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The effects of Modic-III change on the osseointegration in cervical disc prosthesis: an experimental study in caprine models

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Abstract

Objective To quantitatively investigate the effects of Modic-III changes on the porous bone ingrowth at the interface of cervical disc prosthesis using caprine models.

Methods The Modic-III changes were induced at C3-4 level in eight goats by discectomy, followed by the implantation of cervical disc prostheses, while another eight goats served as a control group. Computed tomography (CT) and X-rays of cervical spine were performed intraoperatively and postoperatively at verify implant placement. The vertebral specimens were examined by micro-CT for histomorphometric quantification, including bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th). Methylene-blue/acid fuchsin staining, standard hematoxylin and eosin staining, and Masson staining were used for histologic evaluation. Immunohistochemical staining, including osteocalcin (OCN), alkaline phosphate (ALP), and runt-related transcription factor 2 (RUNX2), were also conducted.

Results All goats were followed for a period of 6 months after prosthesis implantation. The rate of prosthesis complications in experimental group was significantly higher than that in control group (37.5% vs. 12.5%, $P=0.046$). The histomorphometric parameters of experimental group, including BV/TV, Tb.N, Tb.Th, and bone ingrowth percentage were significantly lower than those of control group. The histologic sections of control group showed the excellent bone ingrowth and close contact between bone and prosthesis interface. By contrast, in experimental group, plenty of interfacial gaps were filled up with abundant fibrous tissue. The immunohistochemical sections of control group demonstrated the bone trabecula was surrounded by numerous osteoblasts, compared with the clear and smooth bone trabecula margin surrounded by few osteoblasts in experimental group. Moreover, the experimental group had significantly lower integrated optical density values of OCN, ALP, and RUNX2 staining.

Conclusion The Modic-III changes significantly impaired the osseointegration of artificial cervical disc in caprine models by reducing the number of osteoblasts, BV/TV, Tb.N, Tb.Th, bone ingrowth percentage and down-regulating the expression levels of ALP, Osteocalcin, and Runx-2, possibly leading to more occurrence of prosthesis complications.

Keywords Modic-III changes, Osseointegration, Caprine models, Cervical disc prosthesis

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Introduction

A rapidly established, strong, and long-lasting connection between an implant and bone is essential for the clinical success of orthopedic implants. This tight connection results from two kinds of implant stability [1]. One is the initial stability at the moment of implant surgery, which is usually provided by a press-fit mechanical interaction or acute fixation using keels, teeth, rails, etc [2]. The other is the secondary stability, which is obtained after a given healing period. The immediate post-operative stability is a necessary condition to obtain the implant osseointegration, while the long-term stability of in vivo implant depends on the quality of remodeling phenomena at the bone-implant interface, which induce changes of the multiscale biomechanical properties of bone tissue located in the vicinity of the implant surface [1].

Over the past decade, cervical disc replacement (CDR) has gained popularity among some spine surgeons and has become a viable option to treat the patients with refractory cervical disc degenerative disease. However, despite of the satisfactory results, complications like subsidence, migration, and even dislocation were also reported [3]. Modic-III changes, characterized by the hypointense on both T1-weighted imaging (T1WI) and T2-weighted imaging (T2WI) at degenerative vertebral endplate and subchondral bone marrow, were thought to represent subchondral bone sclerosis [4]. Clinical study noted that preoperative Modic-III changes at instrumented level could result in the significant lower bony fusion rate after posterior lumbar interbody fusion surgery [5], indicating that Modic-III changes might impair the bone ingrowth into prothesis surface. However, to our knowledge, there was no experimental study supporting this view in cervical field.

Our previous study has evaluated the extent of osseointegration at the cervical disc prosthesis-bone interface in caprine models [6]. The purpose of this study was to quantitatively investigate the effects of Modic-III changes on the porous bone ingrowth at the interface of Pretic-I Disc (Trauson) using caprine models.

Methods

Animal research permission

The Institutional Animal Care and Use Committee at the West China Center of Medical Science, Sichuan University granted approval for this investigation. Conduct of experimentation on living animals followed the recommendations of the Guide for the Care and Use of Laboratory Animals [7], and under the close supervision of qualified and experienced persons. All experiments were performed in accordance with relevant guidelines and regulations. The research complies with relevant guidelines (e.g. the revised Animals (Scientific Procedures) Act 1986 in the UK and Directive 2010/63/EU in Europe) and/or ethical approval. We comply with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Prosthesis introduction

The Pretic-I Disc (Fig. 1) consists of two titanium alloy plates (Ti6A14V) and a hemispherical core (ultra-high-molecular-weight polyethylene, UHMWPE). This ball-in-socket design allows the superior plate to move back and

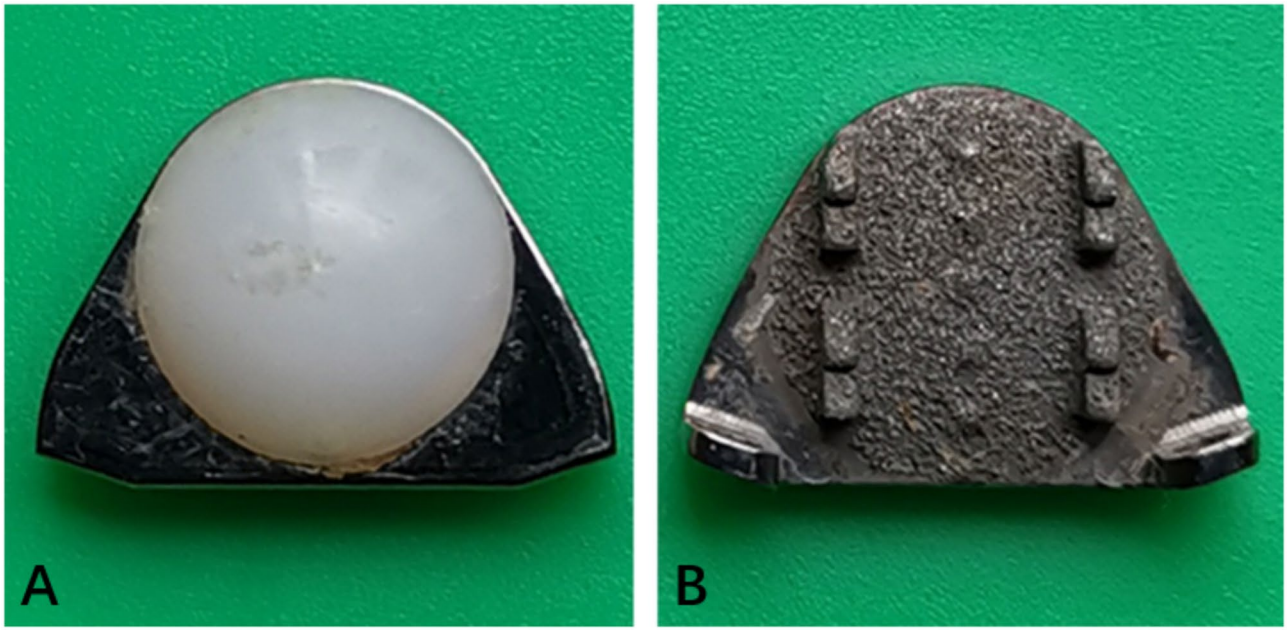

Fig. 1 The structure of the Pretic-I Disc. **A** The inferior image of the cranial end of Pretic-I Disc; **B** The superior image of the cranial end of Pretic-I Disc

forth along a slot in the horizontal direction, resulting in a mobile center of rotation. There are two rows of dentate crests in the back surface of each plate to improve the initial stability of the prosthesis and avoid implant migration. The surface of plate is sprayed with a hydroxyapatite coating to facilitate bone ingrowth [8].

Study design

Sixteen mature goats (the mean age of 14 months old and the mean weight of 18 kg) from the animal center of Sichuan University were used in this study. The Modic-III changes were induced at C3-4 level in eight goats by discectomy (Fig. 2), while another eight goats served as a control group. The detailed procedures for establishing animal model were described in previous research [9]. In control group, after exposure, the anterior longitudinal ligament and posterior longitudinal ligament were cut, and the intervertebral disc at the surgical level was completely removed to achieve a thorough decompression. Then, use a curettage to deal with the cartilage endplate and a high-speed burr was used to prepare the bony endplate and the Pretic-I Disc was implanted into animals. In experiment group, to prepare a model of endplate degeneration, we usually use a curettage to scrape off the lower cartilage endplate of the upper vertebral body. Computed tomography (CT) and X-rays were conducted at 2, 4, 6 months after establishing model to confirm the presence of Modic-III changes at the surgical level. Then, high-speed burr was used to prepare the bony endplate and the Pretic-I Disc was implanted into all animals. All goats were followed for a period of 6 months after prosthesis implantation.

Surgical preparation and procedures

Each animal was sedated with an intravenous injection of anesthetic medications (diazepam 0.2 mg/kg and ketamine HCL 5 mg/kg), followed by endotracheal intubation and general inhalation anesthesia using 1–2% isoflurane with continuous intravenous fluids (range 3–6 mL/lb/h) administered for the duration of surgery. In addition, prophylactic intravenous antibiotics (cefazolin sodium, 1 g) and analgesics (butorphanol 0.1 mg/kg) were administered before and after surgery.

The anterior Smith-Robinson approach to the cervical spine was adapted to the goat through a right-sided longitudinal incision with the length of 6–8 cm. Once the anterior cervical vertebral elements were exposed [10], the C3-4 intervertebral disc was radiographically identified, and a standard anterior cervical discectomy and decompression of the spinal canal were performed. The Pretic-I Disc was then implanted at the C3-4 level under fluoroscopic control. Blood loss, operating time, and intraoperative and perioperative complications were quantified.

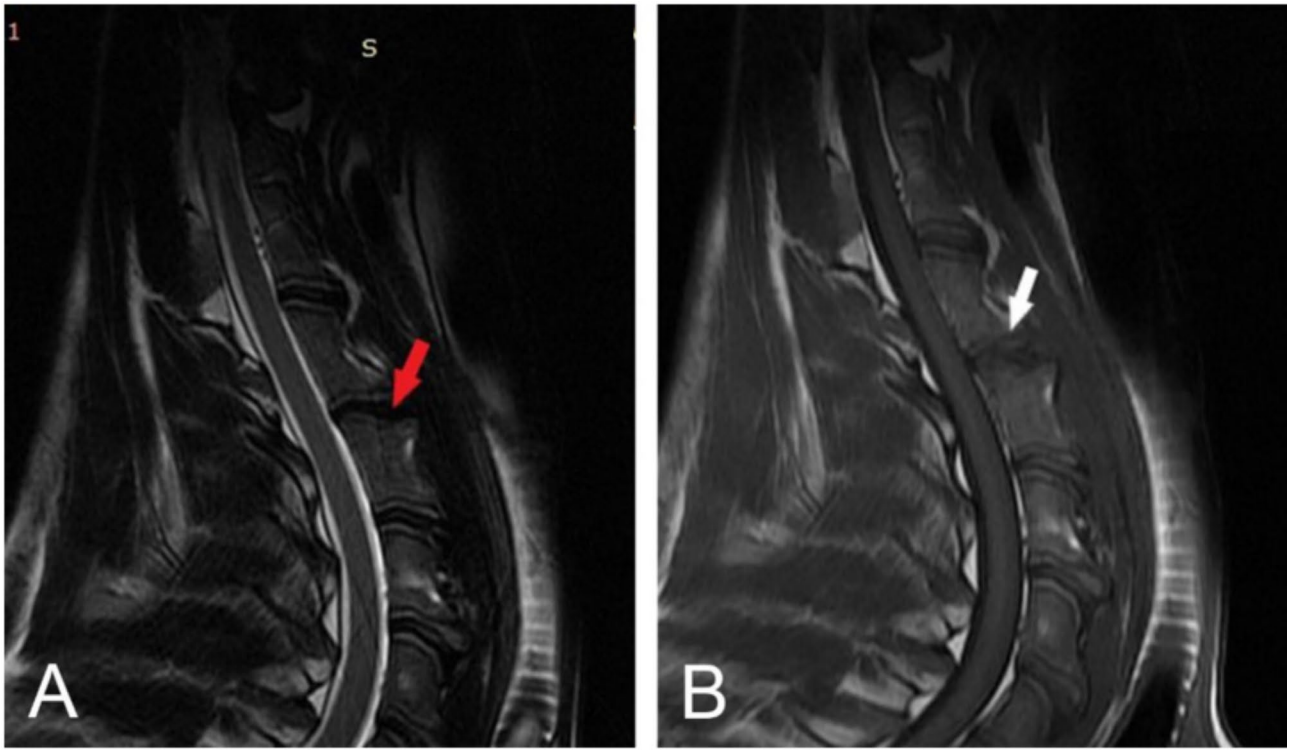

Fig. 2 MRI at 4 months after discectomy showing the Modic-III changes at vertebral endplate. **A** Hypointense on T1-weighted imaging (red arrow); **B** Hypointense on T2-weighted imaging (white arrow)

Observations of ambulatory activities and wound healing were monitored daily, and all animals received analgesics and prophylactic antibiotics for the first 10 days after surgery. X-rays and CT of cervical spine were obtained intraoperatively and postoperatively at 1 week, 1, 3 and 6 months to verify implant placement. Then, euthanasia was performed for each animal using an overdose (150 mg/kg) of concentrated pentobarbital solution (390 mg/mL) at 6 months after prosthesis implantation. The spinal column then was carefully dissected, immediately placed in double-wrapped plastic specimen bags, and frozen at -25 °C for subsequent radiographic and histologic evaluation.

Histomorphometric evaluation

The operative segments were examined by Micro-CT to obtain histomorphometric parameters, including bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th). The Micro CT scanning parameters are set to voltage of 90KV, current of 88uA, resolution of 90 μm, scanning time of 14 min, and 360 degrees rotation (Micro CT small animal imaging system, PerkinElmer Quantum GX, provided by Sichuan University Public Experimental Platform). The DICOM format data obtained after scanning is imported into the Micro CT/Small Animal Live Imaging Data Analysis System (device model: Analyze 12.0) for analysis, and Micro CT has limitations in morphological analysis when approaching metals. Using RadiAnt DICOM Viewer, the prosthesis surface in contact with vertebral endplate was traced manually and expressed as a total endplate area pixel count. The regions of trabecular contact were subsequently traced, quantified in pixels, and expressed as a percentage of the total endplate area (% ingrowth = bone contact area/gross total endplate area). The overall bone ingrowth percentage was calculated by averaging the ingrowth percentage of sagittal and coronal Sect. [11] (Fig. 3).

Histologic and immunohistochemical evaluation

The specimens were fixed in 10% neutral buffered formalin solution, then dehydrated in a series of graded alcohol which was later substituted by dimethylbenzene and embedded in paraffin. Using thin-sectioning microtomy, the paraffin embedded sections were cut into 3–5 μm thickness, and then evaluated by 3 staining techniques: methylene-blue/acid fuchsin staining, standard hematoxylin and eosin staining, and Masson staining. Immunohistochemical staining including osteocalcin (OCN), alkaline phosphate (ALP), and runt-related transcription factor 2 (RUNX2) were also conducted, which was analyzed by Image-Pro Plus 6.0.

Statistical analysis

Quantitative data were presented as mean ± standard deviation. Chi-square and independent t tests were used to compare categorical and continuous variables, respectively. All data were analyzed using SPSS (version 19.0; SPSS Inc., Chicago, Illinois). $P < 0.05$ was considered statistically significant.

Results

All 16 animals survived the surgery and postoperative period without incidence of vascular, neurologic or infectious complications. The average operating time was 78.6 ± 18.6 min (range 60–100 min), with an estimated blood loss of less than 50 ml. All animals were ambulatory after 24 h and experienced normal wound healing.

X-ray and CT analysis

X-rays showed a case of anterior migration was recorded in control group at 6 months after surgery, while a case

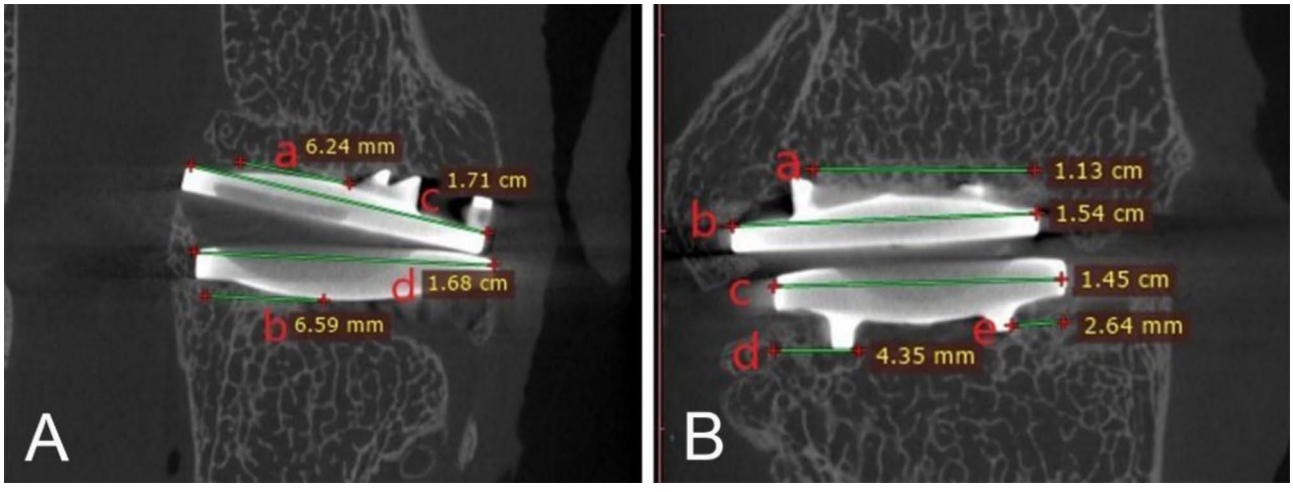

Fig. 3 The measurement of bone ingrowth rate. **A** Sagittal bone ingrowth rate = (a + b)\(c + d); **B** Coronal bone ingrowth rate = (a + d + e)\(b + c)

of prosthesis dislocation in experimental group at 3 months after surgery (Fig. 4). Micro-CT images showing endplate sclerosis, prosthesis dislocation and gaps in bone-prosthesis interface in experimental group, while the close contact of prosthesis-bone interface in control group. (Fig. 5). In the last follow-up, 3 cases of prosthesis dislocation in experimental group and a case of anterior migration was recorded in control group. The rate of prosthesis complications in experimental group was significantly higher than that in control group (37.5% vs. 12.5%, $P = 0.046$).

Histomorphometric

The histomorphometric parameters of 2 groups were presented in Table 1. Overall, the histomorphometric parameters of experimental group, including BV/TV, Tb.N, and Tb.Th, were significantly lower than those of control group ($P < 0.001$). Also, the histomorphometric analysis at the prosthesis-bone interface demonstrated a significantly lower bone ingrowth percentage in experimental group (44.26% ± 6.55% vs. 75.83% ± 4.73%, $P < 0.001$).

Histologic and immunohistochemical analysis

Overall, the histologic sections of control group showed the excellent bone ingrowth and close contact between bone and prosthesis interface, without the presence of particulate wear debris or significant histopathologic changes. By contrast, in experimental group, plenty of interfacial gaps were filled up with abundant fibrous tissue (Fig. 6). The immunohistochemical sections of control group demonstrated the bone trabecula was surrounded by numerous osteoblasts, compared with the clear and smooth bone trabecula margin surrounded by few osteoblasts in experimental group (Fig. 7). Moreover, the experimental group had significantly lower integrated optical density values of OCN, ALP, and RUNX2 staining (Fig. 8).

Discussion

To the best of our knowledge, this study quantified the negative impacts of Modic-III changes on the osseointegration of artificial cervical disc in caprine models for the first time. The histomorphometric data demonstrated the preoperative Modic-III changes led to significantly less bone volume density, trabecular number, trabecular thickness, and mean porous ingrowth, in comparison with normal controls. Histologic sections showed the wide gaps between bone and prosthesis interface, which were filled up with abundant fibrous tissue after Modic-III changes. Immunohistochemical sections also indicated the clear and smooth bone trabecula margin surrounded by few osteoblasts in experimental group. These results collectively proved that Modic-III changes remarkably impaired the osseointegration at bone-implant interface. This poor bone production and maturation might attribute to the high rate of prothesis complications in experimental group (37.5% vs. 12.5%, $P = 0.046$). The important reason is that during the formation of Modic III changes, bone sclerosis under the endplate and endplate deflection continues to develop, the inflammatory response increases, the water content in the endplate decreases, and the degeneration process is accelerated after intervertebral disc resection. Endplate sclerosis and endplate deflection have a significant impact on the growth and insertion of bone trabeculae, which greatly hinders subsequent bone healing. In addition, the causes of Modic III changes also include biomechanical changes in the intervertebral space, which have a significant impact on the osteogenic ability after arthroplasty surgery.

Modic changes, which comprise vertebral endplate and bone marrow lesions seen on MRI, were systematically described and classified by Modic et al. in 1988 [12]. The pathophysiology of Modic change was complex. In their original study, Modic-I changes were associated with disruption and fissuring of endplates and formation of a fibrovascular granulation tissue, representing bone marrow edema and inflammation; Modic-II changes were involved in the conversion of normal red hemopoietic bone marrow into yellow fatty marrow, as a result of marrow ischemia; Modic-III changes demonstrated cicatrization phase of disc degeneration [12]. It appeared that Modic changes were dynamic markers of the normal age-related degenerative process. These lesions could convert from one type to another with time, with mixed-type changes probably representing the intermediate stages in this conversion [13]. However, Modic-III changes, the sclerotic stage of degenerative disk disease, were supposed to be much more stable than type I and type II changes [4]. This stability of Modic-III state ensured the feasibility and reliability of successful animal model establishment. During the long waiting period, there may be some changes in the local area of the cervical spine. Firstly, after the removal of the intervertebral disc, there will be biomechanical changes in the local area, resulting in changes in the force on the endplate during this period and may impact on subsequent intervertebral disc placement. However, due to the limited stress of the intervertebral disc can withstand and the strong compensatory ability of the goat cervical bone structure, the biomechanical properties are not significantly affected. Secondly, during the gradual degeneration of the endplate, local inflammatory factors accumulate, leading to an increased possibility of local infection. After surgery, we used antibiotics to prevent infection in goats with a tendency towards infection, and ultimately no goats experienced infection. Finally, surgical modeling makes

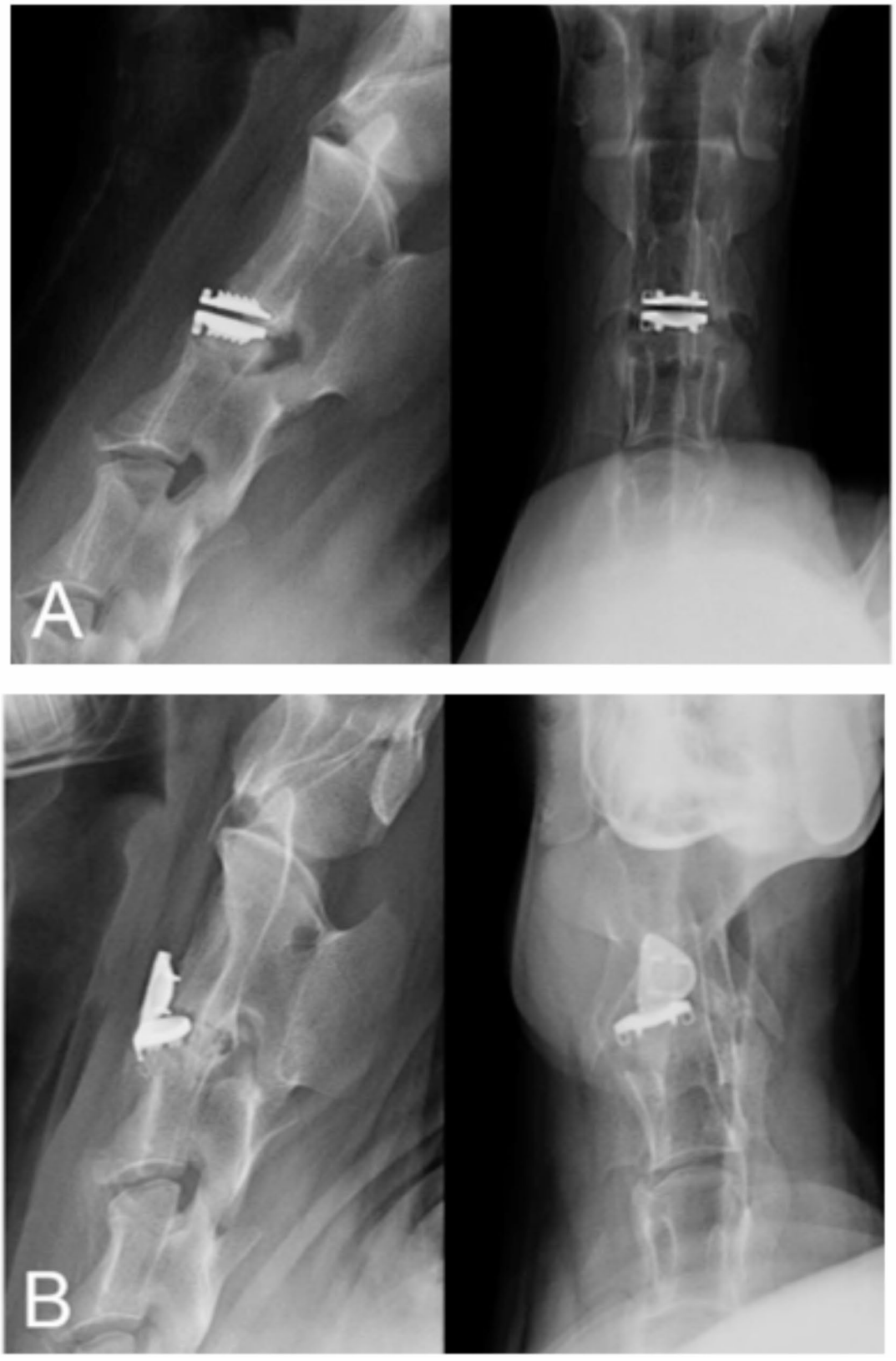

Fig. 4 Lateal and coronal X-rays. **A** A case of anterior migration was recorded in control group at 6 months after surgery; **B** A case of dislocation in experimental group at 3 months after surgery

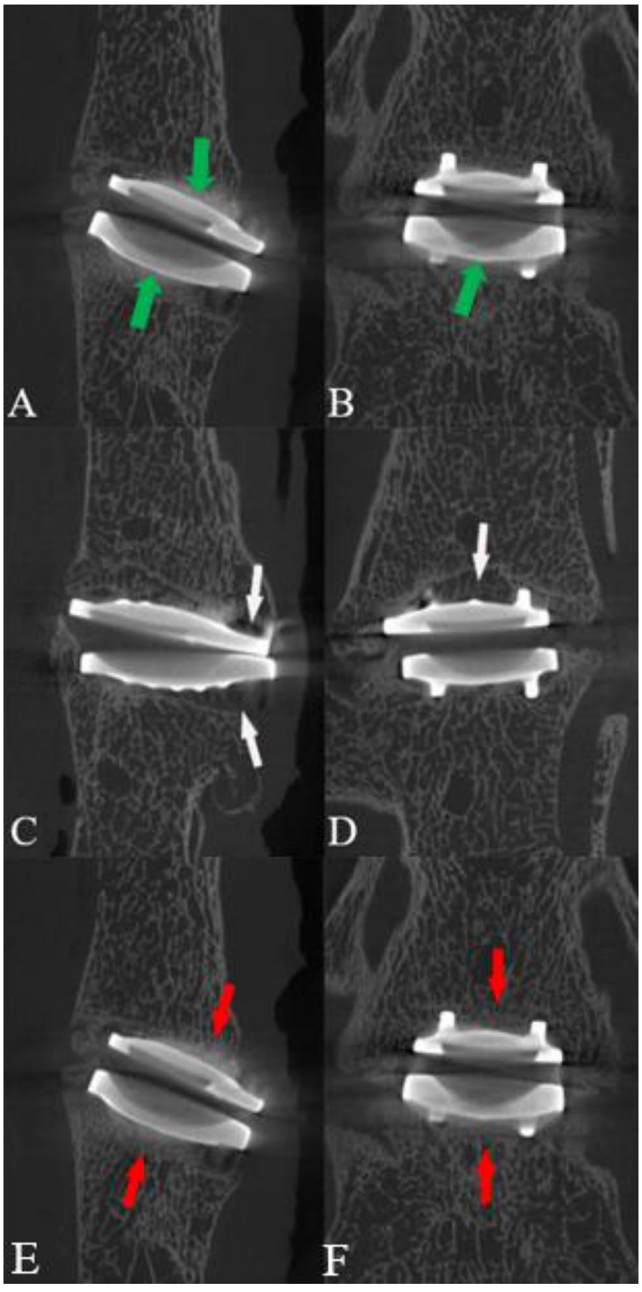

Fig. 5 Micro-CT images. **A**, **B** Case presentation in experimental group showing endplate sclerosis (green arrows) and prosthesis dislocation; **C**, **D** Case presentation in experimental group showing gaps (white arrows) in bone-prosthesis interface; **E**, **F** Case presentation in control group showing the close contact (red arrows) of prosthesis-bone interface

Table 1 The histomorphometric parameters of 2 groups at 6 months after surgery

Variables	Control group	Experimental group	*P* values
BV/TV (%)	73.19 ± 4.64	53.22 ± 6.54	$P = 0.001$
Tb. N (mm^{-1})	1.70 ± 0.13	1.15 ± 0.22	$P = 0.001$
Tb. Th (mm)	0.41 ± 0.07	0.28 ± 0.08	$P = 0.001$

the local tissue sticky, which poses difficulties for the subsequent intervertebral disc insertion operation. During the process of inserting intervertebral discs into the experimental group, we did find some tissue to be sticky and edematous, but it did not cause significant trouble for us to continue inserting intervertebral discs. We believe that the potential impact of a six-month waiting period on surgery can be ignored, and there is no significant difference between the experimental group and the control group. In addition, antibiotics are also a key

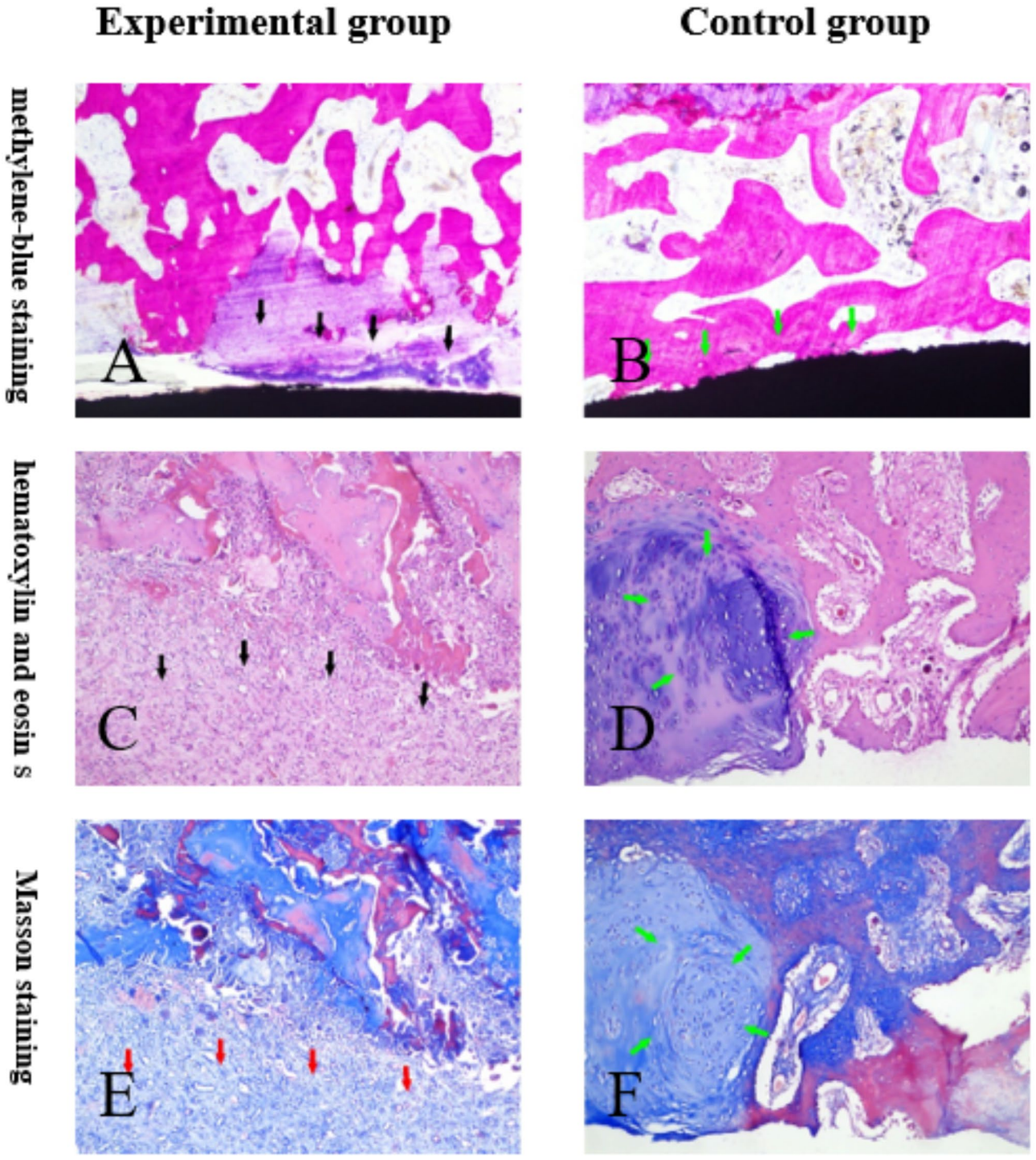

Fig. 6 Histologic sections (×100) at 6 months after prosthesis implantation. **A** Methylene-blue/acid fuchsin staining showing the gap between bone and prosthesis interface filled up with fibrous tissue (black arrows) in the experimental group; **B** The excellent bone ingrowth rate and close contact between bone and prosthesis interface (green arrows) in the control group; **C**, **E** Hematoxylin and eosin staining Masson staining showing the abundant fibrous tissue without apparent bone ingrowth (black and red arrows) in the experimental group; **D**, **F** The formation of woven bone (green arrows) in the control group

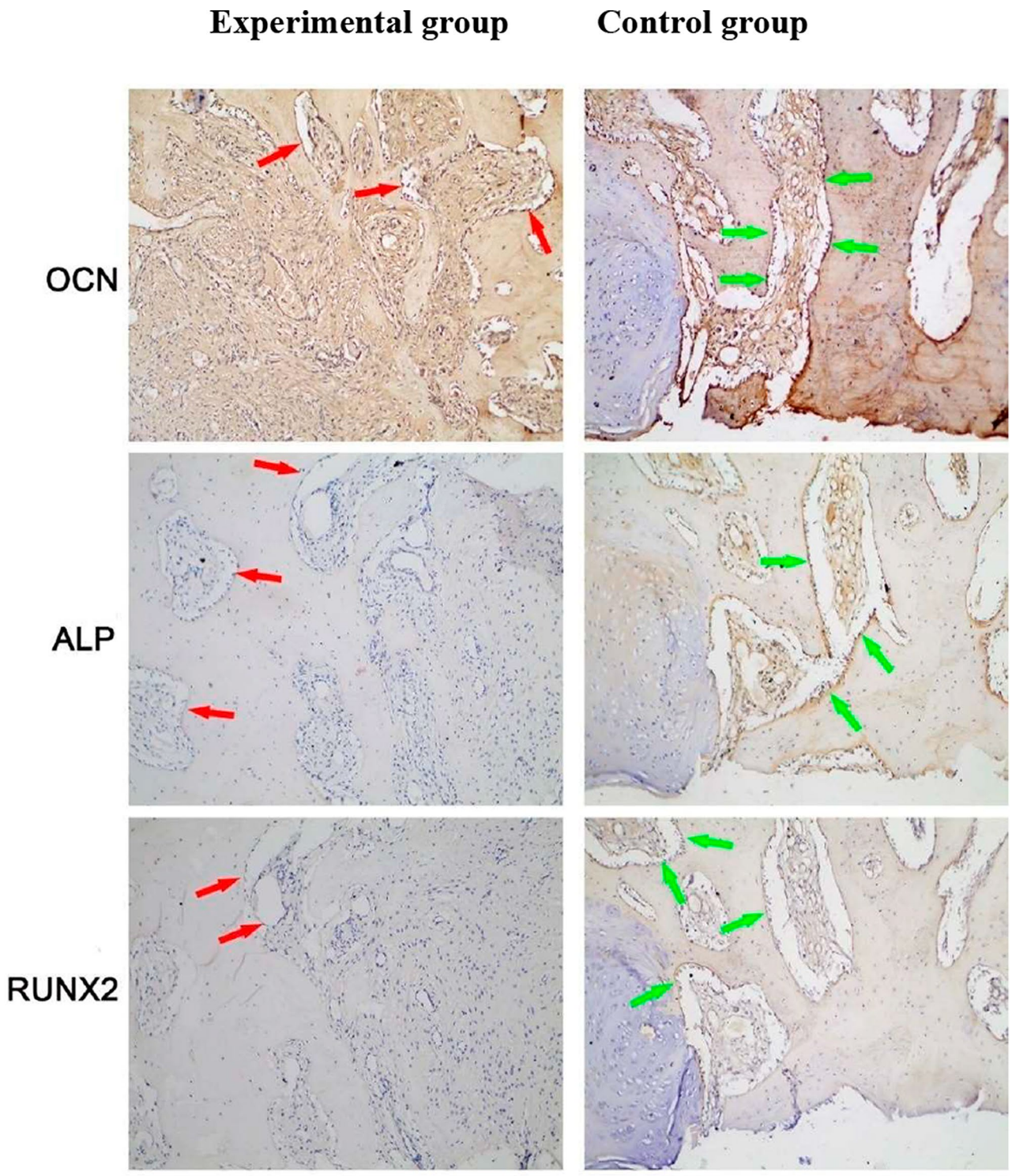

Fig. 7 Immunohistochemical sections (×100) at 6 months after prosthesis implantation. The bone trabecula was surrounded by numerous osteoblasts in control group (green arrows). However, in experimental group, the margin of bone trabecula was clear and smooth, surrounded by few osteoblasts (red arrows)

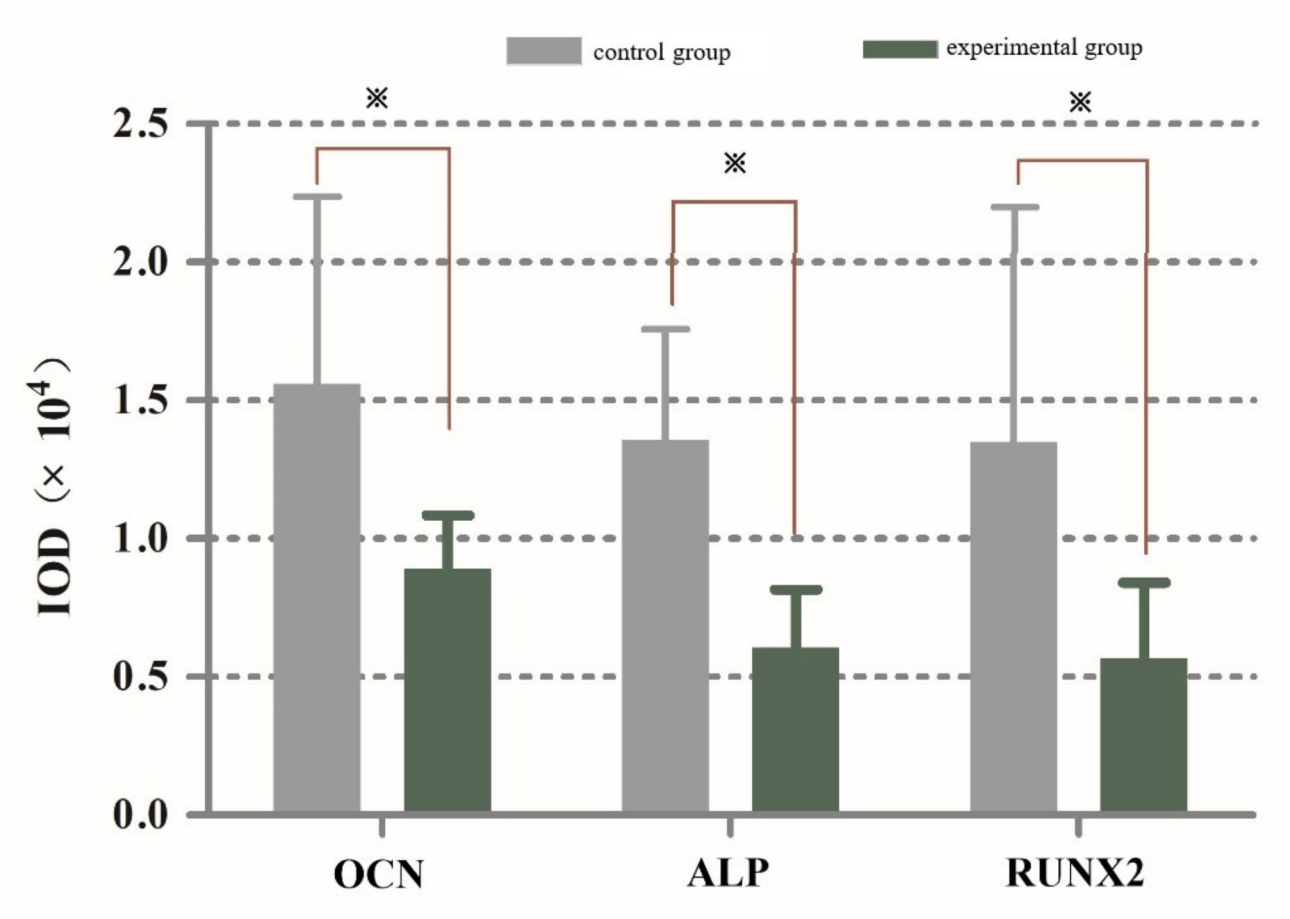

Fig. 8 The bar chart showing the experimental group had significantly lower integrated optical density (IOD) values of OCN, ALP, and RUNX2 staining

consideration factor. Antibacterial drugs can cause a significant loss of calcium in the body after use, which may lead to bone destruction and induce osteoporosis. After bone damage, it can trigger bone hyperplasia. However, we believe that the drug concentration in the intervertebral disc is also quite low within the effective blood drug concentration time, because intervertebral disc tissue is a tissue organ without blood vessels. Therefore, the effect of antibiotics on intervertebral disc metabolism is an interesting question, and we will continue to explore this issue in future research.

Implant osseointegration consists in the time evolution of bone structure to obtain a direct, structural, and functional connection between living bone and the loaded implant surface. Previous study [6] reported the excellent porous osseointegration at the prosthesis-bone interface in the Prestige LP Disc (Medtronic Sofamor Danek, Memphis, TN, USA), which were in agreement with the findings of control group in this study. On the one hand, the serrated crests provide the better primary press-fit fixation, resisting pull-out or excessive micromotion. On the other hand, the porous hydroxyapatite spray coating facilitates bone ingrowth in the long term. Both factors encourage the implant osseointegration, where newly formed bone tissue is in intimate contact with the implant surface so that at the microscopic level, no interposition of fibrous tissue occurs [14]. This osseointegration process was important because the biomechanical properties of bone-implant interface are the key determinants for the long-term implant stability as well as for the evolution of the implant status [15]. However, the bone-implant interface properties are not only determined by the quantity and quality of the implant surface, but also largely influenced by the physiological function and biomechanical traits of bone tissue around the interface [1]. This study found that the Modic-III changes at vertebral endplate could significantly undermine the implant osseointegration and increase the risk of prosthesis loosening or migration.

We speculated the following factors might contribute to these results. First, Modic-III changes were usually accompanied by endplate sclerosis and endplate deflection, both of which might compromise the mechanical competence of vertebra [16]. This assumption was also supported by the significantly decreased bone volume density, trabecular number, and trabecular thickness

of experimental group in this study. Second, the shape mismatch between inferior endplates and flat-footprint prosthesis are prevalent. Such morphological mismatch, if left untreated, would result in limited contact between cervical endplates and artificial discs, eventually causing interfacial gap as well as increased contact stress [17]. In the case of irregular endplates, which were common in Modic-III changes, we had to mill more endplate bone to maximize the contact area. However, more endplate bone removal meant inevitably less endplate strength. Cheng et al. reported a significant loss of endplate strength when 1 mm of endplate (44% loss) or 2 mm of endplate (52% loss) was removed [18]. These two factors resulted in substantially declined endplate strength, which might impair the initial stability and cause excessive micromotion after the prosthesis insertion, eventually leading to poor bone ingrowth at the interface [14]. On the other hand, the Modic-III changes or endplate sclerosis could significantly reduce the blood and nutrition supply to the endplate [19], exerting a negative impact on the bone modeling and osseointegration on the prothesis surface in the long run.

There were several limitations in the present study. First, only the effects of Modic-III changes on osseointegration were investigated because there was no feasible and reliable method to create stable Modic-I and Modic-II changes in animal models. Modic-I and Modic-II changes could convert from one type to another with time [13]. Second, although the C3-4 segment of sheep was widely used as a model in cervical spine research, it could not completely stimulate the biomechanical and physiological properties of human cervical spine [20].

Conclusion

The Modic-III changes significantly impaired the osseointegration of artificial cervical disc in caprine models by reducing the number of osteoblasts, BV/TV, Tb.N, Tb.Th, bone ingrowth percentage and down-regulating the expression levels of ALP, Osteocalcin, and Runx-2, possibly leading to more occurrence of prosthesis complications.

Abbreviations

CT	Computed tomography
BV/TV	bone volume fraction
Tb.N	trabecular number
Tb.Th	trabecular thickness
OCN	osteocalcin
ALP	alkaline phosphate
RUNX2	runt-related transcription factor 2
CDR	cervical disc replacement
T1WI	T1-weighted imaging
T2WI	T2-weighted imaging

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Author contributions

LC and ZX provided equal contributions to this study, both of them collected all data and prepared the manuscript. XH performed statistical analysis and interpreted the data. Analysis of radiographs was performed by ZX. YY helped in the statistical analyses. HL designed the study and was a major contributor to the preparation of the manuscript. The authors have read and approved the final manuscript.

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Data availability

Datasets are available from the corresponding author on a reasonable request.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval and consent to participate

The Institutional Animal Care and Use Committee at the West China Center of Medical Science, Sichuan University granted approval for this investigation. Conduct of experimentation on living animals followed the recommendations of the Guide for the Care and Use of Laboratory Animals, and under the close supervision of qualified and experienced persons. All experiments were performed in accordance with relevant guidelines and regulations. The research complies with relevant guidelines (e.g. the revised Animals (Scientific Procedures) Act 1986 in the UK and Directive 2010/63/EU in Europe) and/or ethical approval. We comply with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Consent for publication

Not Applicable.

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