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A polycaprolactone-tricalcium phosphate composite scaffold as an autograft-free spinal fusion cage in a sheep model

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ABSTRACT

Titanium (Ti) based spinal fusion cages are frequently used in the clinics for the treatment of spinal degeneration and related diseases, however, their further clinical application is generally harassed by several drawbacks such as stress shielding, non-biodegradability and additional bone grafting procedure. Our earlier work has demonstrated the efficacy of a biodegradable macro-porous polycaprolactone-tricalcium phosphate (PCL-TCP) composite scaffold in promoting bony tissue ingrowth as well as its ability to sustain mechanical loads upon implantation into an orthotopic defect site. In this study, we investigated the use of PCL-TCP scaffold as an autograft-free spinal fusion cage in a preclinical sheep model over 12 months, and compared the fusion efficacy against Ti cages incorporated with autografts. Results showed that despite PCL-TCP scaffold as an autograft-free cage attaining a slower fusion rate at early stage (6 month), it achieved similar degree of spinal fusion efficacy as Ti cages aided with autograft at 12 month post-operation as evidenced by the radiographic and histological evaluation. PCL-TCP cages alone demonstrated better bone ingrowth with 2.6 fold higher bone/interspace ratio (B/I) and more homogeneous bone tissue distribution compared with that of the Ti cages (88.10 $\pm$ 3.63% vs. 33.74 $\pm$ 2.78%, $p < 0.05$) as seen from the histological and micro-CT analysis. Moreover, besides the bone tissue ingrowth, a quantitative approach was illustrated to accurately evaluate the osteointegration of fusion cage with surrounding bone tissue, and showed a 1.36 fold higher degree of osteointegration occurred in PCL-TCP cage group than Ti cage group (CS/PC: 79.31 $\pm$ 3.15% vs 58.44 $\pm$ 2.43%, $p < 0.05$). Furthermore, biomechanical analysis showed comparable mechanical strength of fused segments in both groups in terms of the range of motion and stiffness at 12 month ($p > 0.05$). The degradation profile of the PCL-TCP cages was noted to increase in tandem with new bone ingrowth into the pores, while maintaining good structural integrity necessary for supporting the spinal interbody segments. Therefore, with the better osteointegration, more bone tissue ingrowth as well as its favorable biodegradable and radiolucent properties, PCL-TCP cage has been demonstrated to be a promising candidate as an autograft-free fusion cage for clinical application.

1. Introduction

Spinal diseases caused by degeneration, deformity or trauma remain to be a major clinical burden as patients inflicted by chronic pain and significant activity limitation [1] would require surgical intervention. Spinal fusion procedure is often performed to address problems such as degenerative disc disease and recurrent disc herniation, to preserve neurological function and maintain the spine stability [2].

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Several spinal fusion strategies have been developed over the years. Autologous iliac bone graft transplantation strategy was used for the treatment of spine interbody fusion in the beginning [3]. This approach is however, fraught with problems of post-operative collapse of the bone graft due to insufficient mechanical strength of autologous bone graft and involves donor-site complications such as secondary fracture, vascular tears and chronic pain [4,5]. The use of titanium cages could lead to shorter period of hospitalization, less donor-graft site complications, and faster recovery. However, its clinical application has been limited by several inherent problems such as relatively lower bioactivities, radiological interference during post-operative X-ray or computer tomography (CT) examination. Stress shielding effects due to the mismatch of mechanical properties between titanium and natural bone tissue may also lead to pseudarthrosis, peri-prosthesis osteolysis and implant migration in some clinical scenarios [6–9]. Moreover, this methodology still requires the additional harvest of autologous bone grafts to facilitate spinal fusion and remains permanently inside the body [10].

In our previous study, we have established a three dimensional (3D) scaffold fabrication system using fused deposition modeling (FDM) technique for the development of a biodegradable polycaprolactone-tricalcium phosphate (PCL-TCP; 80/20 composition) scaffold with a unique honey-comb interconnected structure [11–14]. Compared against the gold standard of a titanium cage, we envision that the PCL-TCP would function as an advanced spinal fusion cage for the following reasons: (1) They are radiolucent and will not interfere with post-operation radiographic imaging; (2) They are biodegradable leaving behind no foreign material within the human body over time; and (3) They possess similar mechanical properties as cancellous bone, hence minimizing concerns relating to stressing-shielding [15]. In addition, our previous studies have demonstrated its osteoconductive properties in facilitating bone tissue regeneration and promoting osteointegration [16-19].

In this study, we aim to investigate the long term performance of PCL-TCP scaffold as an autograft-free spinal fusion cage to promote the spinal fusion in the preclinical sheep model, as compared with traditional Ti6Al4V cages. Specifically, the efficacy of spinal fusion was evaluated from the perspectives of bone tissue ingrowth, bone cage integration and mechanical strength of fusion with host tissue. Furthermore, besides the conventional bone ingrowth volume calculation, we illustrated an approach to quantitatively evaluate the osteointegration of fusion cage with surrounding bone tissue, by analyzing the perimeter of bone/cage contact surface.

2. Materials and methods

2.1. Animals and experimental design

All animal experiments were performed in strict accordance with protocols approved by the animal ethics committee of the Fourth Military Medical University. All procedures were performed under the international guidelines for protection of animals. Adult female Small Tailed Han Sheep (Age: 16–24 months; Weight: 36–48 kg) were used for this study ($n = 18$) and randomly assigned to three time points (6, 9, 12 months, $n = 6$ sheep per time point)(Fig. 1A). Sheep were acclimatized to the laboratory environment for a week before operation. Each sheep underwent anterior cervical discectomy and fusion with a PCL-TCP cage or Ti cage at C2/C3 or C3/C4 level randomly. For the Ti cage, autologous bone tissue was harvested from the iliac crest and used to fill up the central hole of cage. For experimental evaluation, the animals were subjected to X-ray examination at 0, 6, 9 and 12 months post-operatively. Micro-CT and histology were conducted at 6, 9 and 12 months post-operatively. Mechanical properties were tested at 6 and 12 months ($n = 3$), with three fresh sheep cadaveric cervical spines used as controls.

2.2. Scaffolds preparation

The PCL-TCP cages (Fig. 1B) were fabricated from 80% PCL with 20% TCP filaments using a fused deposition modeling technique as previously reported [20]. It has a lay-down pattern of 0/60/120° and porosity of 75%, with TCP particles homogeneously distributed onto the rods of PCL. The PCL-TCP scaffold was cut into cylinders, with a radius of 6 mm and a height of 5 mm. The cages were immersed in 5M sodium hydroxide for 3 h at room temperature, washed with sterile water three times, and then centrifuged at 1500 rpm for 30 min to remove water. These scaffolds were subsequently immersed in 70% ethanol for 24 h for sterilization and then rinsed in sterile water three times prior to implantation. The metallic cages (Fig. 1C) were constructed using electron beam melting technique using Ti6Al4V. The cylindrical metallic cages have a radius of 6 mm and a height of 5 mm, with a 2 mm radius hole in the middle (porosity: 68%; elastic modulus: 2.5 GPa). The Ti cages were cleaned ultrasonically for 5 min, and autoclaved before implantation.

2.3. Surgical procedures

Sheep underwent fasting for 24 h before the operation. Surgeries were performed in an aseptic operation room. Anesthesia were achieved using Xylazine Sailaqin Zhusheye (Jilin Huamu Animal Health Product Co. Ltd., China) 0.1 ml/kg. The neck area was shaved and scrubbed with iodophor, followed by 70% ethyl alcohol.

Before the spinal fusion surgery, a $10 \times 10 \times 5$ mm^3 of autologous bone was harvested from the iliac crest using a 10 mm oscillating saw. To perform anterior cervical discectomy and fusion, a 10 cm incision was made longitudinally 2 cm left to the midline. Esophagus and trachea were retracted to the left and protected using moistened gauze, while the carotid artery was retracted to the right. A midline incision was made at the longus colli muscle situated at the anterior surface of the vertebral column. The ventral surface of the discs at C2/C3 and C3/C4 were exposed for discectomy. After the removal of those discs, all endplates were sawed to induce bleeding. An intervertebral space was then created in each sheep to accommodate the PCL-TCP or Ti cages in the position of C2/C3 or C3/C4 randomly. Ti cages were implanted with the central holes filled up with autologous iliac crest bone grafts. After implantation, an anterior titanium plate and cortex screws were used to stabilize cervical vertebrae. The incision was sutured up in layers and patched up.

Post-operatively, Ceftriaxone Sodium (CeftriaxoneSodium For Injection, Sanjing Pharmaceutical Co.,Ltd., 1 g/day) and buprenorphine (Buprenorphine Hydrochloride Injection, TIPR Pharmaceutical Responsible Co., Ltd, 0.01 mg/kg) were administered intramuscularly for 4 days and every 8 h for 3 days respectively. The sheep were euthanized at 6, 9 and 12 months by exsanguination upon anesthesia. The spinal segments of interest were harvested, with plates and screws as well as neighboring soft tissue carefully removed leaving all ligamentous and bony structures intact. The specimens were then sawed to proper length prior to storage in −20° fridge until analysis.

2.4. X-Ray evaluation

Lateral x-ray imaging was conducted at 6, 9 and 12 months post-operatively. A radiographic scoring system was adopted to evaluate fusion performance as previously described [21]: Grade 1: not fused, clear radiolucency across the disc space area; Grade 2: partly fused, evidence of bridging of bone with some radiolucent lines across the disc space; Grade 3: fused, a solid bridge of bone with no radiolucent lines in between.

2.5. Micro-CT analysis

The microstructure changes of specimens were evaluated using Siemens micro-CT system (Inveon™ CT scanner, Siemens, Germany). Scan was performed at 80 kV, 500 μA, and a spatial resolution of 30 μm. From each specimen, a $15 \times 15 \times 15$ mm^3 volume of interest (VOI) was selected centering on fusion cage. The scanning data were processed by the Inveon Acquisition Workplace (IAW) (Siemens, Germany) to reconstruct 3D images, which in turn were used to analyze quantitative bone volume versus total fusion mass volume ratio (BV/TV) and the PCL-TCP cages volume versus total fusion mass volume ratio. Ti whole region and Ti Ring region stands for the whole volume of the Ti cage and the volume of Ti cage without the central hole region respectively. Bone volume fraction of each region was calculated accordingly.

2.6. Mechanical testing

Mechanical studies were performed on 12 experimental specimens at 6 and 12 months (24 motion segments) and 3 normal cervical specimens (6 motion segments). These frozen specimens were thawed immediately before use and performed at room temperature within 4 h. Specimens were loosely wrapped with saline moistened gauze to prevent drying out. The caudal end was fixed in polymethylmethacrylate to gain rigid fixation on the spine simulator.

The Range of Motion (ROM) of flexion/extension and left/right lateral bending were tested with WDW 100C testing apparatus (HUALONG Test Instruments CO., Ltd, ShangHai, China), and left/right axial rotation was tested at a constant loading rate (1.0°/s) with WNJ 1000 testing apparatus (HUALONG Test Instruments CO., Ltd, ShangHai, China). Pure moments of ±5Nm were applied without any preload. The moments and angular displacement of flexion/extension, left/right lateral bending, and left/right axial rotation of each segment were tested for 4 cycles. After 3 cycles of pre-conditioning, the data from the fourth cycle were used for statistical analysis [22]. The ROM was marked as the degree of triaxial displacement at maximum load and stiffness k was calculated as moment (Nm)/rotation angle (°).