Bone In Growth with 3D-Printed Soft Titanium® Scaffold

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Abstract

BACKGROUND: Bone growth into implants is an essential factor in fusion impacting device performance and patient outcomes. 3D printing methods enable fabrication of complex scaffold geometries to promote bone growth and may provide substantial improvement over traditional devices.

METHODS: Bilateral, transcondylar implantations were made in adult New Zealand white rabbits. Four groups (n=2/device/time) were implanted: Soft Titanium® or PEEK at 4 and 8 weeks post-operative retrieval. Devices underwent micro-CT imaging and histomorphologic quantification. All implants contained graft windows and received comparable autograft from the implant site.

RESULTS: Micro-CT revealed substantial peri- and intra-implant hard tissue growth in Soft Titanium® scaffolds (22.1 and 30.5%), with markedly less formation adjacent to or within PEEK devices (8.9 and 23.0%; average by volume at 4 and 8 weeks respectively). Histomorphometry revealed substantially greater bone volume in Soft Titanium® devices (10.01 and 7.13%) as compared to PEEK counterpart (2.86 and 3.08%) at both 4 and 8 weeks, respectively.

CONCLUSIONS: Soft Titanium® devices clearly demonstrated bone growth throughout the scaffold and at a greater rate than PEEK, despite comparable autograft-packed windows. Internal porosity formed new bone tissue faster than the graft window.

CLINICAL RELEVANCE: Increased bone attachment and infiltration can result in reduced migration and subsidence in spinal fusion, ultimately improving biomechanical stability with the potential to enable improved patient outcomes.

Keywords: 3D Printing, Titanium, Spine Implant, Bone Growth, In Growth, In Vitro, Osseointegration

Introduction

Implants and porous titanium structures have been studied extensively in order to optimize growth of bone, resulting in identification of appropriate pore sizes particularly for surface penetration [1-4]. This attachment and osseointegration for implants are intended to promote early fixation, overall implant stability, and reduction in implant mobility, critical for spinal fusion [5].

Titanium devices have been evaluated against PEEK devices, which have been shown to incite inflammation and fibrous tissue growth potentially inhibiting bone formation [6]. This response in PEEK is augmented by the hydrophobic nature of the surface in traditional implants giving rise to attachment issues between bone and risk of pseudarthrosis [7].

Further, to achieve bone growth throughout porous implants, pores must be oriented to allow fluid and tissue commutation, preventing restriction of growth [8]. It is also desirable to create an environment where load-bearing bone tissue may grow to promote healthy tissue formation [9].

Thus, a scaffold was designed including both small and large pores to facilitate bone attachment and in growth. Further, the scaffold enables formation of bone tissue within a structure which will share the load with maturing bone and reduce the stress on surrounding tissue.

The purpose of this study was to overview the design of and evaluate bone in growth into such a device. We examined the Soft Titanium® porous 3D-printed titanium scaffold developed by HD LifeSciences® and collected measures of bone growth into transcondylar Soft Titanium® implants in rabbits. Data collected from this study suggests performance of Soft Titanium® as applied in spinal implants in humans, and examines whether the scaffold promotes greater bone formation at earlier time points compared to PEEK.

Methods

Scaffold Design

Soft Titanium® interfacing pores are designed in the range of 300-900µm with the average pore diameter about 400µm. Internal pores are up to 1100µm in diameter. Open cell channels from endplate to endplate maximize bony in growth potential.

Total porous volume is designed to be 70% of the scaffold volume.

A surface treatment is applied throughout each implant to optimize the environment for bony on growth. This area is magnified by the porosity, providing surfaces for attachment throughout a device with area dramatically greater than traditional devices.

PEEK Control Design

PEEK control devices were made with the same external dimensions as the Soft Titanium® samples but featured several necessary design adjustments.

The PEEK devices did not have any porosity, and therefore needed small circumferential ridges to prevent migration as shown in Figure 1. In addition, a lack of porosity in the PEEK devices meant that they featured significantly less volume in which bone could grow.

In Vitro Study

To examine the efficacy of Soft Titanium® as compared to PEEK, both devices were implanted in the distal femur (condyle) of skeletally mature New Zealand white rabbits.

In this study, we evaluated bone growth in the setting of a 3D-printed Soft Titanium® implant versus a PEEK implant.

Skeletally mature New Zealand white rabbits are widely used to evaluate bone in growth into porous biomaterials. They can regenerate bone in approximately 6 weeks, versus larger species such as sheep, dogs, or pigs, which occur at weeks 12, 14 and 20, respectively. Hence, investigators can evaluate new material performance within a relatively short period of time.

Implantation

Implantations were made in skeletally mature New Zealand white rabbits and subsequently removed at 4 and 8 weeks. The protocol was reviewed and approved by Harvard/MGH and Pine Acres Rabbitry Farm IACUC prior to this study.

Four of each implant type (PEEK and Soft Titanium®) were implanted. The devices consisted of 5 mm diameter x 12 mm depth rods (Figure 1) with graft windows. These devices were placed into matching defects created in the distal femur (transcondylar; Figure 2). Defects were made with a 4.9mm drill to achieve a press fit between implant and bone tissue, and a reamer was used as necessary for appropriate fit. Autograft from the implant site was used to fill the graft window of the implants but not the porosity of the scaffold (Figure 3).

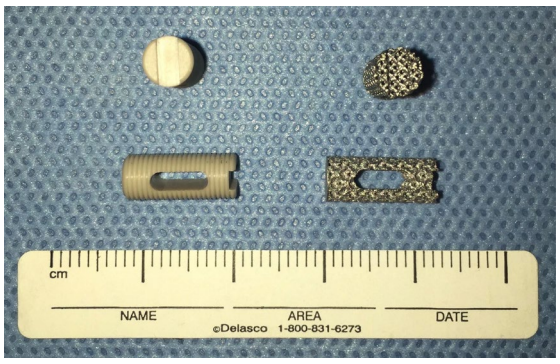

Figure 1 - PEEK and Titanium (Ti-6Al-4V ELI) HD Soft Titanium® with lumen.

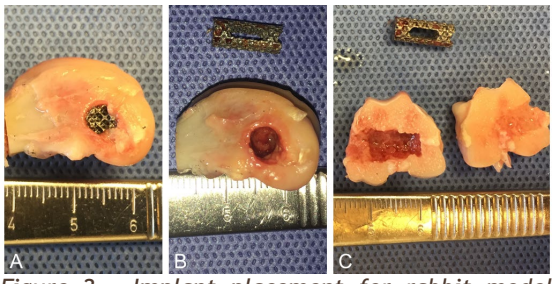

Figure 2 - Implant placement for rabbit model implantation. HD LifeSciences Soft Titanium® implant shown. (A) lateral view with implant inserted via lateral femoral condyle, (B) lateral view with implant removed, (C) axial view cross-sectioned to visualize defect.

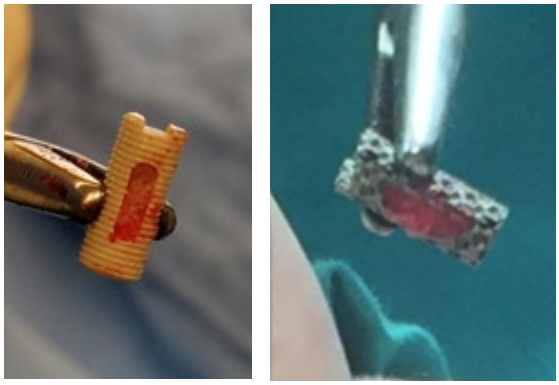

Figure 3 - PEEK and Soft Titanium® implants each with autograft-filled lumen; pores were not packed on the Soft Titanium® device.

One of each implant type (PEEK and Soft Titanium®) was implanted per rabbit and was randomly assigned to the right and left hind leg.

This study was compliant with all relevant laboratory animal wellbeing guidelines and regulations. All procedures were performed after induction of general anesthesia and surgical site prepping (Betadine or chlorhexidine gluconate 4%) and draping in sterile fashion.

Following implantation (Figure 4), the surgical site was closed, and the rabbits were returned to their cages. No other procedures (surgical OR non-surgical) were performed.

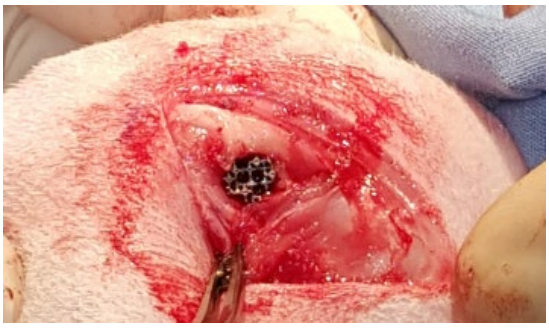

Figure 4 - Soft Titanium® following transcondylar implantation.

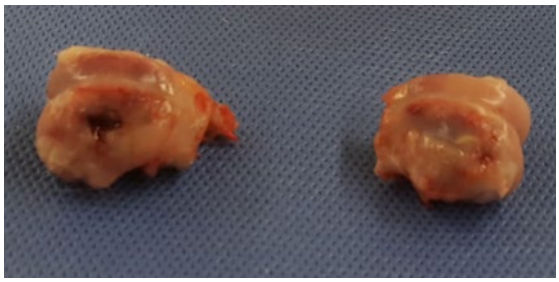

Figure 5 - Implants in distal femur following explant and prior to analysis.

At the 4th or 8th week following implantation, devices and surrounding tissue were removed (Figure 5) and subsequently embedded in 70% EtOH for transport to histology or 10% buffered formalin in preparation for micro-CT.

Micro-CT images were taken of all samples (55 kVp, 145 µA, 1x200 ms; Voxelsize 15.0 µm) with implants encased in tissue and suspended in water and low-density foam for stability and orientation. Image collection was conducted at the Harvard Forsyth Institute.

Implants were sent for hard tissue histology (methyl methacrylate embedding) and quantitative histomorphometry.

Production implants composed of Soft Titanium® were additionally imaged in light microscopy (Keyance VHS-6000).

Analysis

Device performance was assessed via micro-CT imaging and histomorphometry, as well as measurement of production pore size.

Micro-CT

Images were initially formatted and aligned in DataViewer [10] before processing for bone volume quantification in MicroView [11]. For consistent comparison of bone contact and permeation of each implant, we created a rectangular 1mm envelope around each device (Figure 6, orange box).

Volume was calculated for the complete window and totaled for all regions where bone was detected above the surrounding tissue threshold (Eq 1).

$$V_N = \frac{\text{Volume of Bone}}{\text{Envelope Volume}} \qquad (1)$$

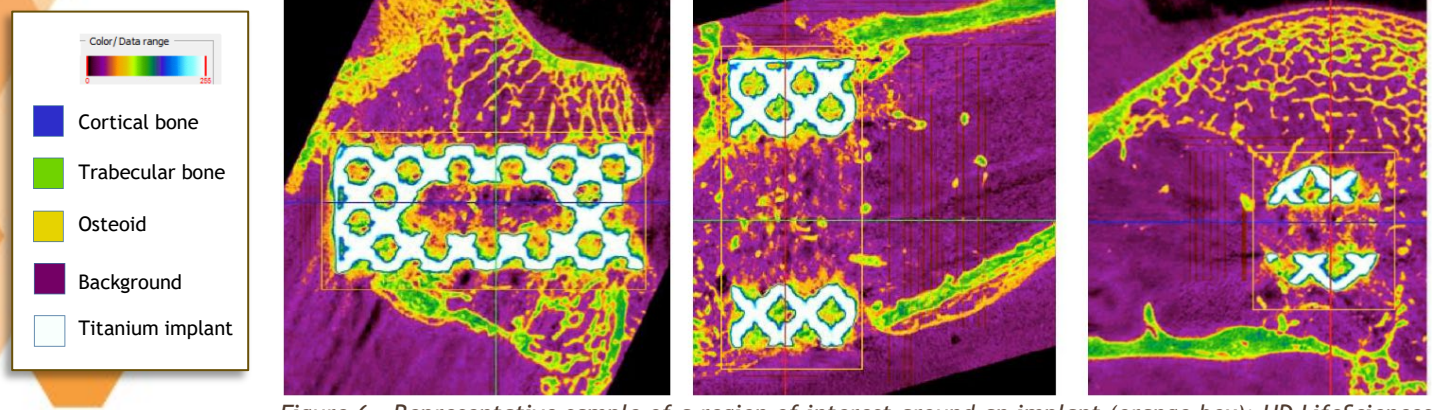

Figure 6 - Representative sample of a region of interest around an implant (orange box); HD LifeSciences Soft Titanium® implant shown. External border of bone adjacent to the implant (+1mm) was included for all quantifications.

Histomorphometry

PEEK and HD Soft Titanium® samples were divided between cross-sectional slices along the length and across the width of each implant (square and round cross sections) for diverse histological assessment, with multiple slices of one type taken from each implant.

For all samples, we quantified the amount of bone, implant material, cellularity, and the total volume in the sample. The difference between these equals the extra matter and interstitial space. These quantities were represented in the data as percentages of the total sample section. Mean data was compared between HD Soft Titanium® and PEEK devices at 4 and 8 weeks. Additional measures compared the percent composition within the internal (non-implant) space by dividing each individual volume by the total internal volume.

Pore Measurement

Pore geometry was quantified via software-enabled area and diameter measures to compute mean pore size. Pore morphology was also captured via SEM.

Results

Micro-CT

Each pore in the HD 3D-printed Soft Titanium® device showed growth, with demonstrated commutation from external surfaces to internal pores as well as between internal pores (Figure 7). Interface surfaces of the graft window showed formation of bone, while the expanse of the window showed bone formation varying from implant-to-implant and lagging formation in the porous structure.

PEEK devices showed little growth at peri-implant surfaces in the graft window, but bone did appear within the window in some cases. Most external implant surfaces on PEEK devices showed no tissue formation, although in some cases bone appeared external to the implants between some sections of teeth.

The HD Soft Titanium® scaffold grew substantial bone within and adjacent to the implant quickly, with 22.1% volumetric bone formation at 4 weeks, as compared to 8.9% in PEEK (Table 1, Figure 8).

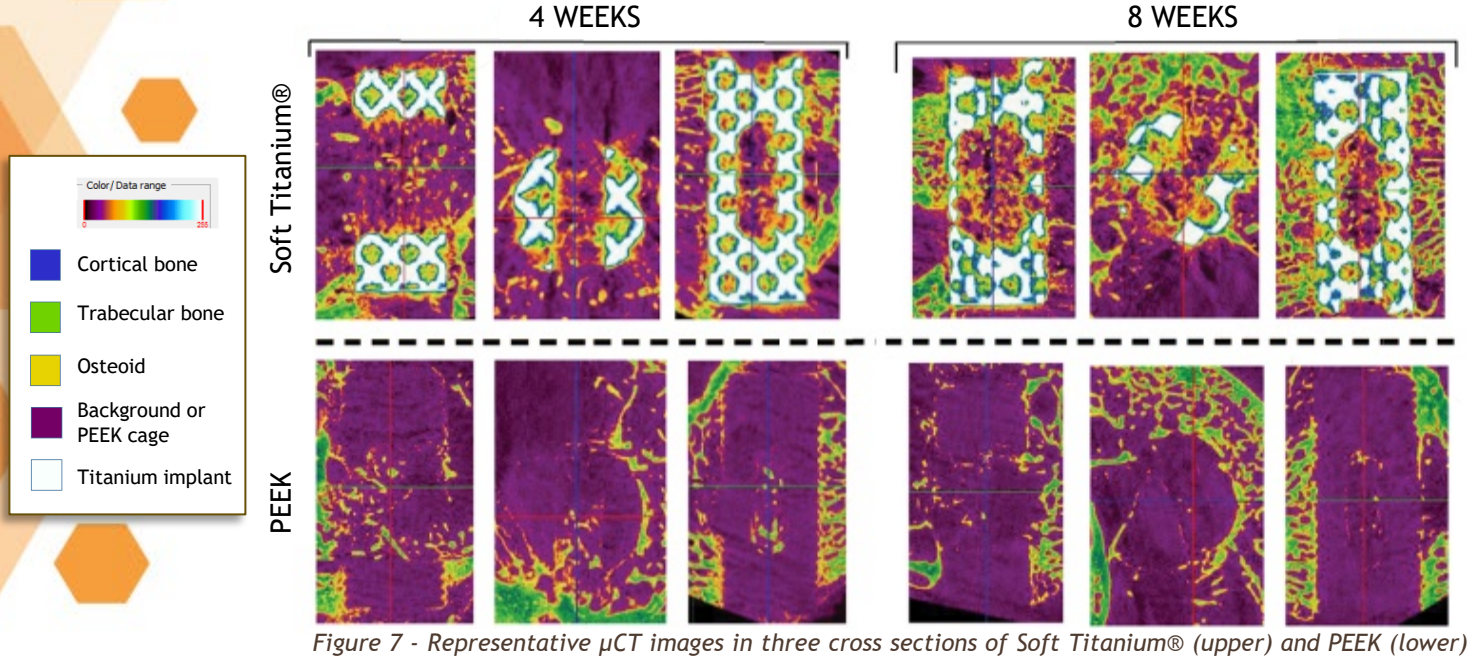

Figure 7 - Representative µCT images in three cross sections of Soft Titanium® (upper) and PEEK (lower) samples at 4 (left) and 8 (right) weeks demonstrating dramatically increased growth into Soft Titanium® devices as compared to PEEK. Cortical and trabecular bone formations are visible in Blue/Green, with void and implant material as Purple/White.

Table 1 - Normalized bone formation within each implant as a ratio of total bone volume as compared to total volume within each implant, demonstrating substantially more bone growth in Soft Titanium®.

	Bone Volume/Total Volume (%)
Soft Titanium® 4w	22.1
PEEK 4w	8.9
Soft Titanium® 8w	30.5
PEEK 8w	23.0

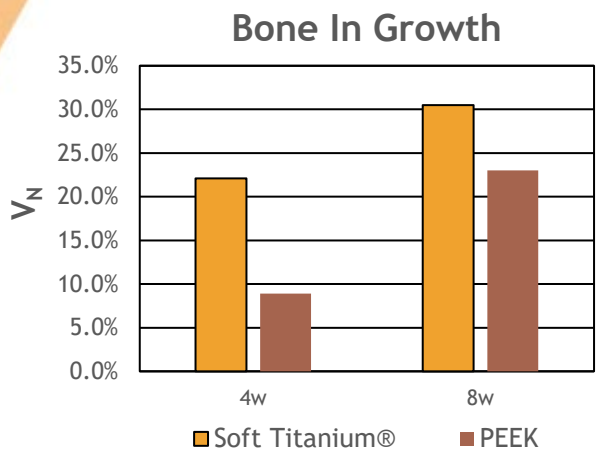

Figure 8 - In growth into Soft Titanium® and PEEK samples at 4 weeks and 8 weeks demonstrating substantial growth into Soft Titanium® devices as compared to PEEK.

High density bone formation (blue, Figure 7) was visible in the HD Soft Titanium® samples adjacent to the implant surfaces in all 4-week samples.

The trend continued with 30.5% bone formation on average in the HD device at 8 weeks and PEEK reaching 23%.

Histomorphometry

Cellular results demonstrated a substantial increase in bone volume in HD Soft Titanium® devices (10.01 and 7.13%) as compared to PEEK counterpart (2.86 and 3.08%) at both 4 and 8 weeks respectively (Table 2).

Assessment of total internal volume to scaffold volume in cross section confirmed ~70% porosity of Soft Titanium®, as compared to roughly 22-26% in PEEK implants (Table 3).

Normalized to internal volume across implants, the tissue composition demonstrated 10-14% bone, with the highest occurring at 4 weeks in Soft Titanium®.

Cellularity composition was consistent across most implants and time points at 51-52%, however dropping off in PEEK to 37% at 8 weeks.

Table 2 - Tissue composition within each implant as a percentage of implant volume.

	Bone Volume (BV) (%)	Residual Scaffold (%)	Cellularity (%)	Extra (%)
Soft Titanium® 4 Wk	10.01	30.64	35.48	23.86
PEEK 4 Wk	2.86	77.87	11.43	7.84
Soft Titanium® 8 Wk	7.13	31.29	35.02	26.56
PEEK 8 Wk	3.08	73.91	9.67	13.34

Table 3 - Relative proportions of tissue and volume within each implant.

	Internal (mm^3)	Internal / Implant (%)	BV / Internal (%)	Cellularity / Internal (%)	Extra / Internal (%)
Soft Titanium® 4 Wk	18.29	69.36	14.44	51.16	34.41
PEEK 4 Wk	12.00	22.13	12.92	51.67	35.42
Soft Titanium® 8 Wk	22.13	68.71	10.38	50.97	38.65
PEEK 8 Wk	8.96	26.09	11.79	37.08	51.13

Similarly, void, lymphocytes, and fibrous tissue comprising the Extra space made up roughly 34-39% of non-implant volume across most implants and time points, but increasing in PEEK to 51% at 8 weeks.

Pore Measurement

Implant small pores were measured to be in the target range of 300-500µm (Figure 9). Average small pore diameter was 393µm.

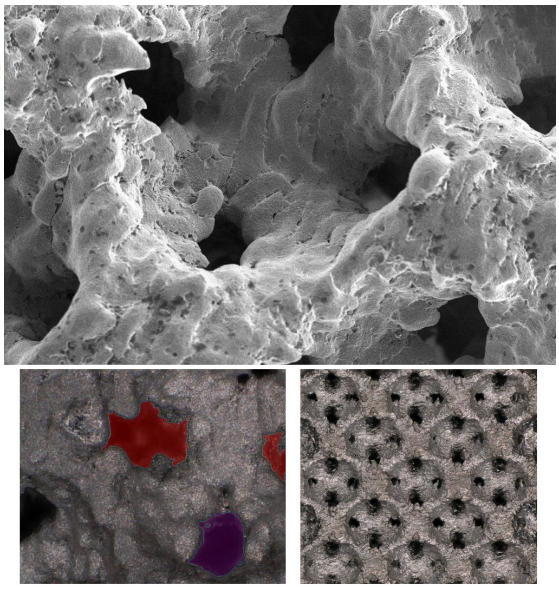

Figure 9 - Pore size measurement of Soft Titanium® via SEM and light microscopy indicated small pore sizes of average diameter 393µm.

Discussion and Conclusion

Soft Titanium® demonstrated bone formation and interdigitation throughout the porous structure, even as early as 4 weeks, and exceeded PEEK performance for promoting bone growth in each measure. Tissue formation in the porous structure appeared more rapidly than formation in the graft window of either device. Bone in growth is essential for implant performance in reducing the risks associated with implant mobility, subsidence, stress shielding, and other modes of clinical failure as observed in competitive devices.

Soft Titanium® devices saw a strong conversion of tissue into bone.

PEEK devices exhibited a reduction of cellularity in exchange for an increase in void.

Well-matched cellular composition between PEEK and Soft Titanium® devices indicated that both promote early fibrous infiltration and the Soft Titanium® scaffold infiltrated and grew bone throughout the porous volume. The presence of this trend at 4 weeks indicated that bony interdigitation begins early after implantation. While Soft Titanium® devices saw a strong conversion of tissue into bone throughout the scaffold, PEEK devices exhibited a reduction of cellularity in exchange for an increase in void at 8 weeks.

Soft Titanium® implants saw a substantial increase in total bone formed. There was a reduction in bone per volume at 8 weeks in Soft Titanium® attributable to limited bone formation within the device lumen as compared to within the porous structure.

High bone density abrupt to the implant and presence on all surfaces of the implant indicated a strong surface adhesion throughout the porous structure, promoting early attachment between tissue and implant surfaces and greater bone formation. This early attachment may reduce implant mobility and improve device fixation.

An increase in bone growth as observed in the Soft Titanium® implants has the potential to provide for a quicker, more robust fusion, resulting in a reduction in cost of care, reoperation rate following non-union, and revision surgery.

Bone penetration throughout the implant and potentially more robust bone formation bode well for the long-term implantation and survivability of Soft Titanium® devices.

Some variability between data and time points was observed as a result of this pilot study including relatively few samples and slices for histology.

Author Contributions

This work was conducted, analyzed, and authored by Christopher Jones, surgery and micro-CT analysis performed by David Bichara, with guidance on study design and analysis, and surgery performed by Jason Tinley, and clinical analysis and assessment by Jason Toy.

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Connect

For further information on HD LifeSciences' Soft Titanium® technology, please visit www.HDLifeSciences.com or contact info@HDLifeSciences.com.

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