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Original Article

Analysis of bony ingrowth in novel cervical disc prosthesis

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Abstract: A study was carried out to investigate the porous ingrowth and histologic characteristics at the prosthesis-bone interface of cervical disc replacement with a novel cervical disc prosthesis (Pretic-I). Eight mature male goats underwent C3-C4 total disc placement with the novel disc prosthesis through an anterior surgical approach. The specimens were examined using microcomputed tomograph, proceeded by undecalcified histologic technique and routine paraffin processing. Histologic and histomorphometric analyses were used to evaluate the porous osseointegration at the prosthesis-bone interface. There were no cases of prosthesis migration, loosening, subsidence, or neurologic or vascular complications. Gross histologic analysis of the novel disc prosthesis illustrated excellent ingrowth at the prosthesis-bone interface, without significant histopathologic changes. Histomorphometric analysis at the prosthesis-bone interface indicated that the mean porous ingrowth was 42.5%±8.4%. The total range of ingrowth was 32.5% to 54.6%. Histomorphometric analysis of porous ingrowth at the prosthesis-bone interface was more favorable for cervical disc replacement with the novel disc prosthesis than the historical reports of peripheral total joint arthroplasty. These findings in the present study provide a foundation for ongoing clinical investigations.

Keywords: Cervical disc replacement, animal model, porous ingrowth, histomorphometry, disc prosthesis

Introduction

The biomechanical and biological factors affecting the success of cervical disc replacement implants have mainly focused on two fundamental strategies: initial stability and long-term stability, which are still the hotspots in basic and clinical research. From a biomechanical perspective, the initial stability of cervical disc replacement implant is mainly attributed to the mechanisms of acute fixation, which are accomplished by endplate modifications involving the use of keels, teeth, and serrations providing increased acute fixation strength as previously reported [1]. However, acute fixation does not ensure the long-term stability of cervical disc replacement implants, which are mainly subjected to biological osseointegration at the prosthesis-bone interface. Hence, the design concepts of the most current used cervical disc prostheses are as much as possible to meet, and improve, the initial and long-term stabilities of arthroplasty devices.

To date, there are a variety of intervertebral disc prostheses with various design concepts, but only a few can be used in clinical or experimental research. Moreover, most of the current widely used artificial cervical disc prostheses have a flat endplate surface, which is inconformity with the sophisticated morphology of the cervical vertebral endplate [2-4]. This inconformity may lead to the occurrence of prosthesis subsidence [5, 6]. Therefore, we designed a novel artificial cervical disc prosthesis (Pretic-I) based on the physiological curvature of the cervical endplate. Using an *in vivo* caprine model, the current study was undertaken to investigate the initial stability and the biologic porous ingrowth characteristics of the novel artificial cervical disc prosthesis, with success criteria based on radiographic analysis and quantitative histomorphometry.

Material and methods

Animal research permission

The Institutional Animal Care and Use Committee at the West China Center of Medical Sciences, Sichuan University, Chengdu, Sichuan granted approval for this eight caprine animal

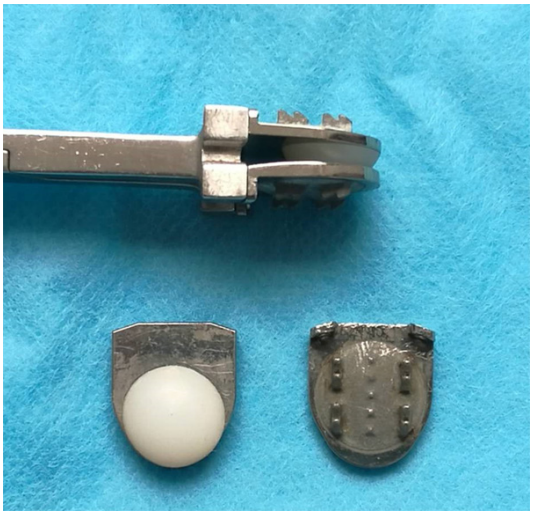

Figure 1. The novel cervical disc prosthesis (Pretic-I) contains two cobalt-chrome alloy end plates, an ultra-high molecular weight polyethylene core, and a unique titanium/calcium phosphate hydroxyapatite coating.

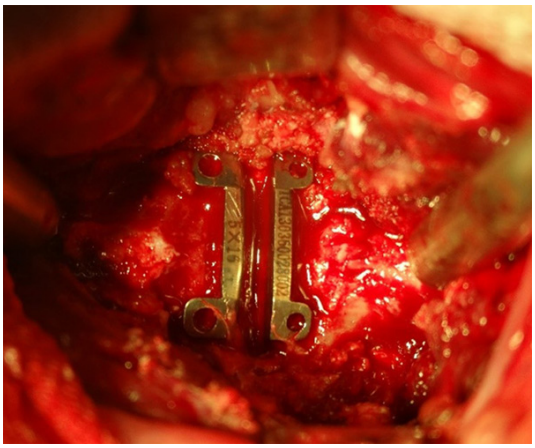

Figure 2. Anterior intraoperative view. The novel disc prosthesis is implanted at the operative segment.

project. Surgery, perioperative care, housing, sanitation practices, husbandry, and veterinary care followed the recommendations of the Guide for the Care and Use of Laboratory Animals [7]. Animal care personnel were qualified through training and experience to perform all required duties.

Device design

The Pretic-I cervical disc prosthesis contains superior and inferior end plates (Ti6A14V), an ultra-high molecular weight polyethylene (UHMWPE) core, and a unique TiCaPHA coating (**Figure 1**). The primary endplate bearing surface contains two layers of pure titanium, with a pore size of 75-300 μm. The titanium coatings consist of a special adhesive layer (<90 μm) and a cover layer (<180 μm). The UHMWPE-bearing surface attached to the superior endplate permits itself to move back and forth along a slot in the horizontal direction. Hence, the prosthesis is consistent with the principle of a mobile-bearing or unconstrained arthroplasty. Theoretically, the mobile-bearing, unconstrained characteristic of the prosthesis not only markedly diminishes the stress concentration at specific points on the UHMWPE core [8], but also reduces the stresses at the prosthesis-bone interface and leads to more favorable porous ingrowth than a fixed bearing or constrained disc prosthesis. A CaP coating (approximately 20 μm thick) is electrochemically bonded to the serrated titanium surface of the implant, which serves to optimize mineralized anchorage at the vertebral endplates [9]. The procedure for electrochemical coating of hydroxyapatite (HA) retains the open cell structure of the underlying pure titanium coating to optimize implant bonding.

Animal model and surgical preparation

A total of eight mature male goats (2-3 years old, mean weight 30 Kg) were included in this study, and followed for 6 months after surgery. The goats were randomly numbered and handled with the same procedures, without other control groups. 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 with isoflurane (1% to 2%) with continuous intravenous fluids (range 3-6 mL/lb/h) administered for the duration of surgery. Prophylactic intravenous antibiotics (cefazolin sodium, 1 g) and analgesics (butorphanol 0.1 mg/kg) were administered pre- and post-operatively.

Surgical technique and postoperative evaluation

The anterior Smith-Robinson approach to the cervical spine was adapted to the goat model through a right-sided longitudinal incision with length 6- to 8-cm, and the C3-C4 intervertebral disc underwent a standard anterior cervical discectomy. The endplate surfaces were pre-

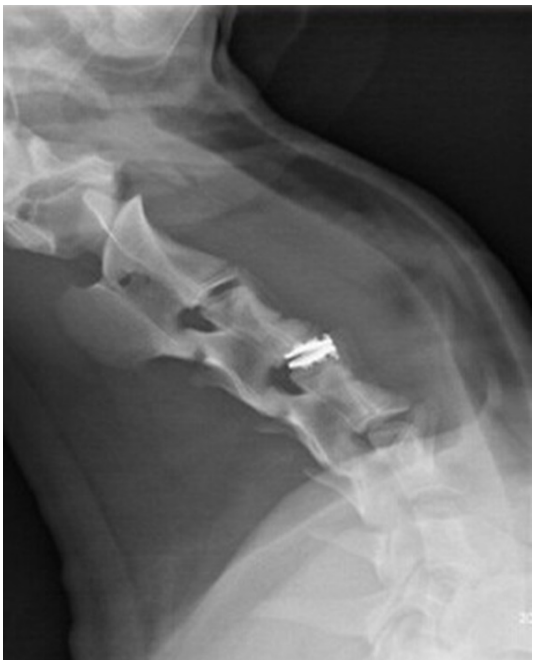

Figure 3. Lateral X-ray film of the caprine cervical spine demonstrates that the disc prosthesis is in place.

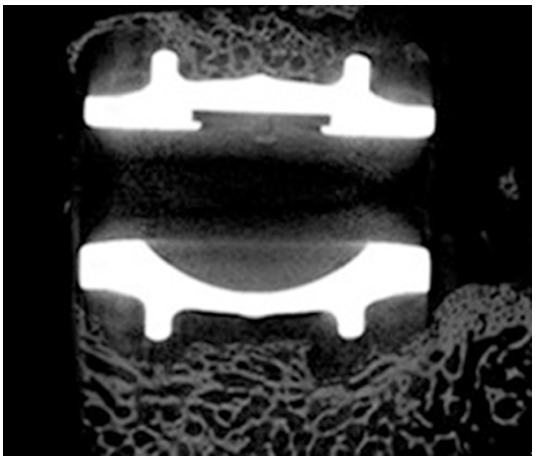

Figure 4. Microcomputed tomograph of the operative segment. Excellent ingrowth is seen at the prosthesis-bone interface 6 months after surgery.

pared using curettage and a high-speed burr. The novel prosthesis was then implanted at the operative segment (**Figure 2**). Blood loss, operating times, and intraoperative and perioperative complications were quantified.

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. Lateral X-ray films of the cervical spine were obtained intraoperatively and after surgery to verify implant placement (**Figure 3**). All animals were humanely killed at 6 months after surgery using an overdose (150 mg/kg) of concentrated pentobarbital solution (390 mg/mL). The spinal column then was carefully removed and frozen at -25°C in double-wrapped plastic specimen bags.

Histology and histomorphometry

Each of the eight operative segments was examined using microcomputed tomograph (Micro-CT) for histomorphometric quantification of trabecular bone area at the prosthesis-bone interface. The regions of trabecular contact were expressed as percentage of the total endplate area (% ingrowth = apparent bone contact area/gross total endplate area).

Eight of the sixteen vertebral specimens, selected randomly, were processed using undecalcified histologic technique. After slide preparation of these specimens, the sections underwent histologic preparation including dehydration in 100% ethanol, undecalcified solution processing, and embedding in polymethylmethacrylate. Using the EXAKT Micro-grinding Device (EXAKT Technologies, Oklahoma City, OK, USA), the embedded sections were cut from 250 to 300 μm thick, ground and polished to 100 μm, and then stained using standard toluidine blue stain. The other eight vertebral specimens were fixed and underwent routine paraffin processing and slide preparation. Using thin-sectioning microtomy, the paraffin embedded sections were cut (3-5 μm in thickness), slide mounted, and stained using standard hematoxylin and eosin.

Statistical analysis

Statistical analysis was performed using SPSS version 19.0 software (SPSS Inc., Chicago, Illinois). All data are shown as mean ± standard deviation. Histomorphometric data were presented as the percentage of trabecular bone in contact with the novel disc prosthesis (titanium endplates) and statistically compared with historical reports of peripheral total joint arthroplasty using an analysis of variance (ANOVA) with Student-Newman-Keuls test. Significance was indicated at $P<0.05$.

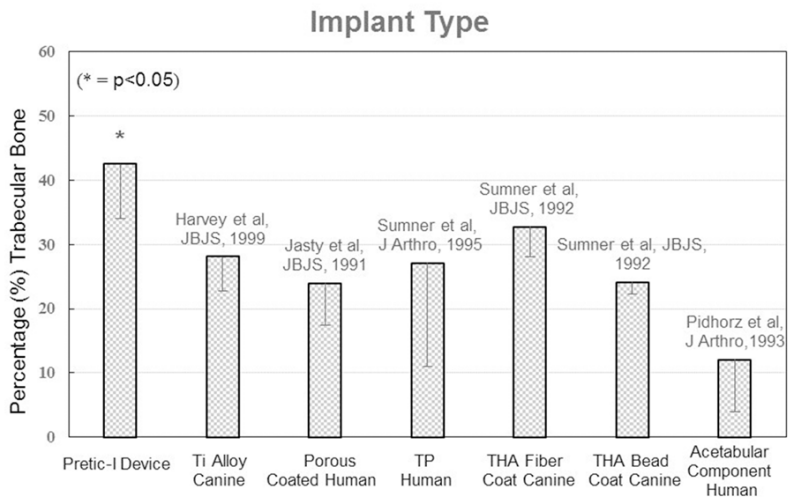

Figure 5. Porous ingrowth. Percentage of ingrowth-bone contact on the endplate surface. The bar graph illustrates porous ingrowth for the novel disc prosthesis that is more favorable than that reported for total joint arthroplasty in the peripheral skeleton.

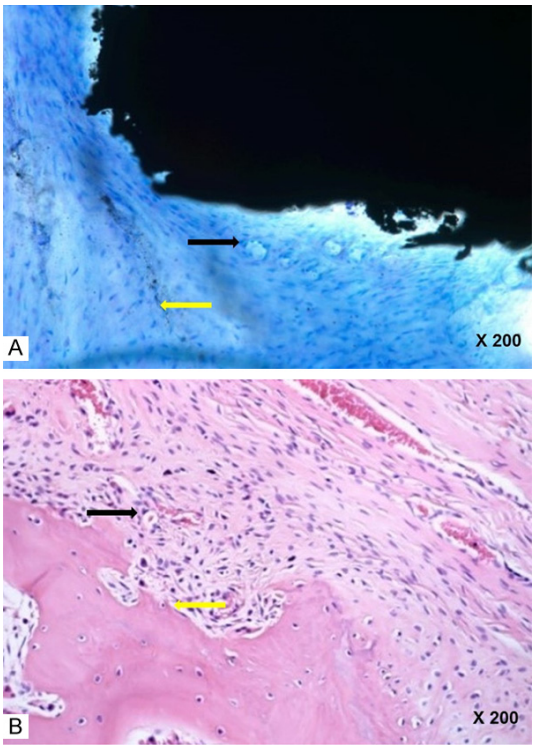

Figure 6. Sections using toluidine blue stain (A). Sections using hematoxylin and eosin stain (B). The proliferated osteoblasts (black arrow). The interface between regenerated osseous tissues and mature trabecular bone (yellow arrow).

Results

All animals survived during and after surgery, without incidence of vascular, neurologic or infectious complications. The operating times averaged 70.5±18.5 minutes (range 57-96 minutes), with an estimated blood loss of less than 50 ml. All animals had resumed normal behavior by 1 week after surgery. Based on anteroposterior and lateral plain films, there was no evidence of prosthesis migration, loosening, or subsidence. Gross histologic analysis of the novel disc prosthesis illustrated excellent ingrowth at the prosthesis-bone interface, without evidence of particulate wear debris or significant histopathologic changes.

Histomorphometry

Micro-CT of the operative segments demonstrated excellent osseointegration at the prosthesis-bone interface (**Figure 4**). Histomorphometric analysis at the prosthesis-bone interface (apparent bone contact area/gross total endplate area) indicated that the mean porous ingrowth was 42.5%±8.4% (total range: 32.5% to 54.6%) at 6 months, which was higher than that reported for porous ingrowth found in the peripheral skeleton (**Figure 5**).

Bone histology

Based on undecalcified histologic technology, eight verterbral specimens were dehydrated, embedded, and underwent slide preparation and staining using toluidine blue stain. In addition, the remained eight vertebral specimens were fixed and underwent routine paraffin processing and slide preparation. The paraffin embedded sections were cut, slide mounted, and stained using standard hematoxylin and eosin. As a result, there were both plenty of proliferated osteoblasts and regenerated osseous tissues in some regions of the prosthesis-bone interface (**Figure 6A**, **6B**).

Discussion

As an alternative to standard anterior discectomy and fusion, cervical disc replacement has been widely used in the surgical management of cervical degenerative disc diseases. An arti-

ficial cervical disc serves to replicate the function of the entire degenerative disc. To this end, the implanted disc prosthesis should not only restore the disc space height, preserve the motion function of the target segment, but also encourage osseointegration at the prosthesis-bone interface for long-term survivorship of the disc device. Hence, it is essential to evaluate the extent of porous ingrowth in a novel cervical disc prosthesis.

In the current study, radiographic analysis showed no evidence of prosthesis migration, loosening, or subsidence. Based on the histomorphometry data, the mean porous ingrowth was 42.5%±8.4%. This demonstrated excellent osseointegration at the prosthesis-bone interface for the novel disc prosthesis, similar to that reported in previous studies [10, 11]. Moreover, the mean porous ingrowth was much higher than that reported for porous ingrowth found in peripheral total joint arthroplasty (only 20%-30% ingrowth) [12-16]. One possible reason for the improved extent of porous ingrowth in cervical disc replacement implant is the unique TiCaP coating on the serrated surface of the novel disc prosthesis. The serrations permit a primary press-fit fixation, facing toward the bony endplates to resist pull-out, and the special coating significantly encourages osseointegration in the long run. In addition, we postulate that another reason for the more favorable porous ingrowth is ligamentotaxis causing long-term and sustained compression across the prosthesis-bone interface.

In terms of the quantification methods for osseointegration, there is controversy regarding the most accurate method of measuring the porous ingrowth of cementless prostheses [17, 18]. The three most widely used methods are microradiography, stained histology, and backscattered electron imaging-scanning electron microscopy (BEI-SEM) [1]. In the present study, we adopted microradiography and stained histology to evaluate the porous ingrowth at the prosthesis-bone interface. It turned out that there was evidence of excellent osseointegration at the prosthesis-bone interface. Using the three methods to compare the porous ingrowth of acetabular cups, previous study found that BEI-SEM and histologic sections possessed comparable results, whereas microradiography underestimated the porosity of the porous coating by a mean of 17% and simultaneously overestimated the amount of bony ingrowth by a mean of 0.8% [19].

Device wear can occur at any interface, especially at the bearing surfaces but also at the host-implant or implant-implant interfaces [20]. Device debris, a common device-related complication, can lead to bone loss, implant loosening, heterotopic ossification, implant failure, and subsequent revision [21, 22]. Moreover, wear production differs resting upon the materials adopted and mechanisms of biomechanical stress applied to the device. There is no evidence of particulate debris in this study, because the novel disc prosthesis had a high wear resistance, as verified by a previous study [23].

To our knowledge, as the first comprehensive *in vivo* study of this novel disc prosthesis, the present project establishes a successful animal model for cervical disc replacement and documents excellent osseointegration at the prosthesis-bone interface. However, the caprine model reported in this study used only a 6-month follow-up period and a small sample size. It is expected that longer follow-up evaluation with greater numbers of subjects would be required to obtain a more reliable measure of the porous osseointegration of cervical disc replacement with this novel prosthesis.

In summary, histomorphometric analysis of porous ingrowth at the prosthesis-bone interface was more favorable for cervical disc replacement with the novel disc prosthesis, compared to historical reports of peripheral total joint arthroplasty. These findings in the present study provide a foundation for ongoing clinical investigations using the novel cervical disc prosthesis.

Disclosure of conflict of interest

None.

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