A Novel Extreme Lateral Approach for Needle Puncture Model of Lumbar Disc Degeneration in SD Rats.

Ziying Cheng
Sun Yat-Sen Memorial Hospital
Yuxi Li
Sun Yat-Sen Memorial Hospital
Weike Zeng
Sun Yat-Sen Memorial Hospital
Yuwei Liang
Sun Yat-Sen Memorial Hospital
Junshen Huang
Sun Yat-Sen Memorial Hospital
Jiajun Huang
Sun Yat-Sen Memorial Hospital
Shixin Lu
Sun Yat-Sen Memorial Hospital
Ming Li
Sun Yat-Sen Memorial Hospital
Lin Huang (huangl5@mail.sysu.edu.cn)
Sun Yat-Sen Memorial Hospital

Technical advance

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Abstract

Background: Various animal models have been proposed to investigate several aspects of intervertebral disc degeneration (IDD). As for needle puncture model, literatures have mentioned the needle puncture in percutaneous posterolateral approach, anterior ventral approach. Our research is mean to describe a novel, extreme lateral invasive approach without posterior paraspinal muscle for needle puncture rat model.

Methods: 16 adult female SD rats aging 12 weeks old was used to perform this research, which was randomly divided into two groups. We used the extreme lateral approach from the skin to lumbar intervertebral disc and advancing the 18G needle into the intervertebral disc in the experimental group and without needle puncture in the control group. Disc degeneration was confirmed by magnetic resonance imaging (MRI)and histologic evaluation.

Results: All rats survived the duration of the experiment. 60 days after operation, the puncture discs of experimental group showed a significant decrease of signal intensity in the T2-weighed magnetic resonance imaging comparing with the control discs of control group. Histological analysis demonstrated the boundary between the nucleus pulposus (NP) and annulus fibrosus (AF) was not distinct, even the connective tissue nearly replaced the position of NP and proteoglycan content decreased within the punctured discs compared to the control discs. Histological score showed statistical significant difference between injury discs and control discs.

Conclusions: Our study demonstrated a novel, reliable and safe approach for the needle puncture model of lumbar disc degeneration in SD rats, which provides a potential experimental model for the research on IDD without posterior paraspinal muscle injury.

Introduction

Lumbar intervertebral disc degeneration has been one of the most important public health problems and common causes of low back pain [1, 2]. Up to 80% people suffer from the disturb of the low back pain along with the and lead to an enormous financial burden due to treating this symptom [3, 4]. According to the duration of the low back pain, it can be divided into three types: acute low back pain, subacute low back pain, chronic low back pain. Luoma et al (2000) [5] concluded that chronic low back pain was associated with disc degeneration.

To explore the mechanism of the disc degeneration and reverse the process of the disc degeneration, various animal models have been discovered or developed. As expected, animal models of intervertebral disc degeneration play an increasingly crucial part in clarifying pathomechanism and testing novel therapeutic strategies [6]. Animal models range from small rodents such as mice [7] to rats [8], rabbits [9], dogs [10], goats [11], sheep [12], and primates [13, 14]. In addition, referring to the mechanisms, these models can be divided into three types: spontaneous models, mechanical models and structural models. As its simplicity and less damage, the needle puncture method to obtain the experimental animal model

of disc degeneration has been widely adopted. The first needle puncture animal model was used in rabbits in 2005[9, 15, 16]. Whereas, the first needle puncture rat model was in 2008[17], but mainly in rat caudal disc. As for lumbar intervertebral disc needle puncture or stab incision, literatures have reported the ventral approach [18, 19, 20, 21], and percutaneous posterolateral approach [22, 23]. Well, lumbar disc degenerative diseases are a complex process influenced by different components, including cellular, matrix, endplate, the neurovascular and paraspinal muscles. Recently, the research on the disc degeneration has transformed from disc itself to paraspinal muscle, especially the erector spinae and multifidus [24, 25].

This article introduces a novel approach without posterior paraspinal muscle injury - extreme lateral approach. The idea was discovered from the treatment of the lumbar degenerative diseases in the clinic. As we all known, the purpose of surgical treatment for lumbar disc degeneration or herniation is decompression and fusion. In clinic,various approaches have been found to reach the operative area, including anterior approach(ALIF), oblique lateral approach(OLIF), extreme lateral approach(XLIF), transforaminal approach(TLIF), posterior approach(PLIF), etc. Comparing and contrasting the merits of different approaches, we found extreme lateral approach (Fig. 1) show greater advantages in the exposure the operative area, blood loss and muscle injury. Thus, we tried to use this method in the experimental animal model, and the model was validated by magnetic resonance imaging and histological analysis. Delightedly, the result reached our expected purpose.

Materials And Methods

Experimental animals

Forty 12-week-old female SD rats, weight 260-320g, were obtained from the animal house of the Sun Yat-Sen University (Guangzhou, China) and randomly select 16 rats into our study, which were randomly divided into two groups. Before the experiments, the rats were housed in cages of 4 and kept at a temperature of 23 ± 1℃ on a 12h light-dark cycle and acclimatized to the environment in the animal house for one week. Food and drinking water were available ad libitum. All experiments were approved by the Committee on the Ethics of Sun Yet-Sen University.

Inclusion and exclusion criteria

Every grow-up healthy rat could be involved in the study. The rats were excluded if there is abnormal development of limbs and spine in the rat.

Anesthesia and Skin Preparation

Rats were anesthetized with an intraperitoneal injection of phenobarbital sodium salt (3mg/100g, Merck, Shanghai, China) half an hour before surgery. If necessary, additional anesthetic was administered during surgery. The left ventral side and dorsal side was then shaved from approximately the lowest rib to the iliac crest level.

Positioning and Disinfection

Each rat was put in right recumbent position and expose the left ventral and dorsal side (Fig. 2A). The skin was prepared for aseptic surgery via a 70% of medical alcohol. The rat was draped to isolate the prepared area.

Localization and Incision

Touch the approximately lowest rib and the iliac crest level, choose the midpoint as the midpoint of the incision (Fig. 2B). The dorsal side is approximately the lateral margin of the erector spinae.

A 1.5cm ventral lateral skin incision was made between the rib and iliac crest level (Fig. 2C).

It is noteworthy that the length of incision is not less than 1.5cm. Otherwise, the intervertebral disc was difficult to expose because of the occlusion of paravertebral muscles and retroperitoneal tissues.

Exposure of the lumbar intervertebral disc

Firstly, the epidermis and dermis were then incised layer by layer for entry into the musculofascial layer. Secondly, continue to incise the musculofascial layer until expose the external oblique muscle of abdomen (Fig. 3A) and then internal oblique muscle of abdomen and transverse abdominal muscle (Fig. 3B). After that circumstance, you will reach the extraperitoneal adipose layer which covers the surgical field. Thirdly, we used a piece of sterile gauze about 0.5cm wide to retract ventral tissues, and you will see the dorsal side is the lateral margin of erector spinae, and the next layer is psoas major (Fig. 3C). Last but not the least, try to touch the transverse process of upper and lower vertebral body and bluntly separate the psoas major step by step with the help of tweezers and scissors (Fig. 3D). Well, after all the circumstances are completed, you will see a white area, that is, lumbar intervertebral disc (Fig. 3E).

Needle puncture

After the exposure of lumbar intervertebral disc, a 18G needle was inserted at the level of the disc, crossing the nucleus pulposus up to the contralateral annulus fibrosus in the experimental group (Fig. 3F). After all penetration, the needle was rotated 360°twice and held for 30s. The depth of needle penetration was not controlled necessarily. As for control group, we didn’t conduct this step. Due to needle puncture under direct vision, the experimenter could not be blinded to whether the disc was injured or not.

Closure and Recovery

Besides the epidermis, it was necessary to close the muscular fascial layer. The rats were allowed cage activity ad libitum, and the diet, incision, and posterior limb activity were carefully observed after the surgery.

MRI Acquisition

MRI was performed on 16 rats 60 days after lumbar intervertebral disc needle puncture. Rats were anesthetized as describe before so that they could maintain immobile throughout the entire MRI examination. Images were acquired with 3.0T MRI machine (Achieva; Philips Medical Systems, The Netherlands) using a 50mm×50mm 4-channel phased array rat coil (Chengguang Medical Technologies Co., Shanghai, China). After being anaesthetized, the rats were placed in the supine position and the four limbs of each animal were positioned symmetrically. The imaging acquisition included T2-weighted imaging (T2WI) in sagittal and transverse positions. Sagittal images were obtained in the longitudinal plane parallel to the path of the spine and transverse image acquisition plane is perpendicular to the path of the spine. T2WI was acquired using a turbo spin echo sequence with the following parameters: repetition time [TR]/echo time [TE], 3000/110 ms; flip angle, 900; echo train, 9; section thickness, 0.5 mm; no intersection gap; number of averages, 6; field of view = 200 mm×160 mm; in plane resolution, 0.35 mm; 10 sagittal slices. It is well-known that the reduction of water content means intervertebral disc degenerates and the mean signal intensity decreases in MRI. Thus, we could conclude whether the injured disc degenerates or not after comparing the mean signal intensity of the control disc.

Histological Analysis

Rats were sacrificed at 60 days after the needle puncture by excess anesthesia with phenobarbital sodium salt. The puncture disc and the adjacent intervertebral disc were dissected carefully. Tissue was fixed in 4% paraformaldehyde, for 24h and decalcified in 15% ethylenediaminetetraacetic acid (EDTA) for 15 days, paraffin-embedded, and sectioned to 5-μm thickness with a microtome. The sections were stained with hematoxylin and eosin (H&E) and alcian blue for changes of structure and proteoglycan content histologically, under a light microscope at 40× magnification.

Statistical analysis

The statistical significant differences among means of the data on AF score, NP score and the total score of AF and NP were calculated. Independent-sample *t* test was used to analyse the difference between injured disc and control disc. All statistical analysis were performed on IBM SPSS 25.0 and the significant level was defined as p 0.05.

Results

All rats survived the duration of the experiment with no complications. One of the rat's result was showed as follows.

Magnetic Resonance Imaging

The sagittal T2-weighted magnetic resonance imaging showed degeneration of the lumbar intervertebral disc 60 days after surgery. The punctured discs showed a significant decrease of the MRI signal compared to control discs (Fig. 4).

Histological Analysis

After 60 days, comparing with H&E staining results of the control disc (Fig. 5A), the boundary between the NP and AF was not distinct and the number of cells decreased (Fig. 5C), even in other plane of the same disc (Fig. 5E) showed that the connective tissue mostly replaced the position of NP. Comparing with alcian blue staining results of the control disc (Fig. 5B), blue stain area decreased in NP and AF (Fig. 5D*&F*).

Statistical analysis

The structure changes in H&E stain grading showed significant statistical differences between the injured discs and the control discs (p□0.05). The results were showed in table 1 and 2.

Conclusions

Our study has demonstrated a reliable and safe approach without posterior paraspinal muscle injury for needle puncture model in SD rats: extreme lateral approach. The model's key point aims to injure the intervertebral disc through a relatively safe and easy access. In addition, it establishes a novel therapeutic approach for treating degeneration or delivering drugs and optimizes the potential adhesions after an open operation.

Discussion

Lumbar intervertebral disc degeneration (IDD) is one of the causes of low back pain. The purpose of the research on IDD mainly lies in repairing the injured AF or slow down the process of NP degeneration. Recently, some researchers' focus has transformed from disc itself to the correlation between IDD and paraspinal muscles, such as the erector spinae and multifidus. It is noteworthy that our novel approach did not injure the posterior paraspinal muscle, which presents a potential experimental model. Our study is a randomized comparative trial with 16 female SD rats. Experimental group is needle puncture, control group is sham operation. The histology and imaging were evaluated at 60 days after operation.

As we described in the introduction, animal models range from various species. Currently, the mechanisms can be divided into three types: First, spontaneous models. The representative animals are sand rats [26, 27, 28], genetically modified mice [7, 29], chondrodystrophoid dogs [10], baboons and macaques [12, 30]. Second, mechanical animal models. Jeong et al (2010) study [31] showed that the relevance between disc degeneration and exposure of the spine to the force. The classic methods mainly used in laboratory are bending of the rat tail [32] and resection of the facet joint and spinous processes [33]. Third, structural models. The method can be classified as chemical and physical. The chemical mainly is chemonucleolysis, such as chymopapain [34]. The physical methods mainly include the endplate injury [35, 36], stab incision [21], and needle puncture [17, 37].

Referring to the needle puncture, there were clinical findings in some literatures initially. A ten-year matched cohort study by Carragee et al (2009) [38] reach a conclusion: using small gauge needle and limited pressurization resulted in accelerated disc degeneration, disc herniation, loss of disc height and

signal and the development of reactive endplate changes. Likewise, Kang et al (2009) [39] study showed that there is a 3-fold increase in risk of developing adjacent level disc degeneration in incorrectly marked discs after ACDF at short-term follow-up.

Well, to explore the mechanics and biology of the needle puncture animal models, Adam et al (1991) [40] studies showed 18G needle punctures led to significant changes in disc mechanics and resulted in inward bulging of the AF, lamellar disorganization, and cellular changes. Similarly, Casey et al (1996) [41] study concluded that needle puncture caused a rapid decrease in dynamic modulus and increase in creep during 1-hour loading, but no changes were detected in water content, disc height, or proteoglycan lost to the media.

Comparing with other animal models of needle puncture, rodent models (e.g., rats) have significant merits with regard to price, access and application. However, limitations for such animals lie in persist notochordal cells and significantly different size from the human ones. As for the notochordal cells, Alini et al (2008) [6] found that the number of these cells decreases rapidly following birth in humans and notochordal cells are completely absent from the NP by early adulthood, that limits the potential therapeutics. The persistence of notochordal cells is an important consideration as these cells have a significant influence on the intervertebral disc by influencing proteoglycan metabolism [42, 43], hyaluronan production [44], and possible progenitor cell function [6].

Comparing with the needle puncture in the rat tail, our study performed on the lumbar intervertebral discs directly, not on the caudal discs. Comparing with the anterior ventral invasive disc needle puncture, our study decreased the blood loss significantly, reduced the disturbance to abdominal organs and decreased the possibility of nerve injury which may cause by the depth of needle penetration. And comparing with the percutaneous needle puncture, our study showed that intraoperative fluoroscopy was not required. It is worth mentioning that Masuda et al [33] and Sobajima et al (2005) [16] have also taken this approach in rabbits, but the specific modeling steps were not clearly described.

In addition, from anatomic angle, the position of the rats we choose in our study was right recumbent position, not left recumbent position, because the abdominal aorta is on the left side of the inferior vena cava and the abdominal aorta has good elasticity and is not easy to be damaged comparing with the inferior vena cava.

Which factors of needle puncture can influence the process of intervertebral disc degeneration? The answers are the size of needle, the number of the needle puncture, and the depth of the needle penetration. For the optimal size of the needle, jiale et al (2019) [45] concluded that an 18-gauge needle is the optimal selection to establish the degenerative intervertebral disc model on rats. Likewise, Gun et al (2010) [46] study got the similar conclusion. For the number of the needle puncture, Kim et al (2005) [29] concluded that the 21-gauge 3-puncture technique was superior in producing disc degeneration over a shorter period of time. For the depth of the needle penetration, Bin et al (2008) [17] study showed that full penetrative puncture resulted in a faster decrease in disc height, lower glycosaminoglycan content, and higher grades of histologic degeneration.

Last but not the least, why we choose the time point at 60 days? Tao et al (2018) [47] study concluded that 2-week time point may be better for use in further experiment studies. Whereas, Chen et al (2020) [48] study showed that 8-week time point the half penetrative needle puncture performed a significant decrease signal intensity in MRI.

However,there is limitation in this novel approach. As intraoperative fluoroscopy isn't used in the modeling process, it is difficult to make sure the intervention level of lumbar disc is the same one.

In conclusion, we believe this novel approach could be a better choice for needle puncture, drug delivery and exploring the mutual effect between lumbar disc degeneration and paraspinal muscle injury or atrophy.

Abbreviations

IDD
intervertebral disc degeneration; MRI:magnetic resonance imaging; NP:nucleus pulposus; AF:annulus fibrosus; ALIF:anterior lumbar interbody fusion; OLIF:oblique lateral lumbar interbody fusion; XLIF:extreme lateral lumbar interbody fusion; TLIF:transforaminal lumbar interbody fusion; PLIF:posterior lumbar interbody fusion.

Declarations

Ethics approval and consent to participate

The study was approved by the Animal Ethics Committee of Sun Yat-Sen University.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Authors' contributions

All authors contributed to study conception and design. CZ and LY coordinated, managed all parts of the study and drafted the manuscript. ZW performed the MRI on SD rats and MRI acquisition. LY, HJs and HJj

carried out the literature search. LS conducted data and photos collection. LM and HL provided substantive feedback on the paper and contributed to the final manuscript. All authors read and approved the final manuscript.

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Competing interests

All the authors declare that they have no competing interests.

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Tables

Due to technical limitations, table 1 and 2 is only available as a download in the Supplemental Files section.

Figures

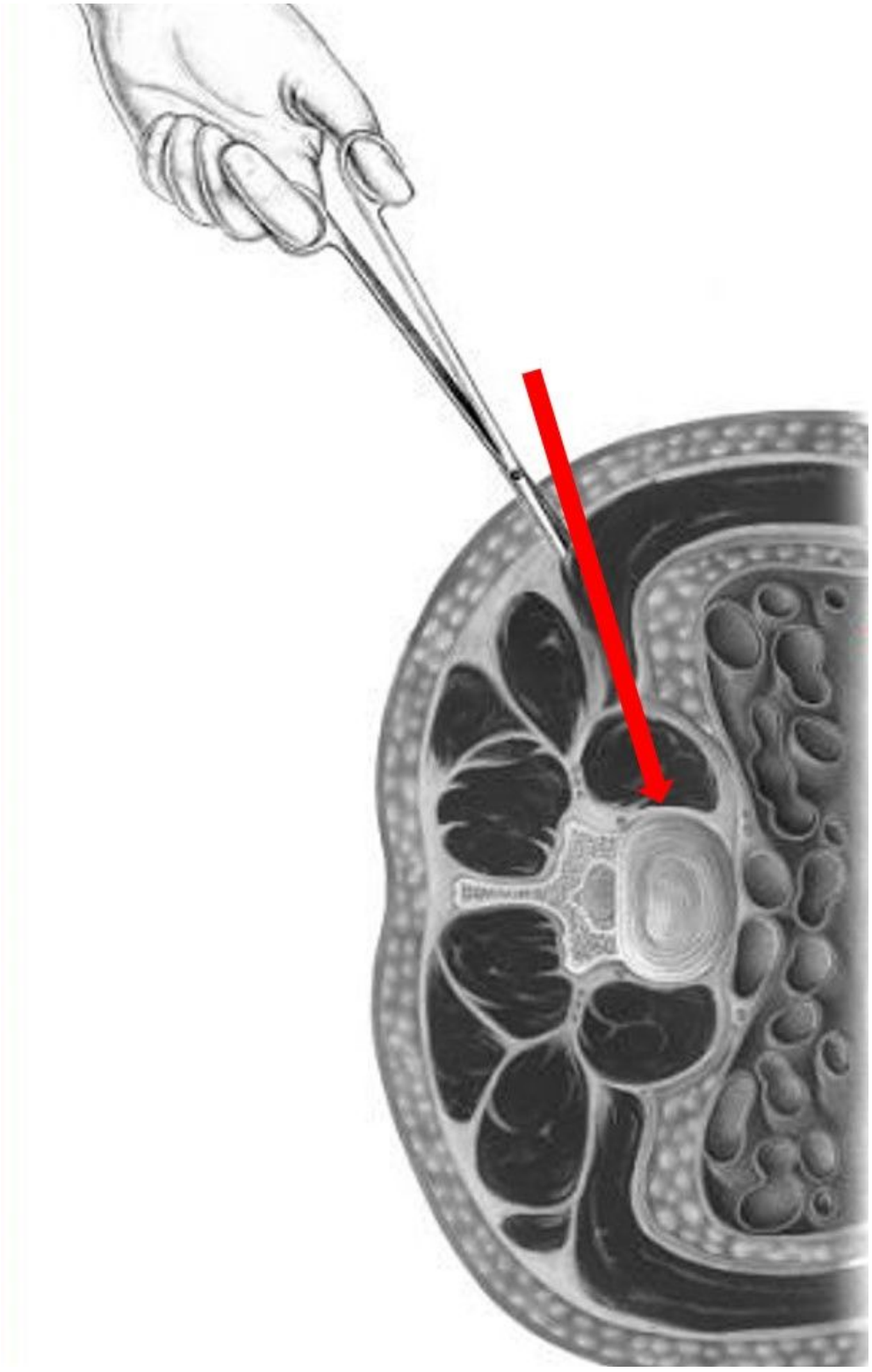

Figure 1

Extreme lateral approach in the clinic (XLIF).

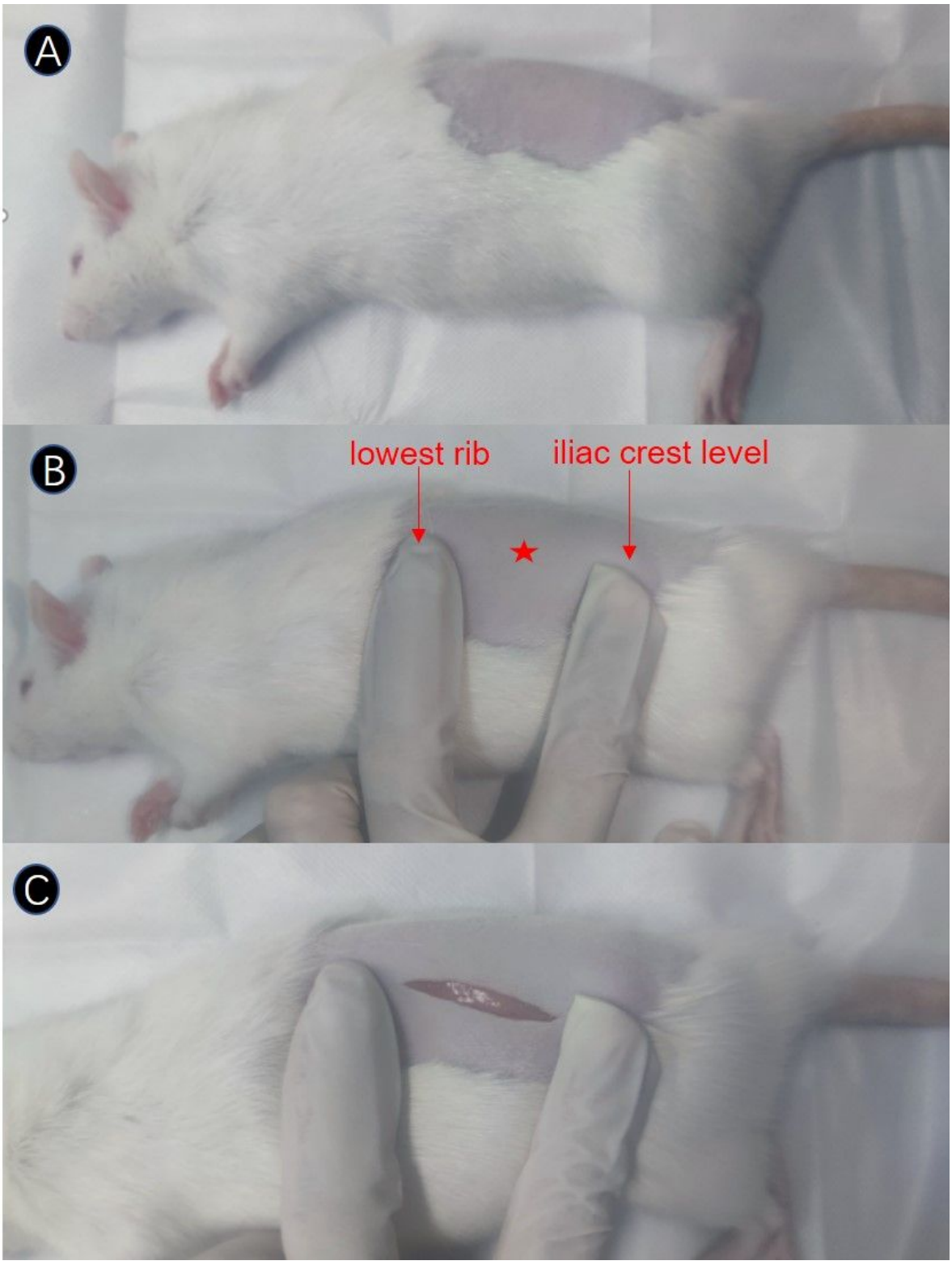

Figure 2

Positioning, Location, and Incision. A Each rat was put in right recumbent position and expose the left ventral and dorsal side. B Touch the approximately lowest rib and the iliac crest level, choose the midpoint as the midpoint of the incision (red five-pointed star represents the midpoint of the incision.). C 1.5cm ventral lateral skin incision.

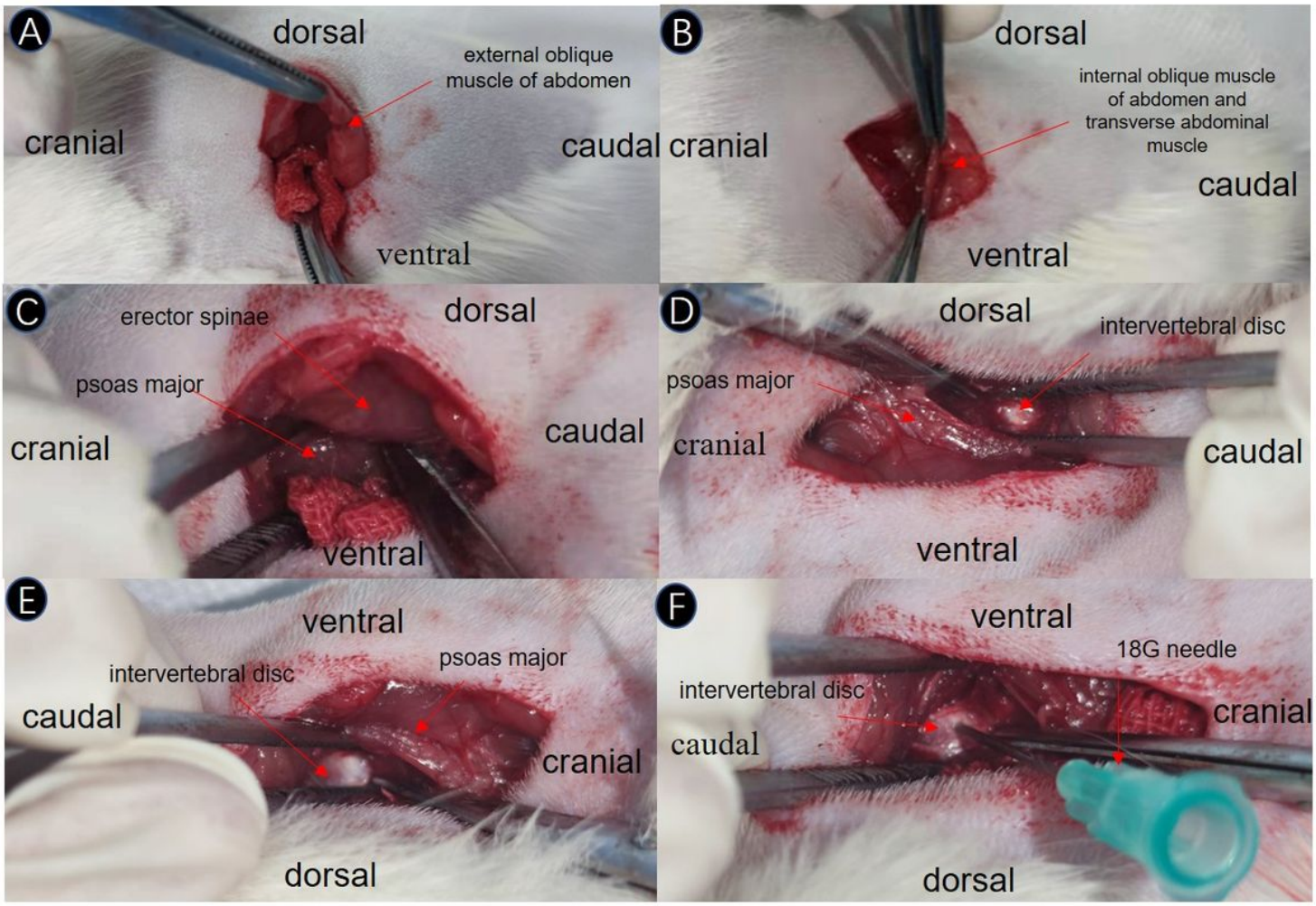

Figure 3

Exposure of lumbar intervertebral disc and needle puncture. A after the musculofascial layer was exposed, incise the musculofascial layer until expose the external oblique muscle of abdomen. B expose the internal oblique muscle of abdomen and transverse abdominal muscle. C After using a piece of sterile gauze to retract the extraperitoneal adipose layer, expose the erector spinae and psoas major. D bluntly separate the psoas major. E expose the lumbar intervertebral disc. F 18G needle puncture.

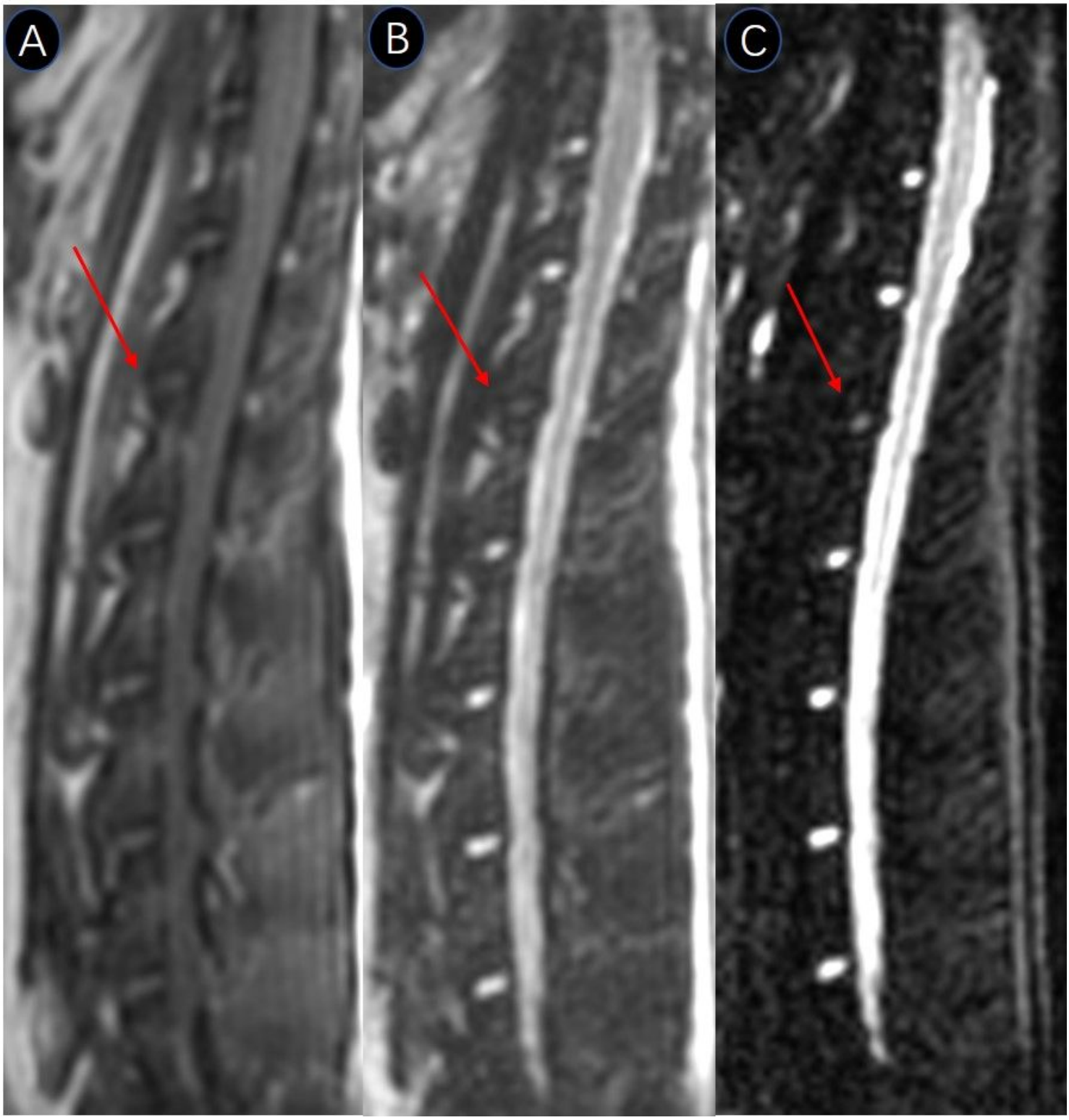

Figure 4

The sagittal T2-weighted magnetic resonance imaging showed a decreased signal in the puncture disc. (red arrows represent the puncture disc. A: T1WI, B: T2WI, C: T2WI-spair)

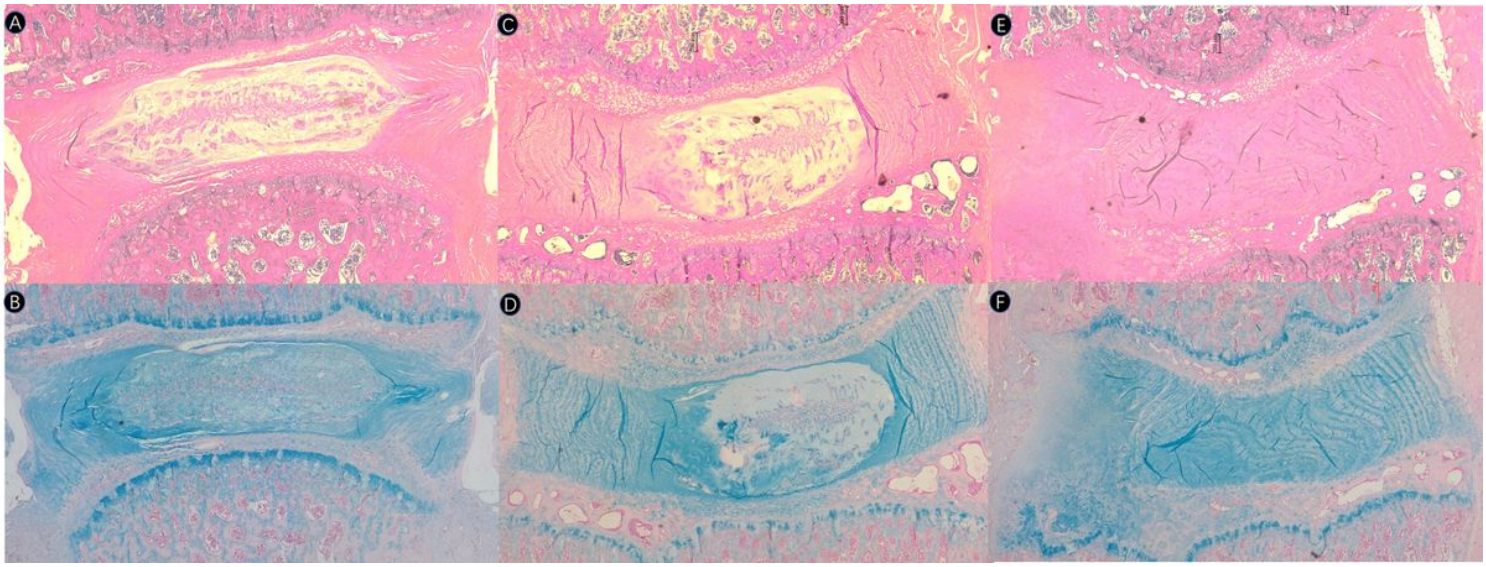

Figure 5

H&E staining (A, C, E) and alcian blue staining (B, D, F) results of control disc (A, B) and needle puncture disc (C-F). A showed the boundary between the NP and AF was distinct and large vacuoles and stellar-shaped nucleus dispersed in the nucleus. C showed the boundary between the NP and AF was not distinct and the number of cells decreased. E showed that connective tissue replaced the position of NP. B showed that deep blue stain in NP and at the border between AF and NP with slow fading in periphery. D showed that some prominent blue stain area in NP with slow fading in periphery within AF. F showed that blue stain area in the connective tissue without NP inside.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- table1.jpg
- table2.jpg