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ORIGINAL ARTICLE

Surgical technique for lumbar intervertebral disc transplantation in a goat model

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Abstract
Purpose Fresh-frozen intervertebral disc transplantation was determined to be an effective treatment for degenerative disc diseases in rhesus monkeys and in humans. Further research in improving different aspects of disc allografts transplantation is needed and will be investigated in large animal models. This study reports the detailed surgical technique of intervertebral disc transplantation without internal fixation and the important notes to ensure success in goats.
Methods Fifty-one male goats were used in this study. Ten goats were used as intervertebral disc allograft donors; the remaining forty-one goats were used to develop the surgical technique for intervertebral disc allograft transplantation. Radiographs, ex vivo MRI and gross observation were used to monitor the stability and healing of the disc allografts at 3 months, postoperatively.
Results Size matching of the disc allograft, preservation of the anterior longitudinal ligament and an appropriate portion of the annulus fibrosus at the recipient site were crucial for stable graft retention. Additionally, a slightly reduced height of the disc allograft compared to that of the recipient slot may avoid graft endplate fracture.
Conclusions Lumbar intervertebral disc transplantation without internal fixation can be successfully performed in goats.

Keywords Lumbar spine · Intervertebral disc · Surgical technique · Allograft · Transplantation

Introduction

Low back pain is one of the most common musculoskeletal disorders and causes disability and suffering globally [1]. Although many underlying factors may result in low back pain, a known determinant is intervertebral disc degeneration [2, 3]. Current treatments for severe degenerative disc diseases are most salvaging approaches (i.e., spinal fusion and artificial disc replacement); there is still no satisfactory way for prevention or reversal.

Recently, an innovative treatment for disc degeneration using fresh-frozen intervertebral disc allograft transplantation was successfully developed in primates [4], and was finally used in the cervical spines of 13 patients, where the allografts have provided acceptable clinical outcomes for up to 10 years [5, 6]. Here, the neurological symptoms, motion and stability of the spinal unit improved significantly, but degeneration of the transplanted disc allograft was observed in some cases at long-term follow-up [5]. To further improve the outcomes of this technology and to understand the mechanism of disc allograft degeneration postoperatively, much further research will need to be done. Nevertheless, in patients who are asymptomatic after disc allografting, it is not ethical to perform a biopsy, as this could accelerate the degeneration of the allografts. Therefore, an animal model for fresh-frozen disc allografting is needed.

J. Xiao and Y.-C. Huang contributed equally to this work.

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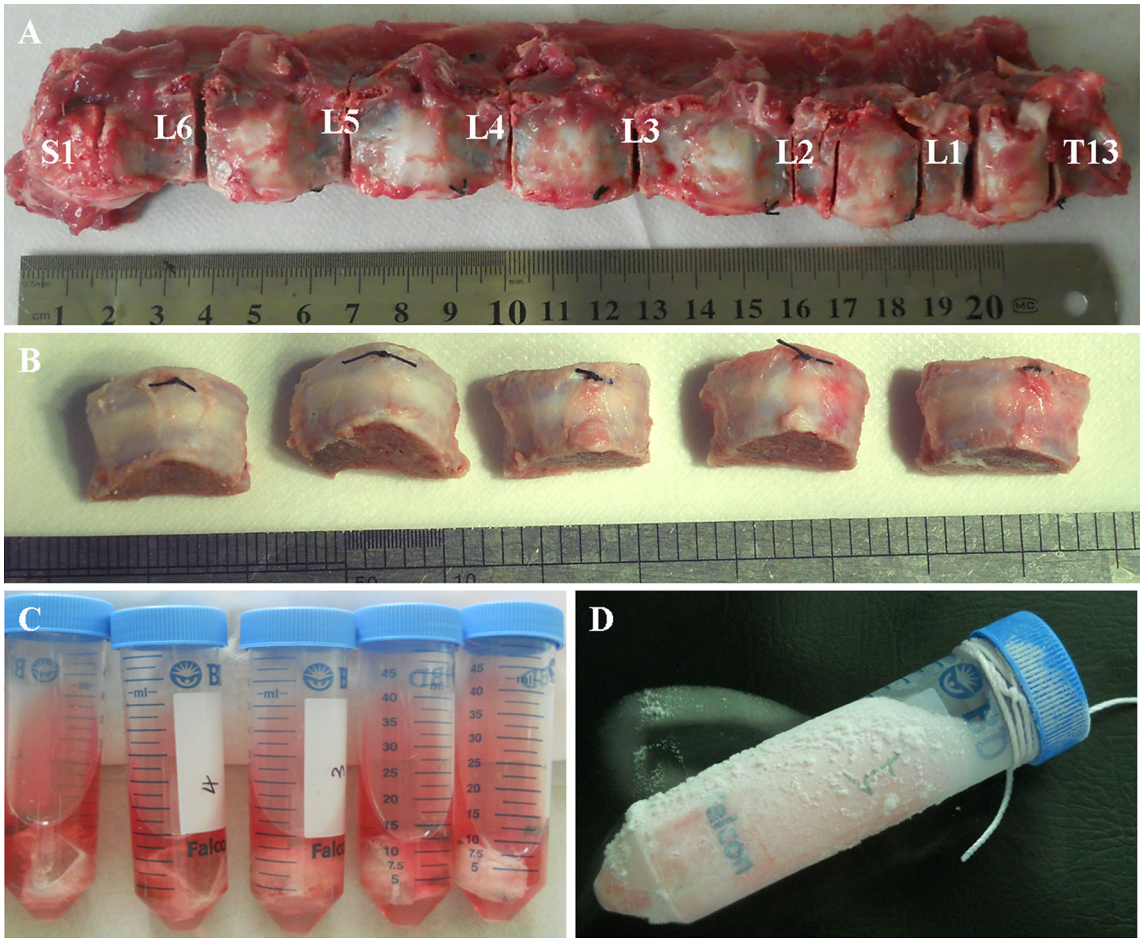

Fig. 1 Intervertebral disc allograft preparation from one donor goat. **a** Spinal column from T13 to S1 was harvested en bloc and then osteotomized at the mid vertebrae into segments. **b** Five lumbar IVDs were prepared as allografts; the endplates together with at least 5 mm of the adjacent vertebral bone were preserved. **c**, **d** The allografts were washed with saline and immersed in the cryopreservative solution at 4 °C for 2 h. The temperature was decreased stepwise to −80 °C over 3 h before being preserved in liquid nitrogen until transplantation

In our previous studies, rhesus monkeys were used for disc autografting, fresh disc allografting and finally fresh-frozen disc allografting [7, 8]. But now rhesus monkeys become largely unavailable and they have even been restricted for experiments in some counties. Hence, a more economical and ethically accessible animal model is preferred for the researchers in this field to replicate. At present, many quadrupedal animals such as mice [9], rats [10], rabbits [11] and goats [12–14] have been used for studying reparative treatments to spine related disorders. For fresh-frozen disc allografting, the goat was chosen because of the following reasons: (1) the lumbar disc of the goat is similar to that of the human in geometry and structure [15, 16]; (2) goats resemble humans in that they may have similar cellular behaviors in the disc [17, 18]; (3) the biochemical and biologic changes observed in the goat disc degeneration model are similar to those in human degenerative disc [19]; and (4) the goat is robust, does not bite and tolerates anaesthesia well [12].

Similar to the previous studies in the monkey [7, 8] and in the human [5], intervertebral disc allografting in the goat lumbar spine should include three steps: disc allograft preparation from the donor goats, lumbar spine exposure and preparation of the recipient slot, and implantation of the fresh-frozen disc allograft. The anatomy of the goat lumbar spine is different from that of primates and humans, especially the psoas muscle. To ensure stable seating of the allograft without internal fixation the surgical details may need to be tailored appropriately. Therefore, the aim of this study was to develop the surgical technique for successful fresh-frozen intervertebral disc allograft transplantation in the goat lumbar spine without internal fixation.

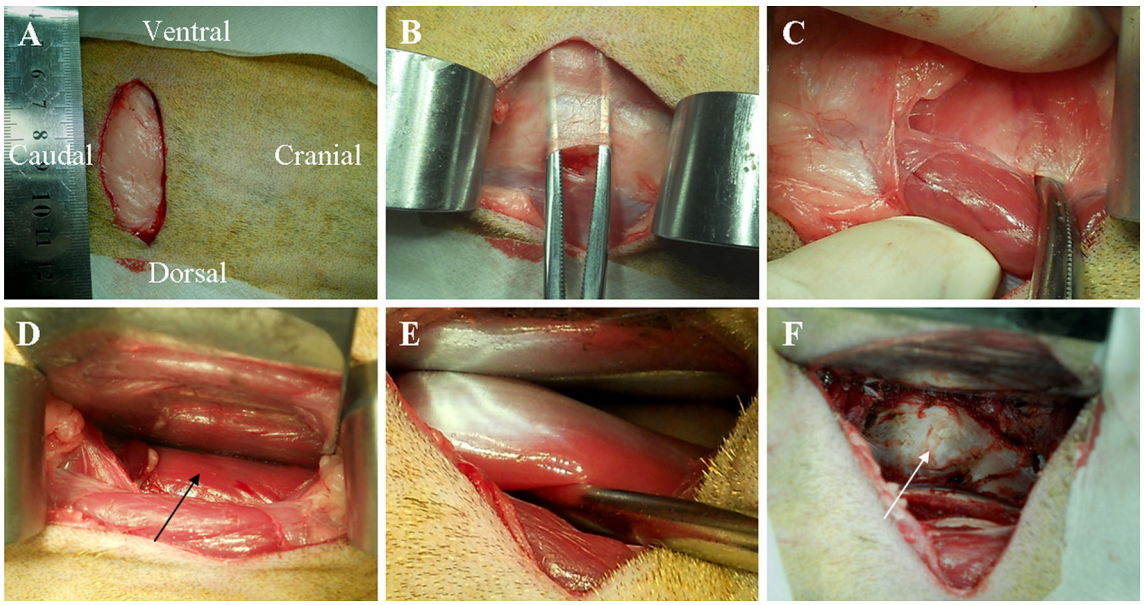

Fig. 2 Surgical technique for psoas muscle and lumbar intervertebral disc exposure. **a**–**d** Orientation of the goats at surgery and the retroperitoneal approach for the psoas exposure (the *black arrow*). **e**, **f** The "retro-psoas" approach for L4/L5 intervertebral disc exposure (the *white arrow*)

Materials and methods

Animals

The research proposal has been approved by Committee on the Use of Live Animals in Teaching & Research, The University of Hong Kong (CULATR1872-09). Totally, fifty-one male goats between 6 and 9 months and weighing between 20 and 27.5 kg were used in this study. Out of these goats, ten goats were used as disc allograft donors and the remaining forty-one goats as allograft recipients.

Preparation of fresh-frozen intervertebral disc allograft

Fresh-frozen disc allografts were prepared according to our previous description [8]. In brief, the donor goats were sacrificed by overdosed Pentobarbitone and the spinal column from T13 to S1 was harvested en bloc (Fig. 1a). After removal of the surrounding muscles, an osteotomy was performed at the mid vertebrae using a power saw. The excessive vertebral body was then removed with a high speed burr preserving at least 5 mm of the vertebral bone adjacent to the endplates. The five lumbar discs from L1/L2 to L5/L6 levels were selected as allografts. The posterior elements of the allografts were removed; a black suture was placed at the cephalic vertebral body to orientate the allografts during implantation (Fig. 1b). The allografts were then washed with saline and immersed in the cryo-preservative solution (10 % Dimethyl Sulfoxide + 10 % calf serum + 80 % DMEM-LG) at 4 °C for 2 h (Fig. 1c). The temperature was then decreased stepwise to −20 °C for 1 h, −40 °C for 1 h and −80 °C for 1 h before being preserved in liquid nitrogen until transplantation (Fig. 1d).

Surgical approaches for lumbar intervertebral disc exposure

All surgeries were performed by the same team. The goats were fasted for about 48 h before surgery. After general anaesthesia with an intravenous injection of a mixture of Ketamine (10 mg/kg) and Xylazine (0.1 mg/kg) and endotracheal intubation, the goat was fixed in the right lateral position and the skin was sterilized using iodine and alcohol. A left flank incision of 6–7 cm in length was made starting from the space between L4 and L5 transverse processes which can be clearly located by palpation (Fig. 2a). The external oblique muscle and internal oblique aponeurosis were cut open following the fibre direction extending to the aponeurosis of the erector spine muscle (Fig. 2b). The junction between the transversus abdominis and the erector spine muscle was exposed (Fig. 2c); the transverse abdominis was then divided up to the tip of the L5 transverse process. The psoas was exposed after mobilizing the peritoneum anteriorly and a retractor was placed medial to the psoas (Fig. 2d). Sequentially, the tips of the transverse processes were located by palpation and exposed by cautery dissection. The psoas was elevated anteriorly by dividing the fibre attachment to the anterior surface of the transverse processes using a blunt dissector

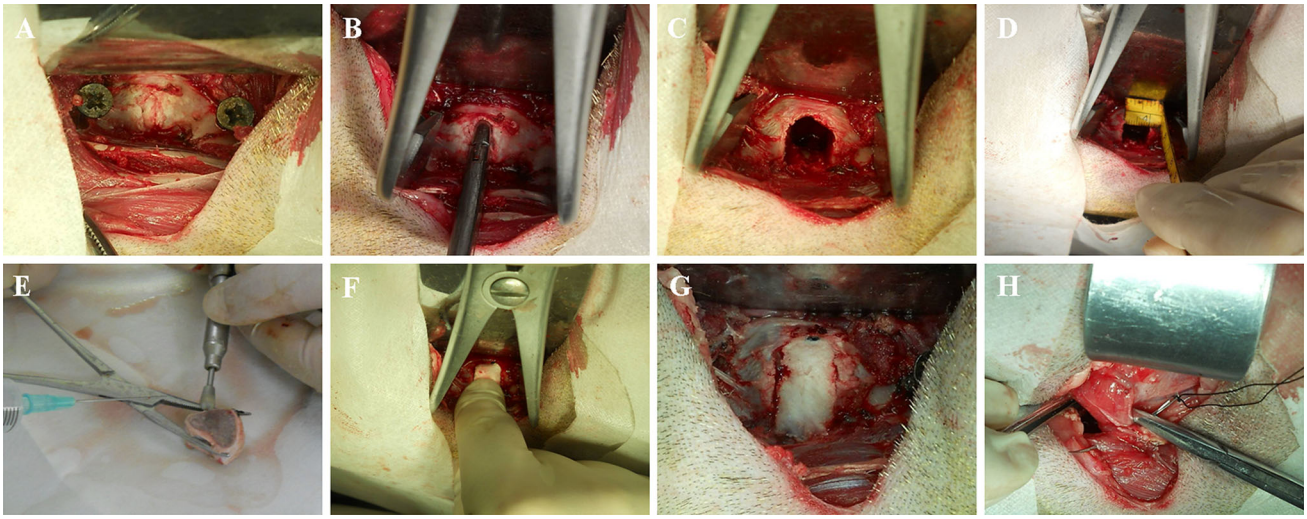

Fig. 3 Surgical technique for lumbar intervertebral disc allograft transplantation. **a** Two screws were inserted into the upper and lower vertebral bodies for distraction. **b**, **c** The posterior annular fibrosus, nucleus pulposus, the cartilaginous and bony endplates were removed; the anterior longitudinal ligament and appropriate portion of the anterior annulus fibrosus were preserved. **d**, **e** The height and the anteroposterior diameter of the recipient slot were measured. A frozen allograft of the most compatible size was selected and thawed. The redundant bone of the allograft was trimmed using a high speed burr. **f**, **g** The allograft was gently thumbed into the recipient slot. The distraction was removed and no internal fixation was required. **h** The psoas muscle was repositioned and the abdominal wound closed

(Fig. 2e). The psoas was then retracted with a narrow "S"-shaped blade placed anterior to the disc fending off the psoas and the peritoneum, the target intervertebral disc was properly exposed without fear of injuring the peritoneum or the intra-peritoneal organs (Fig. 2f). The exposure can be extended in both cranial and caudal directions as necessary.

Surgical technique for intervertebral disc allograft transplantation

After the lumbar intervertebral disc (L4/L5) was exposed clearly using the "retro-psoas" approaches, two temporary screws were inserted into the upper and lower vertebral bodies for placement of a distraction device (Fig. 3a). An osteotomy was made at the vertebral bodies 2.0–3.0 mm above and below the target disc. A 1 cm annulotomy was made lateral to the lateral edge of the anterior longitudinal ligament. This is followed by removal of the nucleus pulposus, posterior annular fibrosus, and the cartilaginous and bony endplates. It is essential to preserve as much as possible the remaining anterior annulus fibrosus and the anterior longitudinal ligament to avoid over laxity of the recipient slot (Fig. 3b, c). The posterior longitudinal ligament in the goat is thin thus care must be taken not to damage the dura and spinal cord while removing the posterior disc components and the posterior annulus. According to the height, lateral and anteroposterior width of the recipient slot, a fresh-frozen disc allograft of the most compatible size was selected and thawed completely at 37 °C. The vertebral bone of the allograft was trimmed appropriately using a high speed burr with saline rinse, avoiding fracture of the bony endplates (Fig. 3d, e); generally, the bony structure with the thickness of 2–3 mm was preserved at both the cranial and caudal ends. Saline rise was then performed to remove the bone marrow components. Sequentially, the allograft was gently thumbed into the slot with only finger pressure aligning the graft with the anterior vertebral margin (Fig. 3f, g). A bony punch must not be used as this will cause micro-fracture of the bony endplates. The distraction was removed and no internal fixation was used. The psoas muscle was repositioned and the abdominal wound was closed in layers (Fig. 3h). The goat was allowed to mobilize freely after recovering from the anesthesia.

X-ray analysis

Left lateral and anteroposterior (AP) X-ray images of the spine were taken immediately after surgery, and at 1 and 3 months postoperatively to monitor the position of the disc allograft and union of the bony surfaces.

Ex-vivo MRI scanning and gross observation

At 3 months postoperatively, the spinal columns from L1 to S1 were harvested en bloc from three goats for ex vivo MRI scanning (Philips Achieva 3.0T, PHILIPS, The Netherlands) with sagittal T1-weighted and T2-weighted fast spin-echo sequences. The T1-weighted sequence comprised the following: TR/TE = 518 ms/9.8 ms; matrix size = 200 ×

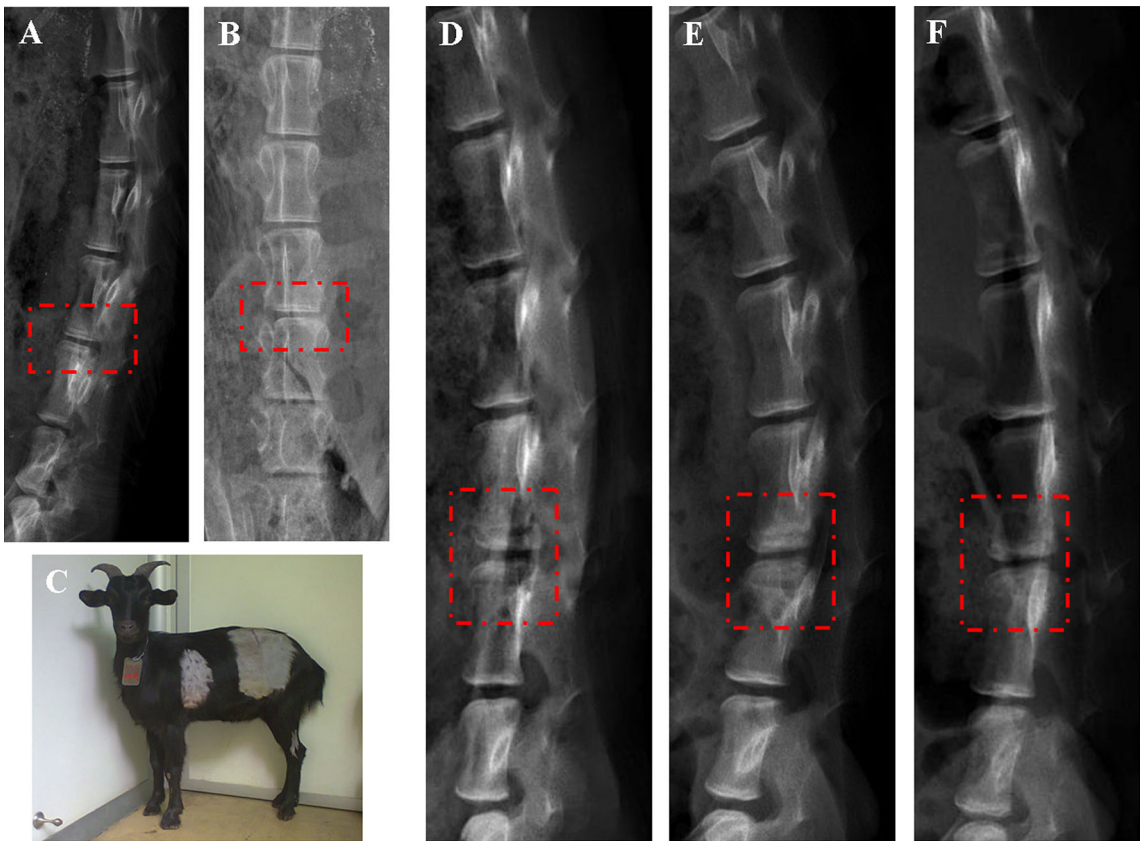

Fig. 4 Left lateral and anteroposterior (AP) X-ray images illustrating the results of the goats immediately after surgery, and at 1 and 3 months post-transplantation. **a**, **b** Lateral and AP view of the lumbar spine after disc transplantation. **c** The goat was allowed free activity after recovering from the anesthesia. **d–f** Lateral X-ray images showed the position of the disc allograft and union of the two bony surfaces (the *red dotted rectangle*) at 3 months postoperatively

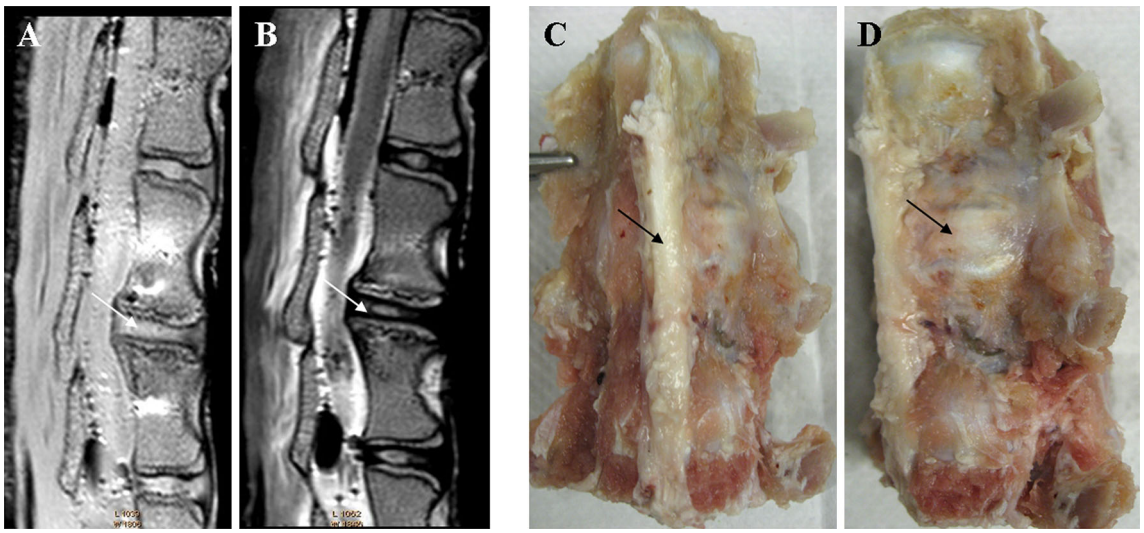

Fig. 5 Ex-vivo MRI scanning and gross observation of the IVD allograft-transplanted lumbar spine at 3 months postoperatively. **a**, **b** Ex-vivo T1-weighted and T2-weighted MRI scanning results of the disc transplanted segment (the *white arrow* shows the disc allograft post-transplantation). **c** Anteroposterior observation of the disc-transplanted segment, *black arrow* denoting the anterior longitudinal ligament. **d** Lateral observation of the disc-transplanted segment, *black arrow* denoting the disc allograft

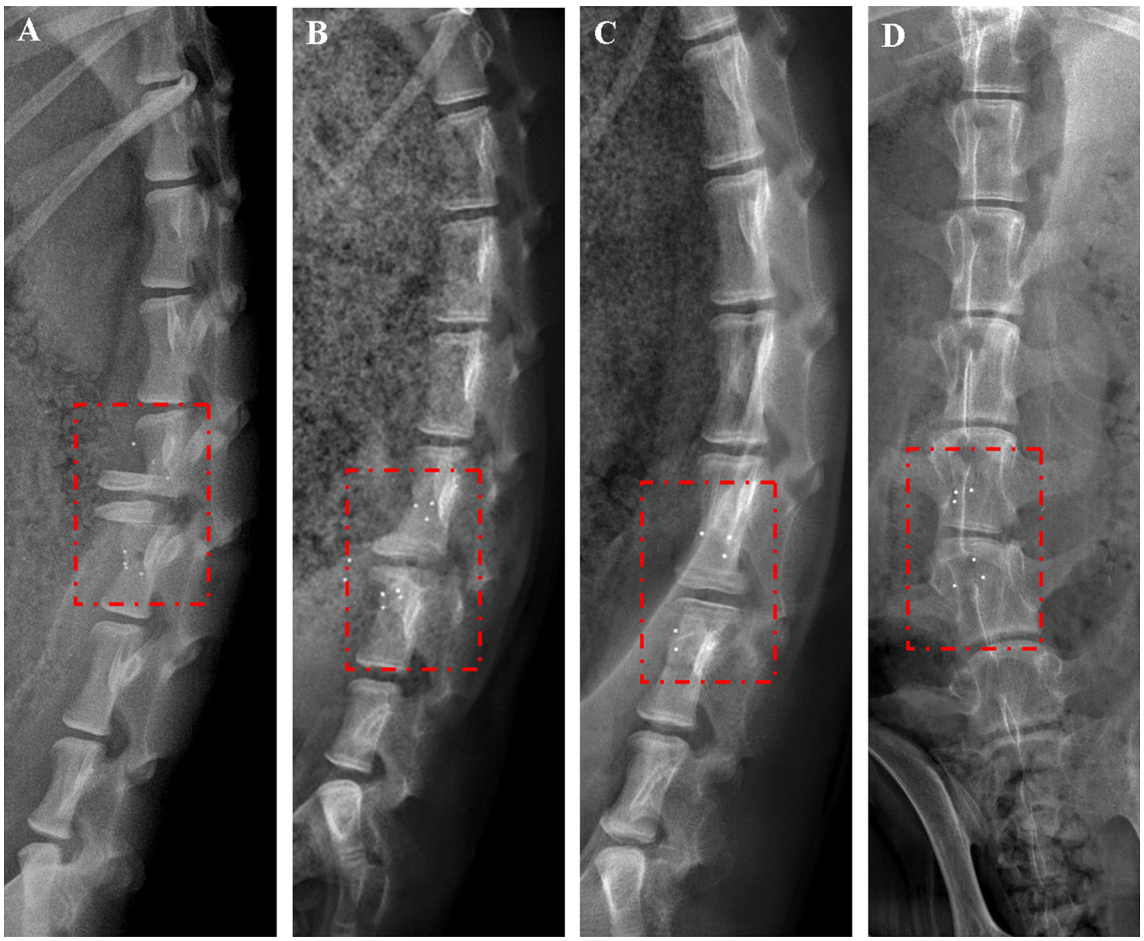

Fig. 6 X-ray images illustrating failures (the *red dotted rectangle*): graft dislocation (**a**), subluxation (**b**), spinal cord compression (**c**) and scoliosis (**d**)

200; FOV = 100 × 100 × 25.2 mm^3; Scan time = 5-min18sec; Slice thickness = 1.2; NSA = 3; TSE factor = 5; Mode = 2D. The T2-weighted sequence comprised the following: TR/TE = 2,854 ms/90 ms; matrix size = 200 × 192; FOV = 100 × 100 × 25.2 mm^3; Scan time = 5min51sec; Slice thickness = 1.2; NSA = 6; TSE factor = 12; Mode = 2D. After scanning, the psoas muscles were removed for gross observation.

Results

The lateral width, anteroposterior width and height of the selected disc allografts after trimming were 20.2 ± 1.1, 13.4 ± 0.5 and 10.0 ± 0.7 mm, respectively. With the use of the "retro-psoas" approach, clear exposure of the lumbar L4/L5 disc was obtained and disc allografts were implanted without internal fixation in 41 goats. As technical proficiency was gained, the operating team was able to reduce the operation time to approximately 90 min with an average of 60 ml blood loss. After the X-ray images on lateral and AP view were taken, the goats recovered from anesthesia.

The results were depicted in Fig. 4. As described in the x-ray images, the disc allograft was implanted at the level of L4/L5 and aligned with the adjacent vertebral bodies. No spinal canal compromise was found (Fig. 4a). Based on the AP view, the disc allograft was well-centered and no scoliosis was seen (Fig. 4b). The goat could move freely after recovering from anesthesia (Fig. 4c). At one month post-transplantation, the disc allograft remained stable without subluxation or dislocation and the two bony surfaces were well-united (Fig. 4d, e). Similar results were seen 3 months after transplantation (Fig. 4f). Based on the T1-weighted and T2- weighted MRI scanning, the disc allograft united well with the host vertebral bones and it was still hydrous at 3-month follow-up (Fig. 5a, b). Gross observation was shown that the anterior longitudinal ligament was well-preserved and disc allograft was positioned well in the recipient slot (Fig. 5c, d).

Nevertheless, a number of graft failures were encountered in the early period of our case series before the surgical technique was refined. There were three graft dislocations and four subluxations amongst the 41 goats (Fig. 6a, b). Spinal cord compression occurred in two cases (Fig. 6c) and scoliosis was observed in two other cases (Fig. 6d).

Discussion

The concept of intervertebral disc transplantation has been proven to be viable in the human cervical spine after many issues including harvesting of the allograft, preservation, implantation surgical techniques and immunoreaction have been carefully studied in primate experiments [5, 7, 8]. However, extending this technique to the lumbar spine remains a challenge. The size of the disc and the magnitude of loading are different from those in the cervical spine. While the disc allograft can serve the mechanical functions of maintaining stability and mobility, progressive degeneration of the transplanted disc of varying speed was seen at long-term follow-up of the human cases. Thus, there are many areas for further research and experiments in large animal model are required to address these gaps. It is therefore, important to develop a technically feasible and reliable surgical protocol that can be easily replicated by other researchers in this field.

At surgery, a 6–7 cm transverse incision that follows the skin crease lines while minimizes skin denervation does not limit extension of the exposure in a cranial to causal direction. The transverse process is an easy landmark to locate and affords accurate identification of the spinal level. Although "pre-psoas" technique for disc exposure was also suggested [20], the "retro-psoas" approach was our approach of choice because of relative ease, minimal bleeding, good exposure of the posterolateral corner of the disc, direct visualization and protection of the dura and exiting segmental roots, and finally the feasibility of cranio-caudal extension when needed.

According to our previous study in primates, size matching and press-fit fixation were essential to achieve primary stability of the disc allograft without internal fixation [4, 7, 8]. Several important points should be noted for success. The disc allograft with most compatible size should be selected to match the lateral and anteroposterior dimensions of the recipient slot; otherwise, graft dislocation, subluxation and spinal cord compression may occur (Fig. 6a–c). The height of the disc allograft after trimming should be about 2 mm less than that of the recipient slot while under distraction so that the disc allograft can be thumbed in easily without forceful insertion. Punching no matter how gentle is not acceptable. This is to avoid microfracture of the bony endplate. Additionally, to improve the stability of the allograft, the anterior longitudinal ligament and appropriate portion of the annulus fibrosus at the recipient site should be preserved. Last, during preparation of the recipient slot, the two bony surfaces should be kept parallel and the soft tension on the two sides should be similar in order to avoid scoliosis after graft insertion as shown on the AP view of an example in Fig. 6d.

After cryopreservation, it was found that more than 60 % of the disc cells kept the metabolic activity; and the mechanical properties and matrix organization of the disc allograft were also maintained [21, 22]. However, the disc allograft was immersed in an ischemic environment until the nutrition was reestablished [23]. Using this newly-developed goat model, it will become of great interest to investigate the nutrient-dependent mechanism regarding the degeneration of the postoperative disc allograft and to develop the reparative strategies.

We believe that the surgical technique of lumbar intervertebral disc exposure is also applicable to other quadruped species that have similar anatomy of large psoas muscles, such as sheep, dogs and pigs. Definitely, the surgical method for disc allograft implantation should also be suitable for tissue engineered disc composites implantation in large animals. But we should acknowledge that the goat as an animal model for disc allograft transplantation certainly does not reproduce the exact anatomy, kinematics and loading of the lumbar spine in the humans because the goats are quadruped [13, 24]. Nevertheless, this is the closest alternative short of the primates. This present study highlighted the technical details and tips for successful intervertebral disc allograft transplantation surgery. It should enable future large animal research in this field.

In conclusion, detailed surgical technique for lumbar intervertebral disc transplantation without internal fixation was successfully developed in a goat model. The "retro-psoas" muscle approach with transverse skin incision was recommended to expose the lumbar spine for intervertebral disc transplantation. Careful selection of size-matched disc allograft and meticulous preparation of the recipient sites were the key elements to successful disc transplantation.

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Conflict of interest None.

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