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A Paradigm for the Development and Evaluation of Novel Implant Topologies for Bone Fixation: In Vivo Evaluation

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Abstract

While contemporary prosthetic devices restore some function to individuals who have lost a limb, there are efforts to develop bio-integrated prostheses to improve functionality. A critical step in advancing this technology will be to securely attach the device to remnant bone. To investigate mechanisms for establishing robust implant fixation in bone while undergoing loading, we previously used a topology optimization scheme to develop optimized orthopaedic implants and then fabricated selected designs from titanium (Ti)-alloy with selective laser sintering (SLS) technology. In the present study, we examined how implant architecture and mechanical stimulation influence osseointegration within an in vivo environment. To do this, we evaluated three implant designs (two optimized and one non-optimized) using a unique in vivo model that applied cyclic, tension/ compression loads to the implants. Eighteen (six per implant design) adult male canines had implants surgically placed in their proximal, tibial metaphyses. Experimental duration was 12 weeks; daily loading (peak load of $\pm 22\text{N}$ for 1000 cycles) was applied to one of each animal's bilateral implants for the latter six weeks. Following harvest, osseointegration was assessed by non-destructive mechanical testing, micro-computed tomography (microCT) and back-scatter scanning electron microscopy (SEM). Data revealed that implant loading enhanced osseointegration by significantly increasing construct stiffness, peri-implant trabecular morphology, and percentages of interface connectivity and bone ingrowth. While this experiment did not demonstrate a clear advantage associated with the optimized implant designs, osseointegration was found to be significantly influenced by aspects of implant architecture.

Keywords

Orthopaedic implant design; Osseointegration; Bone; Mechanical stimulation

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

INTRODUCTION

One strategy to improve prosthetic limb functionality is engineering implantable, bio-integrated devices. A critical aspect in implementing this technology is anchoring the system to remnant bone, which ensures secure fixation under various loading conditions and offers mechanical stability for connections with neural or muscle tissue.

Currently, a small number of amputees use osseointegrated prosthetic limbs, which are generally anchored with an intramedullary stem (Branemark et al., 2001; Hagberg and Branemark, 2009). Complications with these devices have yet to be adequately addressed (Hagberg and Branemark, 2009; Sensinger et al., 2009). One issue is resorption of structurally-critical bone, which may result from infection (Tillander et al., 2010), stress shielding (Tomaszewski et al., 2010; Xu and Robinson, 2008) or both. Furthermore, these devices are only available to amputees with sufficient bone to anchor the intramedullary implant (Branemark et al., 2001; Hagberg and Branemark, 2009). Despite these concerns, little data exists to design alternative implant structures.

Previously, we coupled topology optimization with a finite element (FE) model to develop novel implants that promote secure fixation (Kang et al., Under review). We found optimal structures by distributing limited implant material within a design domain. The FE model comprised a cylindrical design domain surrounded by trabecular bone, and loading consisted of uni-axial forces. The optimization objective minimized compliance of the bone-implant system, which is analogous to minimizing interface deformation. Two designs were fabricated from medical-grade Ti-alloy (Ti-6Al-4V) using selective laser sintering (SLS), a solid freeform fabrication (SFF) technique. Thus, we demonstrated part of a design strategy where optimized implants were developed and then rapidly fabricated.

Evaluating implants within a biological environment is critical to our design strategy. Since functional bone-implant systems undergo daily loading, this requires application of direct, in vivo loads to the implant. Additionally, mechanical loading affects osseointegration (Guldborg et al., 1997b; Leucht et al., 2007; Willie et al., 2010). The cellular processes of mechano-regulated bone adaptation occur locally (Parfitt, 2002); therefore, aspects of implant architecture can influence adaptive changes. Ultimately, peri-implant bone adaptation will affect performance.

The purpose of this study was to: 1) develop an in vivo system that applies controlled, cyclic loads to implants embedded in trabecular bone and 2) use this system to evaluate effects of implant architecture on osseointegration.

MATERIALS AND METHODS

In Vivo Implant System

The in vivo system consisted of the implant in trabecular bone, a percutaneous housing column, a pneumatic actuator that generated tension/compression loads and a control unit with real-time display (Fig. 1). The implant and housing column, placed in the medial, proximal metaphysis of canine tibiae, were aligned perpendicular to the bone's long axis. Prior to loading, co-axial threads were used to simultaneously attach the actuator to the implant and housing column. Implant loads were applied through a connecting rod attached to a diaphragm inside the actuator. Air pressure changes from a pneumatic hose placed positive/negative pressures on the diaphragm, creating compression/tension forces on the implant. A computerized controller and gating mechanism generated cyclic loading (trapezoidal waveform) by alternately exposing the hose to positive and negative air pressures stored within two cylinders (Suppl. Fig. 1). A vacuum-compressor maintained

cylinder pressures (model P251, Gast Manufacturing Inc., Benton Harbor, MI); bleed valves controlled air pressures/force magnitudes. Real-time load display was achieved with a calibrated strain gauge on the actuator's connecting rod, amplifier and oscilloscope (model 123 Industrial ScopeMeter, Fluke Corp., Everett, WA). Following loading, the actuator was removed, and the housing column was covered with a cap.

Surgical Implantation

At surgery, animals were pre-medicated with buprenorphine (0.01–0.02 mg/kg), acepromazine (0.05mg/kg) and glycopyrrolate (0.01mg/kg) administered intramuscularly. An intravenous catheter was placed and thiopental sodium (17–35mg/kg) was given until anesthetic induction. Following intubation, anesthesia was maintained with isoflurane. Under sterile conditions, an incision over the anterior-medial surface of the proximal tibia exposed the sub-periosteal bone. The bone was machined flat with a custom instrument for placing the housing column, which was attached with 8 self-tapping stainless steel screws (size 00×3/16" or 00×1/4", JI Morris Co., Southbridge, MA). The housing column then served as a guide for drilling a 6.4mm diameter hole using a square end mill (series 5-3 flute with 1/4" cutting dia., SGS Tool Co., Munroe Falls, OH); implants were interference fitted into trabecular bone. Soft tissue was closed around the housing column, and bupivacaine (1mg/kg) was administered subcutaneously. Oral antibiotic (cephalexin 30mg/kg twice daily) and anti-inflammatory analgesic (carprofen 2–4mg/kg once daily) were administered the first post-operative week. For the experimental duration, percutaneous housing columns were routinely cleaned with dilute chlorhexidine. Animals were housed individually with unrestricted cage activity; E-collars prevented oral contact with implant sites.

Experimental Design

To examine the influence of implant architecture on osseointegration, two optimized titanium (Ti)-alloy implants were fabricated for in vivo analysis (Fig. 2) (Kang et al., Under review). Both were based upon the same global topology optimization scheme, the objective was to minimize compliance of the bone-implant system, thus minimizing interface deformation. One of the designs had a solid structure derived directly from the global layout of the topology optimization; the other had a porous structure created by converting the design domain into a hierarchical scaffold (Kang et al., 2010). The scaffold was generated by using the layout of the solid design to map low and high porosity microstructure sub-units optimized to be both stiff and permeable (Kang et al., Under review). The low and high density microstructures had porosities of 63% and 33%, respectively; the implant's overall porosity was 50%. The size of the microstructures within the implant was established to generate pores ranging between 300 and 960µm. This range of pore sizes enabled accurate production of the scaffold using SLS technology (Synergeering Group, Farmington Hills, MI), which was utilized for fabricating both optimized implants. A third design, representing a non-optimized control, was also included in this study. This implant had a machined Ti-alloy cylinder with a porous coating formed by sintering a double-layer of commercially pure (CP) Ti beads 420–590µm in diameter (Orchid Coating, Southfield, MI) (Guldborg et al., 1997b). This bead coating had a mean pore size of 160µm and porosity of 32%. Overall dimensions of all implants were 6.4mm diameter by 12.7mm length.

Eighteen skeletally-mature, male purpose-bred beagles (13.3–15.5 months of age and 11.0–12.6 kg at surgery, Covance Research Products, Inc., Kalamazoo, MI) were entered into the study. The same implant design was placed bilaterally in each animal with each design assigned to six animals. Three groups of six animals entered the experiment during a three month period; within groups, each design was assigned randomly to two animals.

Experimental duration for each animal was twelve weeks. Prior to implant loading, there was a six week post-operative healing period for neo-bone formation, mineralization and initial osseointegration (Brunski, 1988; Guldberg et al., 1997a). This healing period was important for two reasons: 1) bone apposition was assumed for the topology optimization and 2) premature loading could generate a fibrous tissue interface (Aspenberg et al., 1992; Brunski, 1988) and implant loosening. Following the initial six weeks, each animal had a loaded and a control implant assigned randomly. Loaded implants received 1000 cycles of $\pm 22\text{N}$ (compression-tension at a rate of 0.5 Hz) five days per week for six weeks (Guldberg et al., 1997a). Euthanasia was achieved using sodium pentobarbital (150mg/kg) administered intravenously three days after the final loading bout. The University of Michigan committee on the use and care of animals approved all surgical procedures and experimental protocols.

Outcome Measures

Following harvest, tibiae were fresh frozen (-20°C). A lathe was used to cut 24mm diameter specimens centered about the implant and extending through the medial-lateral depth of the metaphysis. The most lateral 4mm of bone was removed, generating a surface perpendicular to the implant axis. Specimens were thawed for mechanical testing.

Stiffness of bone-implant specimens was determined using non-destructive mechanical testing (Suppl. Fig. 2). Five cycles of sinusoidal compression-tension loads ($\pm 22\text{N}$) were applied at a rate of 0.05 Hz with a servohydraulic mechanical testing machine (model 858 Mini Bionix II, MTS System Corp., Eden Prairie, MN) under load control. Implant displacements relative to the housing column (affixed to the testing machine's frame) were measured with a LVDT (model 10 MHR, Measurement Specialties, Hampton, VA). Stiffness was calculated from the last two compression-to-tension cycles by averaging the linear slopes of the force-displacement curves.

Peri-implant trabecular morphology was analyzed from micro-computed tomography (microCT) images (Suppl. Fig. 3). Specimens were scanned with an eXplore Locus SP system (GE Healthcare Preclinical Imaging, London, Ontario) using the following parameters: X-ray tube at 80kVp and 80 μamp , 6000ms exposure and 29 μm isotropic voxel size. A copper/aluminum filter reduced beam-hardening artifact (Meganck et al., 2009), and metallic artifacts were minimized by aligning the cylindrical implants with the specimen's rotation axis. Each specimen was scanned with a density phantom for image calibration. A global threshold was applied to the images, and the direct 3D trabecular thickness (Tb.Th.) and bone volume fraction (BVF) were measured. To do this, MicroView (v 2.2, GE Healthcare Preclinical Imaging) was used to segment the metaphyseal trabecular bone. Then, using Boolean operations, a region of interest (ROI) was generated consisting of the trabecular bone within a cylinder centered about the implant. This ROI had a radius and height 3mm greater than the outer surface of the implant, but excluded the implant and bone within 1mm from the outermost implant surfaces. Image artifact caused by metal is a function of composition, amount, scan parameters and filtration (Bouxsein et al., 2010); measuring bone morphology 1mm from the implant, regardless of design, caused little artifact.

Next, specimens were prepared for backscatter scanning electron microscopy (SEM) to measure interface connectivity and bone ingrowth (Suppl. Fig. 4). After dehydration in ethanol and xylenes, specimens were embedded in methylmethacrylate. A diamond saw (IsoMet Low Speed Saw, Buehler LTD, Lake Bluff, IL) was used to cut 800–1000 μm thick longitudinal sections, which were wet polished to 300–500 μm . Specimens were carbon-coated and imaged with an Amray SEM (model 1810, KLA-Tencor Corp., Milpitas, CA). Digital images were thresholded with ImageJ (v 1.40g, NIH, Bethesda, MD) at two grey-

scale values distinguishing implant, bone and soft tissue, followed by conversion to binary format for measuring percentage of available area filled with bone (Sumner et al., 1990). Interfacial bone area (a measure of interface connectivity) was determined from rectangular regions extending 200 μ m from the implants' exterior surfaces, and bone ingrowth was measured within porous spaces. Measurements were averaged from two sections per specimen.

Two sets of statistical analyses were used to compare outcome measures. For all 18 implant pairs, a repeated measures analysis of variance (RM ANOVA) tested for differences in load conditions within pairs and differences in implant design between pairs (significance at $p < 0.05$); a Tukey post-hoc test identified differences between implant designs. Within pair effects of loading measured across all pairs by the RM ANOVA were represented graphically by including a "pooled" group within the data plots. Since the RM ANOVA compared loading effect across all pairs, paired T-tests were used to compare loading within each design (significance at $p < 0.05$). All statistical tests were performed with PAWS Statistics (v18, SPSS Inc., Chicago, IL).

RESULTS

All implants were harvested and analyzed following the 12 week experiment. Cutaneous infections around percutaneous housing columns developed in 8 animals, which were treated with oral antibiotics (cephalexin, clavamox or baytril) and anti-inflammatory (carprofen) medication. All infections responded to treatment and did not affect the bone.

In vivo loading significantly increased the stiffness of the bone-implant constructs by a pair-wise average of 19.3% ($p = 0.040$) (Fig. 3). While no significant difference was detected between designs, the most consistent loading effect was associated with the porous optimized design (20.3% increase). The porous cylinder design had a larger increase (22.5%) but greater variability. The solid optimized design had the weakest loading effect (15.0% increase).

Similar trends were observed in the peri-implant trabecular morphology (Fig. 4). In vivo loading increased (pair-wise) bone volume fraction (BVf) 27.9% ($p = 0.131$) and trabecular thickness (Tb.Th.) by 22.5% ($p = 0.050$). No significant differences were detected between designs for either measure, but the porous optimized design had the strongest loading effect (48.1% and 37.5% increases in BVf and Tb.Th., respectively). The solid optimized design had the weakest loading effect (7.59% and 14.3% increases in BVf and Tb.Th., respectively).

Bone integration patterns were distinct for each implant design (Fig. 5). There was a trend of increased interfacial bone area associated with loading across all designs (pair-wise increase of 18.7%, $p = 0.061$), but this measure was significantly less for the solid optimized design compare to either porous design ($p < 0.01$) (Fig. 6A). This was primarily due to the solid design having little bone contact along its inner, concave surfaces (Fig. 6B); the few trabeculae within this region were thin and poorly connected. In contrast, the two porous designs had robust interface connectivity along outer surfaces. For bone ingrowth, the two porous designs had an increase associated with loading that trended toward significance ($p = 0.062$) (Fig. 7A). However, the cylindrical design had a significantly higher percentage of ingrowth than the optimized design ($p < 0.001$). The optimized design had bone within small and large pores (sizes of roughly 300 and 960 μ m, respectively) near the periphery, but limited ingrowth into deep pores. Therefore, to account for differences in pore depth between designs, ingrowth was compared in the outermost 1mm (Fig. 7B). In this case, loading had a significant effect ($p = 0.011$), but the optimized design still had significantly

less ingrowth than the cylindrical design ($p < 0.001$). Also of note, loading consistently enhanced bone infiltration ($p = 0.004$) in the outermost 1mm of the optimized design.

DISCUSSION

To investigate technologies for bio-integrated prosthetic limb development, our first aim was to develop an in vivo system that applies controlled, cyclic loads to implants in trabecular bone. We found tension/compression loading enhanced osseointegration. Specifically, construct stiffness, a measure of implant fixation, significantly increased with loading. Similarly, loading anabolically affected peri-implant trabecular morphology with a significant increase in trabecular thickness and a mean increase in bone volume fraction. In addition, there was a nearly significant increase in interface connectivity and a significant increase in bone ingrowth. These results support prior studies that demonstrated mechanical stimulation enhances osseointegration (Guldborg et al., 1997b; Leucht et al., 2007; Willie et al., 2010). Since functional implants undergo loading during daily use, our system can test design concepts in a mechanically-mediated, in vivo environment. An important aspect of our system was the incorporation of multi-directional loading. While joint replacements primarily experience compressive forces along articulating surfaces, a bone-anchored prosthetic limb could experience large tensile and compressive forces (as well as shear, torsion and bending).

In the second aim, we used the loading system to evaluate how implant architecture can influence osseointegration. The solid optimized design had little bone contact along its inner, concave surfaces which may negatively affect long-term performance. The porous optimized design was based upon the same optimized layout as the solid design, but included the incorporation of a hierarchical scaffold using microstructure sub-units. Comparing the two optimized designs with respect to osseointegration, the addition of the scaffold significantly enhanced bone apposition, load-induced increases in construct stiffness and peri-implant bone formation. Additionally, the scaffold permitted bone apposition without requiring bone infiltration of the concave regions associated with the solid design. Comparing the non-optimized, bead-coated implant to the two optimized implants, loading resulted in similar increases in fixation and bone integration; therefore, the optimized layout did not represent an advantage. The lack of design-dependent differences could be due to multiple factors. First, implantation time was only twelve weeks with loading during the latter half. Additional weeks of loading would permit further bone adaptation. Second, complete and uniform bone apposition along the outer implant surfaces was assumed for the FE model in the optimization scheme (Kang et al., Under review); this contradicts the in vivo experiment for which interface connectivity varied locally for each implant and varied across implants and animals. Consistent enhancement of in vivo interface connectivity would better match the optimization model and improve in vivo performance.

Osseointegration occurs in two primary stages (Galante, 1985; Hagberg and Branemark, 2009; Hollister et al., 1996). During the initial post-operative period, integration is dominated by a healing process (also called a regional acceleratory phenomenon (RAP) (Frost, 1983)) that is highly anabolic but spatially chaotic (Marco et al., 2005). Following healing, bone is maintained and altered by continual remodeling along established trabecular surfaces (Parfitt, 2002). Bone tissue responds to mechanical stimuli primarily during remodeling (Hollister et al., 1996), but interface connectivity and ingrowth are established during healing. Therefore, the long-term adaptive response of bone is dependent upon early osseointegration. Possible strategies to influence the healing response would be local application of an osteoconductive calcium-phosphate coating (Ducheyne and Cuckler, 1992; Mouzin et al., 2001) or anabolic agent (Khosla et al., 2008; Sumner et al., 2006) along implant surfaces.

The porous cylinder had more bone ingrowth than the porous optimized implant, likely due to aspects of implant architecture. The optimized implant had larger pores and higher porosity (small and large pore diameters of 300 μ m and 960 μ m, respectively, and overall porosity of 47%) than the bead coating (mean pore size of 160 μ m and porosity of 32%). During initial healing, it is possible the optimized design's pores were too large for favorable bone formation due to insufficient surface area for cell adhesion. While optimal pore sizes of 50–400 μ m have been suggested for bone ingrowth (Bobyne et al., 1980), data from an in vivo study of SFF Ti implants with homogeneous pores indicate that this optimal range is not universally applicable (Li et al., 2007). Others have only suggested a minimum pore size of 300 μ m (Karageorgiou and Kaplan, 2005). Alternatively, the differences in bone ingrowth observed in the present study may result from other bone-implant interactions. For example, enhanced permeability of the optimized design may have accelerated bone remodeling following initial ingrowth. This explanation is supported by evidence from another study of SFF Ti implants (Lopez-Heredia et al., 2008). In that experiment, a rabbit model was used to compare osseointegration in implants with 800 or 1200 μ m pore diameters at 3 and 8 weeks post-implantation. For the 1200 μ m pore size only, ingrowth was significantly less at the later time point. To explain this finding, newly formed bone must have resorbed as the integration process transitions from healing to remodeling, and this process was accelerated by the larger pore size. Therefore, in our study, less ingrowth with the optimized implant may indicate the bone is adapting to its mechanical environment rather than a detrimental aspect of the design. This may explain why less ingrowth did not negatively affect the stiffness of the porous optimized specimens. Ultimately, given the biological complexity of the osseointegration process, there is still need for better understanding of critical scaffold design parameters that lead to optimal tissue response (Hollister et al., 2009; Van Cleynebreugel et al., 2006).

Using topology optimization, the global layout of our implants was designed to minimize interface deformation (Kang et al., Under review). This objective function was based on previous research that demonstrated osseointegration is related to the local mechanical environment, which can be affected by implant surface geometry (Simmons et al., 2001a; Simmons et al., 2001b). Additionally, it is known that excessive interface deformation is detrimental to osseointegration (Aspenberg et al., 1992; Brunski, 1988). Nevertheless, there are other mechano-regulatory models to consider. For example, stress-shielding of bone affects implant performance (Huiskes, 1993). Stress-shielding within concave regions of the optimized implants may partially explain limited bone infiltration. To address performance considerations related to interface deformation and stress-shielding, our optimization may require a multi-functional design scheme that incorporates time-dependent load history.

The results of our study must be interpreted within the context of its limitations. First, sample sizes were limited by a large animal model ($n=6$ per design). Canines were selected for their sufficient trabecular volume and similar bone structure to humans (Pearce et al., 2007). Sample size insufficiencies were mitigated by using paired specimens (bilateral implants) and purpose-bred animals with similar ages, weights and genetic backgrounds. Second, destructive mechanical testing (either monotonic or fatigue) would provide more information about implant fixation than non-destructive testing. The same magnitude forces were applied during both in vivo loading and ex vivo mechanical testing, so the bone may have sufficiently adapted to the loading and masked design differences. Non-destructive testing was performed to obtain microCT and SEM measurements from the same specimens. Third, only one experimental time point was analyzed (twelve weeks). A shorter duration would provide information about early healing, and a longer duration would enable more bone adaptation.

CONCLUSIONS

Bio-integrated prosthetic limbs offer the potential of enhanced functionality. A critical step in developing these systems will be establishing long-term, secure fixation to bone in the remnant limb. Given the complex loading environment to be experienced during routine activities, there is little data to design an effective prosthetic anchor. Our *in vivo* system allows us to evaluate novel implant architectures within a controlled, mechanical environment. In this study, we found this system significantly enhanced osseointegration. We also found that multi-scale variations in implant topology enhanced bone adaptation and integration. Specifically, when comparing the two optimized designs, the addition of a hierarchical scaffold significantly increased bone apposition and improved (though not significantly) fixation and bone adaptation. Overall, the results of this study support that our design and evaluation paradigm is an asset for improving osseointegration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
 (A and B) Loading systems were placed in proximal, medial canine tibiae with the implants embedded in trabecular bone. (C and D) These systems consisted of the implant, a percutaneous housing column and a removable pneumatic actuator.

Fig. 2. Three implants were fabricated for in vivo analysis: a solid optimized, a porous optimized and a non-optimized porous cylinder.

Fig. 3. Loading significantly increased stiffness. No significant difference was detected between designs, but the most consistent effect of loading was associated with the porous optimized implant. *Denotes a significant pair-wise increase ($p < 0.05$); # denotes a nearly significant increase ($p < 0.10$).

Fig. 4. Loading increased the mean bone volume fraction (BVF) and significantly increased trabecular thickness (Tb.Th.); no significant difference was detected between designs. The strongest loading effect was associated with the porous optimized implant. *Denotes a significant pair-wise increase ($p < 0.05$); # denotes a nearly significant increase ($p < 0.10$).

Fig. 5. Scanning electron microscopy (SEM) images show examples of bone integration at 10× and 50× magnification.

Fig. 6.

(A) Loading led to a mean increase in interfacial bone area percentage. The solid optimized design had significantly less bone area than either porous design. (B) This limited integration was due to little bone connection along the inner, concave implant surfaces. **Denotes a significant pair-wise increase ($p < 0.01$) and *** ($p < 0.001$); # denotes a nearly significant increase ($p < 0.10$).

Fig. 7.

(A) The porous cylinder had a significantly higher percentage of pore space filled with bone than the porous optimized implant, but the later design had deep (>3 mm) pores. (B) Ingrowth into the outer 1mm was measured to directly compare the designs. Bone ingrowth was significantly increased due to loading, but ingrowth was still significantly greater in the porous cylinder than the optimized implant. The optimized implant also had a consistent increase due to loading. *Denotes a significant pair-wise increase ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$); # denotes a nearly significant increase ($p < 0.10$).