3D-Printed Titanium Cages without Bone Graft Outperform PEEKCages with Autograft in an Animal Model

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Abstract

Background context: Modernization of 3D printing has allowed for the production of porous titanium interbody cages (3D-pTi) which purportedly optimize implant characteristics and increase osseointegration; however, this remains largely unstudied in vivo.

Purpose: To compare osseointegration of three-dimensional (3D) titanium cages without bone graft and Polyether-ether-ketone (PEEK) interbody cages with autologous iliac crest bone graft (AICBG).

Study Design: Animal study utilizing an ovine in vivo model of lumbar fusion.

Methods: Interbody cages of PEEK or 3D-pTi supplied by Spineart SA (Geneva, Switzerland) were implanted in 7 living sheep at L2-L3 and L4-L5, leaving the intervening disk space untreated. Both implant materials were used in each sheep and randomized to theaforementioned disk spaces. Computed tomography (CT) was obtained at 4 weeks and 8 weeks. MicroCT and histological sections were obtained to evaluate osseointegration.

Results: MicroCT demonstrated osseous in-growth of native cancellous bone in the trabecular architecture of the 3D-pTi interbody cages and no interaction between the PEEK cages with the surrounding native bone. Qualitative histology revealed robust osseointegration in 3D-pTi implants and negligible osseointegration with localized fibrosis in PEEK implants. Evidence of intramembranous and endochondral ossification was apparent with the 3D-pTi cages. Quantitative histometric bone implant contact demonstrated significantly more contact in the 3D-pTi implants vs. PEEK ($p<0.001$); region of interest calculations also demonstrated significantly greater osseous and cartilaginous interdigitation at the implant-native bone interface with the 3D-pTi cages ($p=0.008$ and $p=0.015$, respectively).

Conclusion: 3D-pTi interbody cages without bone graft outperform PEEK interbody cages with AICBG in terms of osseointegration at 4 and 8 weeks post-operatively in an ovine lumbar fusion model.

Clinical Significance: 3D-pTi interbody cages demonstrated early and robust osseointegration without any bone graft or additive osteoinductive agents. This may yield early stability in anterior lumbar arthrodesis and potentially bolster the rate of successful

fusion. This could be of particular advantage in patients with spinal neoplasms needing ost￾ablative arthrodesis, where local autograft use would be ill advised.

Keywords: additive manufacturing; interbody cages; interbody fusion; three-dimensional printing; titanium

Running title: 3D-Printed Titanium Cages

Introduction

In the upright position, 80% of spinal loads are transmitted through the anterior column[1]. Ideal interbody implants afford the stability to withstand these loads and permit early mobilization. Currently, interbody cages are commonly comprised of titanium orpolyether-ether-ketone (PEEK); however, neither are ideal materials.

PEEK more closely approximates the elasticity of bone, preventing alteration in load distribution and subsequent undesired osseous remodeling[2]. The material stiffness has been shown to influence fusion: ideal stiffness optimizes the load transfer between the implant and adjacent vertebrae, as well as minimizes stress shielding.[3] PEEK is also radiolucent, allowing for visualization of radiographic fusion. Unfortunately, PEEK is biologically inert and contributes little to osseous integration.[4]

In contrast, titanium implants demonstrate greater bioactivity. Animal studies have demonstrated that porous titanium exhibits in-growth of bony trabeculae as early as 14 days post-implantation.[5] Unfortunately, titanium exceeds the Young's modulus of vertebral bone, potentially facilitating subsidence through vertebral endplates. Furthermore, micromotion may cause the interbody cage to dislodge debris, which may result in a cellular reaction and osteolysis with subsequent loosening at the bone-implant interface. [6] Additionally, radiopaque titanium implants create scatter artifact, limiting the assessment of fusion on imaging.

The development of three-dimensional (3D) printing technology may allow for the production of a more optimal interbody implant—one that is biomechanically stable, biochemically active, and radiographically favorable when compared to non-3D printed titanium interbody implants. Additive manufacturing technology has allowed for the production of titanium based biomimetic structures aimed at improving osseointegration. Unfortunately, the effects of these 3D-printed biomimetic titanium nanostructures on the in vivo process of spinal interbody fusion are largely unknown. This study sought to compare early interbody fusion and osseointegration between 3D-printed titanium cages without bone graft and PEEK interbody cages with autologous iliac crest bone graft (AICBG) in an ovine model.

Methods

Interbody devices

Standard serial implants designed for human implantation and supplied by Spineart SA (Geneva, Switzerland) were utilized. Interbody cages of nanostructured cancellous bone￾like titanium (3D-pTi - Juliet® Ti-LIFE) and PEEK (Juliet®) were compared. The macrostructure 3D-pTi and PEEK cages can be seen in figure 1, while the microstructure of

the 3D-pTi cage can be seen in figure 2. (Figures 1 and 2) Specifications of each interbody implant may be seen in table 1. (Table 1)

Animals and implantation

The animals used were supplied by the farm of [BLINDED FOR REVIEW]. The sheep were between 4 and 5 years of age and exclusively female.The study received prior authorization from the competent body and institutional review board. The sheep were housed in conditions of temperature, humidity, ventilation, and photoperiod controlled according to annex II of directive 86/609/EEC. The period of quarantine was 21 days per the regulatory requirements . The animals were fed with dry feed prepared in pellet form formulated for livestock sheep and dried grass ad libitum. Their weight upon arrival was approximately 40kg. The center's head of animal welfare, staff, and researchers cared for the sheep throughout the experiment.

Surgical technique

After the quarantine period, the animals were sedated with dexmedetomidine (3μg/kg) and morphine (0.2mg/kg) via the internal jugular vein. By the same route, a 20ml bolus of aphysiological serum with morphine, lidocaine, and ketamine (MLK) was administered for further sedation, analgesia, and anxiolysis. This MLK mixture was maintained for duration of the procedure at 40ml/h. Pre-operative broad-spectrum antibiotics were administered (Amoxicillin retard, 15mg/kg intramuscularly (IM)). Once sedated, catheterization of the cephalic vein was performed and intravenous (IV) fluids were administered, as was IV meloxicam (0.025mg/kg).

Anesthetic induction was performed with propofol (2mg/kg IV). Maintenance of anesthesia was performed with 1.5% isoflurane. The surgical site was then widely depilated via electric clippers and thoroughly disinfected with povidone-iodine and ethanol.

A retroperitoneal approach to the lumbar spine was performed following the protocol described by Baramki and colleagues.[7] The sheep were placed in right lateral recumbency and the skin was incised fashion from 2 cm caudal to the costovertebral angle to 5cm cephalad of the stifle fold. The oblique muscles were exposed and cut in the direction of the fibers and the transversus abdominus was incised exposing the retroperitoneal space. The anterior vertebral body was revealed through the interval between the aorta and psoas muscle. The aorta and lymphatic vessels were retracted medially, while the psoas was retracted laterally to expose the intervertebral disk spaces. A thorough discectomy was performed with care to preserve the subchondral endplate. At this point, the devices were placed in intervertebral disks according to manufacturer criteria (Figure 3).

In each animal, two different cages (PEEK and 3D-pTi) were randomized to either L2-3 or L4-5, leaving the intervening space untreated. Thus, each animal served as its own internal control. A total 14 devices were placed in 7 animals.

The wound was closed in layers and povidone-iodine was applied over the approximated incision site. Following anesthetic recovery, the animals were returned to their accommodations, where they continued to abide throughout the experimental period.

Radiographic assessment

All animals underwent a computerized axial tomography (CT) scan at 4- and 8-weeks post-implantation. The 4-week CT scans were performed under deep sedation with medetomidine (15μg/kg), morphine (0.2mg/kg), and 20ml of MLK IV. Unfortunately, during sedation sheep number six died. Thus, this animal underwent autopsy and extraction of thespine at 4 weeks.

After the test period of 8 weeks, the animals were euthanized via IV barbiturate overdose after sedation. The 8-week CT was performed immediately after euthanasia. The thoraco-lumbar spines were then extracted and immersed in a 10% buffered formalin solution for a minimum 2 weeks until the time of processing.

Sample preparation

Once fixed, the samples were dehydrated in increasing concentrations of ethanol (80%, 96%, 100% and 100%) in steps of 3 days. Inclusion was performed on a reciprocating shaker using a resin based of light-curing glycolmethacrylate (Technovit 7200 VLC, Heraeus-Kulzer, Wehrheim, Germany) at increasing concentrations of alcoholic solution in 3-day steps. Polymerization took place using high-intensity blue light. The blocks obtained were prepared for analysis by microCT and subsequently sectioned for histology.

MicroCT analysis

The samples were scanned with a Skyscan 1172 kit (Bruker, Kontich, Belgium). The parameters used for scanning were as follows: 100Kv/100μA, Aluminum / Copper Filter (Al /Cu), 13.58μm pixel size, and 0.4° rotation pitch and 360° rotation of the sample.

The scanned images were reconstructed using Feldkamp’s algorithm with Bruker's NRecon program. All imaging parameters were optimized to decrease image noise and artifact. They were then reconstructed with histogram parameters (dynamic range) 0 to 0.05 for all images.

Histological analysis

After uCT, the samples were subjected to sectioning and roughing techniques using system machinery Exakt (Exakt Aparatebau GMBH, Hamburg, Germany). Each specimen was prepared, obtaining two sections per sample, and reduced to a thickness of about 40 microns. They were then stained by Levai Laczkó staining.

Histological evaluation was carried out by semiquantitative techniques according to annex E, table E.3 of ISO 10993-6: 2007 by trained personnel. This evaluation estimates the degree of compatibility of the biomaterial in relation to a recognized control. An optical microscope (BX51, Olympus, Tokyo, Japan) connected to a digital color camera (DP71, Olympus, Tokyo, Japan) with a motorized plate (Märzhäuser, Steindorf, Germany) was used to capture images. The images were automatically aligned and connected obtaining complete images of the bone and implant up to x40 magnification.

Using the histological images, the proportion occupied by bone, implant, cartilage and soft tissue were differentiated using a digitizing tablet (Cintiq companion, Wacom, Germany). They were colored (Photoshop, Adobe, USA) and calculated using an image analysis program (CellSens, Olympus, Tokyo, Japan).

Two types of histometric evaluations were performed: the percentage of bone implant contact (BIC) and the evaluation of a region of interest (ROI) that encompassed the interior of the implant. (Figures 4 and 5)

Statistical analysis

Statistical analysis of BIC and ROI were performed using Microsoft Excel (Version 16.40, 2020, Microsoft Corporation, Redmond, Washington, United States of America). A two-tailed Welch's unequal variance t-test was utilized in the analysis.

Results

MicroCT analysis

Marked cancellous osseous in-growth in concordance with the trabecular architecture of the 3D-pTi was observed in each implant. There was also de novo osseous formation within the graft window of each implant. (Figure 6)

PEEK cages demonstrated no interaction with adjacent bone in addition to areas of localized fibrosis. The bone graft incorporated within the interbody cage did not improve the osseointegration of the implant. (Figure 7)

Histological analysis

Thin sliced sections demonstrated stark qualitative differences between the PEEK and 3D-pTi cages. The former demonstrated a lack of osseointegration and in-growth about the implant-bone interface, with no inchoate bone present at the graft window. Fibrotic tissue was present histologically near the bone-implant interface. (Figure 8) Osseointegration was present in the 3D-pTi cages with interaction of the native bone and the implant's trabecular architecture. De novo bone formation was present at the center of the cage with both cartilage and collagen present, indicating both endochondral and intramembranous ossificationoccurred. (Figure 9). Greater magnification of the histological slides demonstrated the appearance of woven bone and lamellar bone about the titanium cages, as well as collagen and cartilage (Figure 10).

Quantitative histology demonstrated a significantly greater mean ROI of both bone and cartilage in the 3D-pTi implants vs. the PEEK implants ($p=0.008$ and 0.015 for bone and cartilage, respectively). (Table 2) BIC was found to be significantly higher in the 3D-pTi implants as well ($p<0.001$). (Table 3)

Discussion

PEEK and titanium are popular materials in the composition of interbody implants; the advent of 3D printing has allowed for the development of biomimetic, porous titanium implants. While such devices purportedly augment fusion by providing a nidus for osseous integration within their porosity,[8] there is a paucity of evidence regarding these effects in vivo. We sought to compare the osseous in-growth and integration of 3D-pTi interbody cages without bone graft and PEEK cages with AICBG in an ovine lumbar fusion model.

Titanium is a well-studied material for the construction of intervertebral cages.[9,10] While performing favorably regarding osseointegration,[11] titanium alloys are stiffer than bone, [2] which has led to concerns of subsidence. Indeed, the Young's moduli of titanium andcancellous bone are 50.2 and 3.78GPa,[2] respectively; the 3D-pTi implant utilized in this

study had a Young's modulus of 2GPa, much more similar to that of cancellous bone. Titanium is also radiopaque, limiting radiographic assessment of fusion. Introduced in the 1990s by AcroMed, PEEK is radiolucent and of comparable elastic modulus to bone.[12,13] However, hydrophobicity renders PEEK biologically inert resulting in limited interaction with surrounding native bone.[4] The results of our study recapitulate this, where PEEK cages had negligible osseointegration at 4 and 8 weeks post-operatively, while titanium implants demonstrated robust osseointegration. Despite these differences, recent meta-analyses reported similar clinical fusion rates[13,14] and a greater risk of subsidence in mainly non porous titanium implants.[13]Additive manufacturing steps, such as 3D printing, have been recently utilized to optimize implant qualities.[15] Such benefits include increased radiolucency,[16] decreased stiffness,[17] and the ability to create porous implants.[15,18] The latter is salient for spinal fusion, as this porosity purportedly enhances bony in-growth and osseointegration around the implant,[8,19-22] as well as limits micromotion.[19,21]

In 2004, Fujibayashi and colleagues demonstrated that porous titanium implants have in vitro and in vivo osteoinductive properties due to their structure.[8]Takemoto and colleagues found a fusion rate of 100% in sintered titanium implants with a 50% porosity versus a fusion rate of 60% in non-treated titanium implants at 3 months in a canine model of anterior lumbar fusion. They observed a greater degree of mature lamellar bone and less fibrotic tissue in their bioactive implants.[23] Both studies note the inherent difficulties in constructing such implants, relying on plasma spraying[8] and laser sintering.[23] To create a greater degree of precision in the manufacture of these implants, Wu and colleagues used computer assisted electron beam melting to produce porous implants in 2013. In their ovine model of cervical interbody fusion, they observed a significantly greater degree of BIC with porous titanium, as well a significant decrease in micromotion compared to PEEK cages with bone autograft.[19] 3D printing allows for an even greater degree of refinement in implant manufacturing, allowing the design to be selectively altered where the degree of porosity and strut width can be modified in critical areas of the implant, thus optimizing the biomechanical properties.[20] Indeed, the degree of porosity and pore size are important to osseous ingrowth; Taniguchi et al demonstrated that at 65% porosity and 632μm pore size, there was more optimal fixation at 2 weeks. The 3D-pTi implant was designed to have a pore diameter between 0.6-0.7μm and a porosity of 70-75% to optimize implant characteristics to augment osseointegration.[24] In addition to optimizing implant characteristics, this process also creates a solid implant, thus mitigating the concern of delamination of the surface upon impaction which occurs in plasma-sprayed implants.[25]

The results of our study are similar to what is found within the literature. The novel 3D printed matrix promoted significant osseous in-growth at just 4 weeks post-operatively.At 4 weeks, CT imaging demonstrated osteointegration of the 3D-pTi cages where bone is seen filling the previously void graft window. Contrarily, PEEK cages demonstrated a lack of osseous fusion and loose bone fragments in the graft window. This early interaction at the implant-bone interface observed with the 3D-pTi implants may add stability and limit micromotion in a much shorter time frame, therefore promoting early fusion with early stabilization due to this interaction. McGilvray and colleagues determined that 3D printed porous titanium cages were significantly better at preventing flexion-extension motion and promoted a greater total bone volume at 8 and 16 weeks when compared to PEEK and plasma-sprayed titanium PEEK cages.[20]

We also found the ingrowth and formation of cartilage and bone to be significantly greater in the 3D-pTi cages. Evidence of endochondral ossification was apparent where the accretion of a cartilaginous anlage was noted. The formation of new bone in this manner has been well described with the use of interbody cages.[19,26] The formation of a cartilaginous anlage is in part mediated by micromotion and local hypoxia, and eventually under goes hypertrophy, vascular in-growth, and calcification to form bone.[27] Areas of bone formation without a cartilaginous precursor were also observed, indictive that intramembranous ossification occurred. Given intramembranous ossification occurs with absolute stability, this may be evidence, in concordance with other reports, that 3D-pTi cages indeed limit micromotion.[19,21] While the clinical implication of this is uncertain, the early formation of bone and the osseointegration seen with 3D-pTi porous cages were starkly different than what was observed with PEEK cages. Further differences were noted on an in vitro analysis comparing human mesenchymal cells (hMSC) cultured on 3D-pTi vs scaffolds of 2D titanium and PEEK. It was noted a significantly larger number of hMSCs were found on the 3D-pTi scaffolds compared to PEEK or 2D titanium scaffolds after 14, 21, 28, and 42 days of growth, with a concordant elevation of alkaline phosphatase. 28 These data may indicate that the innate properties of the 3D-pTi cages create an osteoinductive environment fostering the early and robust development of osseous ingrowth seen in this in vivo analysis.

The absence of any material in the graft window of the 3D-pTi cages allowed us to better understand the topographical effects of the implant. Our results concur with the earlier studies that show even without traditional osteoinductive substances, the topography of the porous titanium cage is bioactive by itself.[8] This may be advantageous, especially in situations such as tumor patients, where it may be ill advised to utilize local autograft or osteopromotive allograft. Both allograft and osteoinductive materials such as bone morphogenic protein, are frequently utilized, but are exceedingly expensive. AICBG is the gold standard for grafting purposes, however necessitates a second incision and is associated with post-surgical donor site pain29 and increased surgical times.30 Thus, the osteoinductive and osteoconductive properties of 3D-pTi implants could make them efficacious and cost efficient in the clinical realm.

In light of our findings, there are limitations to be considered. While our data did show a convincingly positive effect regarding osseointegration with 3D-pTi cages, sheep have fundamentally different biomechanics than humans and the implants evaluated were designed for human implantation. Despite this, there are strengths of the ovine model for spinal fusion studies including comparable vertebral size.[31] Additionally, the time interval of the study included only up to 8 weeks, thus our findings do not portray the long-term effect of either material regarding the rate of successful osseous fusion and clinical outcome.

Conclusion

Within sheep, biomimetic 3D-pTi interbody cages are superior to PEEK cages with AICBG regarding bony in-growth and osseointegration with the surrounding native bone as measured radiographically and histologically at 4- and 8-weeks post-implantation. These data are useful in affirming the in vivo effects of 3D printed porosity in lumbar intervertebral cages; however, further translational studies are needed to ascertain the clinical relevance of these findings.

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