sutured discs without fibrin glue developed progressive degeneration as early as 3 weeks after operation. The decrease of T2 signal in residual nucleus pulposus indicates loss of nucleus pulposus hydration and replacement by connective tissue. The interface between annulus fibrosus and nucleus pulposus was no longer obvious. At 12 weeks post-operation, most intervertebral discs were degenerated and blackened on MRI, indicating the loss of nucleus pulposus and the collapse of the intervertebral disc. Pfirrmann grades ranged from Ⅲ to Ⅳ (fig. 3).

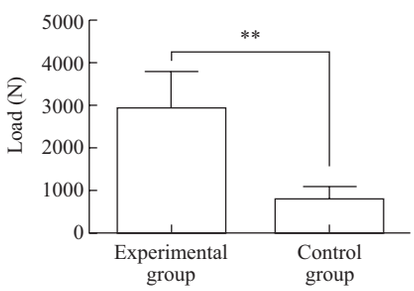

Fig. 2 Comparison of the failure load between the experimental group with fibrin glue and the control group without fibrin glue (10 segments in each group, $^{**}P<0.001$)

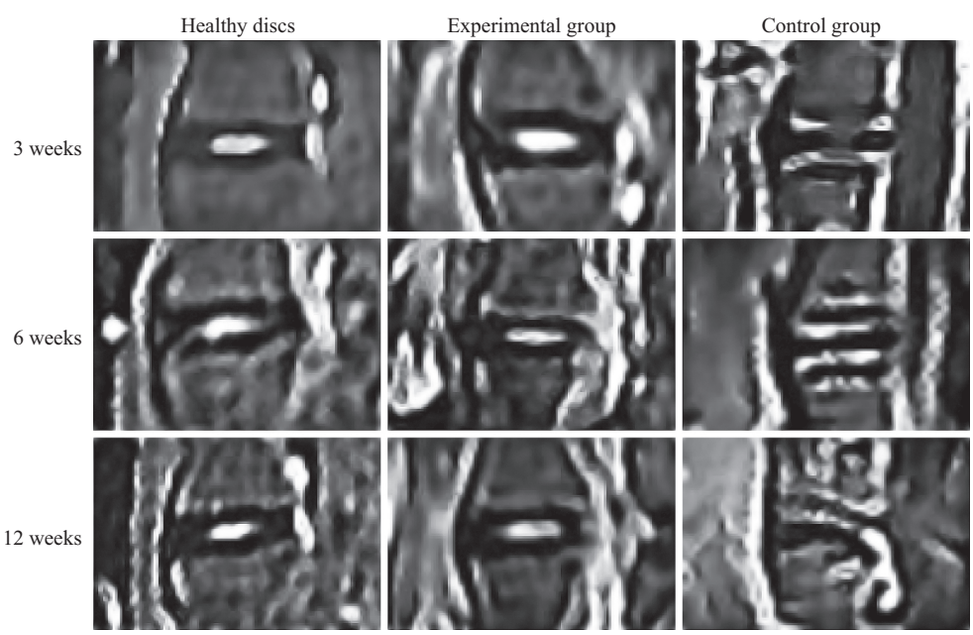

Fig. 3 Results of lumbar MRI in T2-weighted imaging of annulus fibrosus *in vivo*

For accurate quantitation, hydration was assessed by using the T2 relaxation time (T2-RT) measurements of MRI, which was positively correlated with the nucleus pulposus hydration (fig. 4). The average T2-RT of the fibrin glue treatment group decreased slightly 3 weeks after operation, and further decreased at 6 weeks. However, the average T2-RT recovered basically 12 weeks after operation and there was no significant difference between fibrin glue treatment group and healthy intervertebral disc group (67.72±4.03 *vs.* 65.69±2.51 ms, P=0.65). While in the non-fibrin glue treatment group, the intervertebral discs underwent an immediate drop in T2-RT value, showing a significant decrease 3 weeks after operation compared with fibrin glue treatment and healthy intervertebral discs groups (P<0.05). The T2-RT value in the no-fibrin glue treatment group remained very low until 12 weeks after operation.

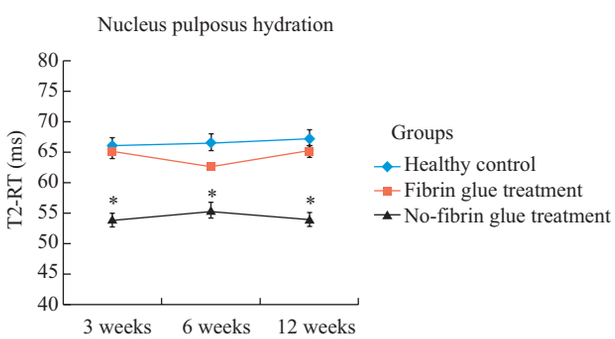

Fig. 4 Comparison of nucleus pulposus hydration according to T2-relaxation time (RT) measurements of annulus fibrosus in the three groups *in vivo* (n=6, $^*P$<0.05)

2.3 Annulus Tissue Response to the Fibrin Glue *In Vivo*

Twenty-four sheep tolerated the surgery and repair, and 12 healthy sheep served as controls. At 3 weeks post-operation, the surgical sutures in the fibrin glue treatment group were hold in place and there was obvious inflammatory reaction around the repair (fig. 5). More importantly, this reaction was not only distributed at the entrance of the annulus fibrosus defect, but also around the repair, where fibrin glue was applied. However, in the absence of fibrin glue, the repairment failed to keep sutures in place, and there was no indication that the outer or inner annulus began to repair. The defect remained widely separated. At 6 weeks post-operation, there was almost no inflammatory reaction in fibrin glue treatment group, and organized collagen fibers were clearly observed in the annulus. A small number of collagen fibers was observed in the outer part of the scar tissue and angiogenesis was seen in the periphery of the annulus fibrosus. No obvious inflammation was found on histological sections. While in the no-fibrin glue treatment group, fibrous tissue gathered to the defect at outer part of the annulus fibrosus. The disrupted inner annulus fibers were not healed themselves.

3 DISCUSSION

Disc herniation is a recognized cause of low back pain[24, 25]. The minimally invasive surgical (MIS) approach of this problem is partial nucleus pulposus discectomy, in which the extruded material is removed.

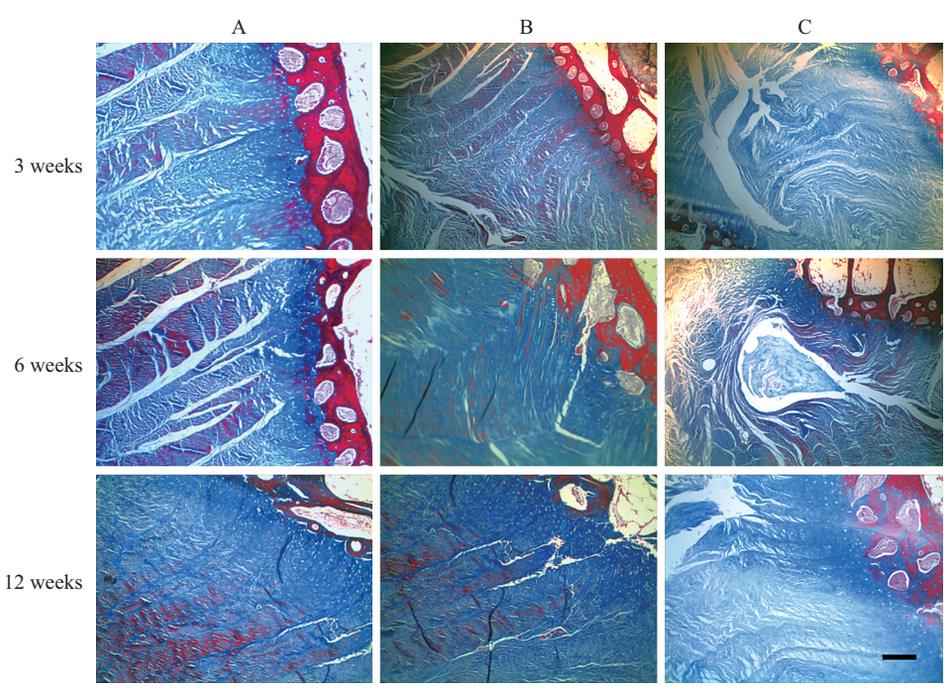

Fig. 5 Representative images of histomorphological examination in healthy control group (A), fibrin glue treatment group (B) and no-fibrin glue treatment control group (C) (scale bar=50 μm)

In most cases, a discectomy successfully relieves symptoms of disc herniation. However, this leaves a persistent defect in annulus fibrosus immediately after operation. Annulus fibrosus defect may be important to consider when the risk of reherniation following lumbar discectomy is recognized[26, 27], which often compromises patient outcomes[28], and requires reoperation[29].

The intervertebral disc is an avascular structure with minimal self-repairing ability[30, 31]. It is difficult to obtain satisfying outcome for the healing response to intervertebral disc injury. This might be due to poor nutritional supply to intervertebral disc[32], low oxygen tension[33], acidic pH[34], low cell density[35], low cell viability[36] and genetic predisposition[37]. Although it is a promising method to repair fibrous rings with cell-seeded porous biomaterials[38], the fixation of biomaterials and the closure of the fibrous defect become particularly important. However, only a few studies have been carried out. In this study, a commercial medical porcine fibrin sealant kit, which is composed of fibrinogen from porcine plasma, coagulation factor XIII and thrombin, combined with surgical suture technology was used to repair the annulus defect in sheep models.

Fibrin glue has two main functions. The first is to speed up the repair and healing of wounds and lesions, thus reducing bleeding and protecting the wounds against bacterial infection[39]. The second is to act as a carrier for extracellular matrix and therapeutic drug scaffold, especially to preserve the biochemical and original properties of the implants[40, 41]. In addition, fibrin has been identified as playing a role in adhesion of the host tissue and exerting an anti-inflammatory effect[42].

The biggest challenge in repairing annulus fibrosus is to overcome its inherent low healing ability. Suture repair of the annulus fibrosus defect is the most straightforward method[43, 44]. However, the long-term effect of mechanical sutures alone may not be good enough, especially in the case with large fibrosus defects[7, 45]. Ahlgren *et al*[46] firstly investigated the effect of repairing annulus fibrosus defects with sutures on the healing strength of the intervertebral disc in sheep. They performed horizontal, cross and window type incision on the annulus, then sewed them up and observed for 6 weeks to compare the healing strength of incisions of different shapes. The results showed that simple sutures of annulus fibrosus defect could not significantly improve the healing in the intervertebral disc. The need for annulus closure methods is increasingly being recognized[47, 48]. It is likely that annulus fibrosus repair will be improved by combination of mechanical and biological strategies. Grunert *et al*[49] used riboflavin crosslinked high-density collagen gel to repair the defect of intervertebral disc annulus, which partially slowed the process of intervertebral disc degeneration. Kang *et al*[23] applied cyanoacrylate medical glue in repairing intervertebral disc annulus defect, which improved closure effect and was non-toxic. Recently, Hegewald *et al*[38] used tissue engineering combined with surgical sutures to repair the defect of annulus fibrosus, and achieved good results in self-repair of annulus tissue. In this study, porcine fibrin glue was applied to enhance the closure effect after suture, and better biological and mechanical function of the intervertebral disc was obtained.

Water content of the nucleus is up to 60%–70%[50] and is essential for the intervertebral disc to maintain its height and proper mechanical function. As shown by the results of MRI, repairing the defect of annulus fibrosus with fibrin glue was helpful to maintain the hydration ability of the nucleus pulposus, indicating that surgical suture combined with fibrin glue could better preserve the water content of the nucleus pulposus. At 12 weeks post-operation, T2-values of the discs treated with fibrin glue were not significantly different from those of the healthy discs. The nucleus pulposus hydration decreased slightly 6 weeks after operation and returned to normal 12 weeks after operation, which further confirmed the effect of fibrin glue on repairing annulus fibrosus. In contrast, the hydration of the nucleus pulposus without fibrin glue treatment decreased significantly 3 weeks after operation compared with that of normal nucleus pulposus. At 12 weeks post-operation, the intervertebral discs without fibrin glue treatment showed obvious hydration loss, and black dehydrated discs. The loss of nucleus pulposus tissue and hydration, as well as MRI and disc histological changes, are the identified indicators for evaluating the degeneration of intervertebral discs. In this study, there was no significant morphological changes in the T2 weighted MRI signal of the intervertebral discs treated with fibrin glue compared with that of healthy discs. In contrast, black discs appeared 6 weeks after operation without fibrin glue treatment, suggesting that intervertebral disc degeneration, intervertebral disc space collapse and dehydration. However, there are some limitations in this study. Firstly, sheep was chosen for animal experiment model, because the lumbar vertebra structure of sheep is similar to that of human, but the stress distribution of human intervertebral disc cannot be completely simulated. Secondly, although mechanical testing, MRI and histomorphology were used to evaluate the healing of annulus fibrosus defect, evidence at molecular level was lacking. Thirdly, it only took 12 weeks to observe the post-operative changes, and long-term follow-up was still needed. Taken together, our study indicates that crosslinked porcine fibrin glue has the potential to close the annulus fibrosus defects and reduce disc reherniation after discectomy. Thus, surgical sutures with fibrin glue application may

be a potential strategy for the treatment of chronic low back pain and intervertebral disc degeneration.

In conclusion, porcine fibrin glue is identified to be able to repair annulus fibrosus defects in sheep models. It can enhance the closure effect of the surgical sutures, and maintain the physiological and mechanical properties of the intervertebral disc *in vitro* and *in vivo*. Although the results of this study are promising, further evidence of molecular mechanisms is needed.

Conflict of Interest Statement

No potential conflict of interest with respect to this article was declared.

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