Journal of Medical Implants & Surgery

Lucca EGR,Kelly C,Gall K, J Med Imp Surg 2021, 6:6

Research Article Open Access

Review of In-Vivo Behavior of Porous Titanium Implants for Orthopedic Applications

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Received date: November 03, 2021; **Accepted date:** November 17, 2021; **Published date:** November 24, 2021

Citation: Lucca EGR, Kelly C, Gall K (2021) Review of In-Vivo Behavior of Porous Titanium Implants for Orthopedic Applications . J Med Imp Surg, Vol.6 Iss.6 No:e119.

Abstract

Porous titanium and titanium alloys are used in various orthopedic implants due to the ability to provide moderate stiffness, high strength, osteoconductive scaffolds to support skeletal reconstruction and guide bone growth. The objective of this review was to analyze preclinical studies conducted on porous titanium implants using a variety of manufacturing processes. The papers revealed an increasing number of published preclinical research studies evaluating porous titanium as a biomaterial for bone implants and more specifically, an increase in the use of additive manufacturing as a fabrication technique since 2010. A wide variety of fabrication methods for porous titanium implants, and preclinical models investigating the bone ingrowth have been reported. The majority of reported porosities for porous titanium implants used in preclinical animal studies had a mean of 61%, and the majority of pore sizes had a mean of approximately 500 µm. Animal models used to evaluate performance of porous titanium implants ranged in model type, time points, and endpoint analyses used. Of the surveyed studies, approximately a third carried out biomechanical testing of the implant. The review includes a discussion of the design and manufacturing of porous titanium implants and the preclinical models used to support translation to clinical applications.

Keywords:

Ti6Al4V, In vivo, Bone ingrowth, Porous, Additive manufacturing

Introduction

One of the critical goals for the design of orthopedic implants is to repair or replace a damaged joint or failed region of bone. Often in these cases, it is favorable for part of, or the entire the implant to osseointegrate with the host bone in order to achieve a stable reconstruction and allow load sharing between the implant and bone. Inadequate integration between bone and orthopedic implants is one of the primary challenges facing orthopedic reconstructions. For example, failure rates in limb salvage procedures range from 24% to 42%, and are typically caused by non-union of the bone to the graft or prosthesis. To overcome such failures, porous metallic implants have been employed to allow for a higher degree of stability in the initial reconstruction towards achieving osseointegration between bone and implant. Porous metallic implants have evolved over recent decades, from sintered beads and wires for arthroplasty components, to porous metallic foams, and most recently additively manufactured (AM) porous scaffolds (Figure 1). Of metallics used for orthopedic implants, the titanium alloy Ti6Al4V is the most widespread given its high strength, as well as superior fatigue and corrosion resistance [1].

Sintered beads offer a surface porosity for integration at the interface with direct apposition of the implant to bone. Their first applications were acetabular cups for hip arthroplasty which improved integration at the bone-implant interface, but were limited in the depth of the pore layer. Advancement of manufacturing techniques such as metal injection molding or sintering metallic fibers enabled manufacturing of volumetric porosity in metal implants, such as commercially pure titanium (CPTi) “foam” implants with highly interconnected pore networks. Further evolution of AM in the past decade has enabled architected porous scaffolds produced from CPTi as well as titanium alloys. Development of volumetric porosity in foams and now AM scaffolds has enabled lower stiffness and “through growth” resulting in improved load sharing between implant and bone. Such implants are particularly adept at repairing or substituting bone as their porosity, pore size, and pore interconnectivity can be adjusted to tune performance for various applications. Furthermore, the topology of the scaffold can dictate the biological capabilities, where the size, shape and interconnectivity of the pores allow for varied oxygenation, transportation of cells and nutrients, and bone ingrowth. Another benefit of fully porous implants includes large void volume and surface area for surface functionalization by compositing with another “graft” material or application of a surface coating throughout. Functionalization of high strength porous metallic implants in such manners can promote osseointegration, microbial resistance, or even allow for localized drug deliver [2].

In fact, AM technologies have become increasingly adopted for use in orthopedic applications due to the ability to create complex porous architectures to allow for bone ingrowth. Of AM technologies for fabrication of metals, powder bed fusion (PBF) technologies have

been widely adopted for manufacture of titanium and other metallic implants due to the ability to produce intricate topological designs and features with high accuracy. PBF techniques include electron beam melting (EBM) and laser powder bed fusion (LPBF). Other AM technologies for fabrication of metals such as laser engineered net shaping or metal binder jetting have seen less adoption for bone scaffold applications to date. AM porous titanium alloys are capable of meeting numerous design objectives for effective load-bearing segmental bone defect substitutes and other orthopedic implants as a result of their exceptional mechanical properties, mass transport properties, surface curvature, ample pore space, and internal surface area. Improvement in reconstruction outcomes for critical sized bone defects has been shown by using porous titanium implants produced by AM. In these cases, the additively manufactured implants were shown to result in higher fusion rates compared to femoral head allograft controls (91.7% vs. 61.9%). While AM is poised to displace traditional manufacturing methods for porous metals, there remains gaps in knowledge regarding the optimization of porous implant design and manufacture which preclinical translational models can help discern [3].

Materials and methods

This review was conducted using the PubMed database to assess the historical research and current state of the art for design, fabrication, and evaluation of porous titanium implants in preclinical bone growth models on June 16, 2020 using the following search string: ((in vivo[Title/Abstract]) OR (preclinical[Title/Abstract])) AND ((titanium[Title/Abstract]) OR (ti6al4v[Title/Abstract])) AND (bone[Title/Abstract]) AND ((porous) OR (scaffold) OR (mesh) OR (foam)). The initial search yielded 341 articles. These were screened with the following exclusion criteria: reviews, studies without in vivo experiments, implants that were not made of titanium or its alloys, screw implants, clinical studies, and implants that were not inserted into bone. Following screening, 160 papers were excluded, and 181 papers pertaining to porous titanium implants produced by both traditional and additive manufacturing methods were reviewed. From each screened paper, information about the publication, the model, the implant, and the results was extracted along with the PubMed identifier number, the first author's name, as well as the year of publication were all collected. Discussion of the design and manufacturing of porous titanium implants, the types of preclinical models employed by researchers, and the strategies used for evaluation of in vivo performance was carried out [4].

In order to synthesize the collected data, classifications were defined. For the implant, the material (Ti6Al4V, CPTi, other alloy), the implant surface treatment (chemical treatment, physical treatment, coating), the manufacturing method (AM, non-AM), the porosity type (surface, volume), the porosity (%), the pore size (μm), and the graft material (none, synthetic, cellular) were identified. The porosity type was determined by whether the implant's exterior surface was porous (surface porosity) or if the entire internal structure was porous (volumetrically porous). Surface treatments were categorized as chemical (i.e., anodization, acid etching, oxidation), physical (i.e., sandblasting, sintering), or coated (i.e., plasma spray, vapor spray). Cellular grafts included bone morphogenetic protein (BMP), polyvinyl alcohol-hydrogels, growth and differentiating factor, arginylglycylaspartic acid peptide, and fibroblast growth factor; and cellular grafts included bone marrow cells, mesenchymal stem cells, fibroblasts, and osteoblasts [5].

For the animal models described, the specific species was recorded and then classified as small, medium, or large. Small animals included mice and rats (<300g); medium animals included turkeys, dogs, cats, monkeys, and rabbits (3kg - 40kg); and large animals were sheep, goats, and pigs (40kg - 300kg). The surgical site (forelimb, hindlimb, joint, spine, cranium/mandible), the duration of the experiment (in weeks), and the defect type (constrained, critical-size, specialty) were also classified. Constrained models are defined as those where a defect is made by osteotomy or drilling, and the implant is implanted in the surrounded by bone; this includes plug and dowel-like models. Critical-size defects, or segmental defects, are defined as large voids in the bone whereby the bone cannot heal itself, typically in which the length of the surgical defect is twice the diameter of the diaphysis of the bone. Specialty defects were categorized as those with specific clinical approaches, including spine and joint arthroplasty models. The methods of each study, including the radiological imaging, histology, histomorphometry, and biomechanical test techniques were compared. Biomechanical tests were grouped as either destructive (i.e., compression, tension, push-out, pull-out, torsion) or nondestructive (i.e., range of motion, lateral bending, flexion).

Results and Discussion

Design and Manufacture of Porous Titanium Implants for Preclinical Models

Preclinical models provide key value in the development and translation of new technologies into medical use. Evaluation of porous titanium implants in orthopedic animal models has been reported since the 1960s, with some of the first studies investigating the effect of electrical stimulation on the interfacial strength between porous titanium implants and cortical bone in dog's femurs, the metal-ion release from porous titanium implants in dog's hindlimbs and forelimbs, and the bony ingrowth into porous-coated titanium implants in turkey's ulnas. Evolution of manufacturing for porous titanium implants has evolved from random sintered beads, fibers, or wires to porous foams, and most recently scaffolds with complex, designed topologies. These trends can be followed in the preclinical literature as well as the commercialization of arthroplasty, arthrodesis, and other orthopedic medical devices over the past four decades (Figure 1).

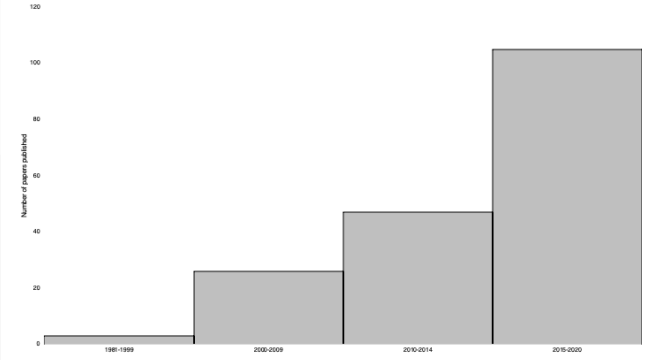


Figure 1 A: Number of publications of papers on osseointegration of porous titanium in vivo models between 1981 and 2020 [8], [9],[13], [16]–[22], [26], [29]–[36], [38]–[40], [43]–[51], [54], [55], [57],[60]–[86], [86]–[206]. B, Evolution of porous titanium implants over time.

Figure 1Bi: Molded and sintered porous Ti fiber specimen. Reproduced from Ducheyne, P. et al. In vivo metal-ion release from porous titanium-fiber material. J Biomed Mater Res. (1984).

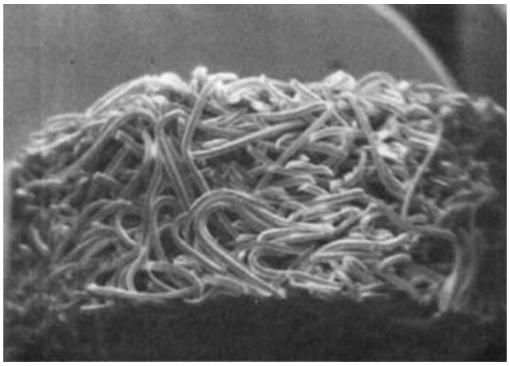


Figure 1Bii: Sintered Ti fiber. Reproduced from Zhang, E. et al. Porous titanium and silicon-substituted hydroxyapatite biomodification prepared by a biomimetic process: characterization and in vivo evaluation. Acta Biomater. (2009). https://doi.org/10.1016/j.actbio.2009.01.014.

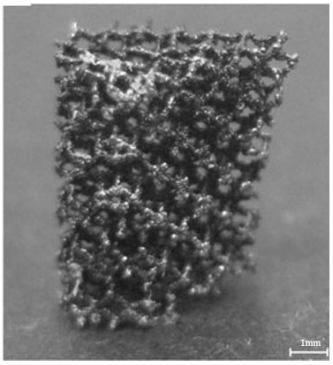


Figure 1Biii: SLM fabricated porous titanium scaffolds. Reproduced from Van der Stok, J. et al. Enhanced bone regeneration of cortical segmental bone defects using porous titanium scaffolds incorporated with colloidal gelatin gels for time- and dose-controlled delivery of dual growth factors. Tissue Eng Part A. (2013). https://doi.org/10.1089/ten.TEA.2013.0181.

Figure 1B iv: 3D printed porous titanium scaffolds with different pore structures. Reproduced from Wang, H. et al. The effect of 3D- printed Ti(6)Al(4)V scaffolds with various macropore structures on osteointegration and osteogenesis: A biomechanical evaluation. J Mech Behav Biomed Mater. (2018). https://doi.org/10.1016/j.jmbbm. 2018.08.049

The implants in the preclinical studies from our search were mostly composed of either commercially pure titanium (CPTi, 52%) or a high-strength alloy (Ti6Al4V, 46%).

While the studies considered in this review broadly captured porous titanium implants, 43% pertained to preclinical models evaluating implants produced by AM (Figure 2A).

AM technologies, such as PBF, have since continued to displace previous processing methods to produce porous metallic scaffolds at lower costs and with increased complexity.

As a result, preclinical studies of porous titanium implants for orthopedic applications have greatly increased over the past decade.

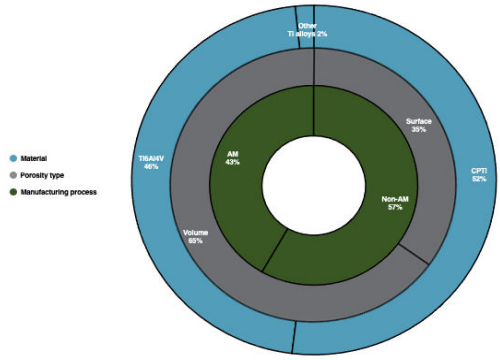


Figure 2 A: Concentric pie charts show the implant material, manufacturing process, and porosity type used for porous titanium in vivo models published from 1979 to June 2020. Ti6Al4V, CPTi, other; volume, surface; AM, non-AM.

Scaffolds and devices that were manufactured via AM were all volumetrically porous, affirming the primary benefit of AM for complex porous implants. Conversely, the majority of those manufactured via non-AM methods were surface-porous (63%), highlighting the challenges in processing volumetrically porous implants with such methods. Takemoto et al. produced porous bioactive titanium implants with a surface porosity of 40% by a plasma spray method combined with chemical and heat post-processing treatment in a rabbit femoral defect model, which yielded enhanced bone “surface” ingrowth and apposition under load-bearing conditions. Hacking et al. manufactured surface porous Ti6Al4V cylindrical femoral intramedullary implants sintered with titanium beads and acid-etched, and found enhanced bone formation at the implant surface (Figure 2Bi).

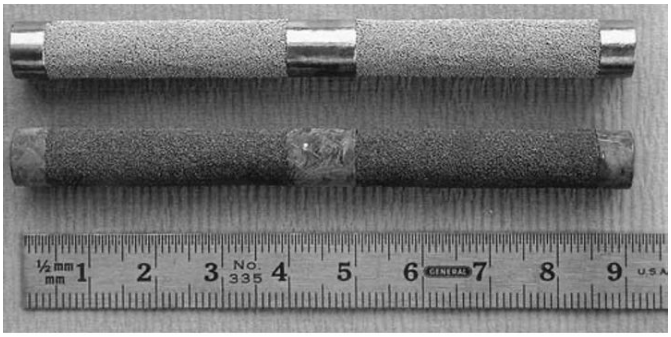


Figure 2 Bi: Non-AM surface porous Ti6Al4V cylindrical femoral intramedullary implants. Reproduced from Hacking, S.A. et al. Acid-etched microtexture for enhancement of bone growth into porous-coated implants. J Bone Joint Surg Br. (2003). https://doi.org/10.1302/0301-620X.85B8.14233.

Wieding et al., on the other hand, produced a volumetrically porous titanium scaffold via LPBF for large segmental bone defects, and determined that it was a suitable alternative to autologous or allogenic graft (Figure 2Bii).

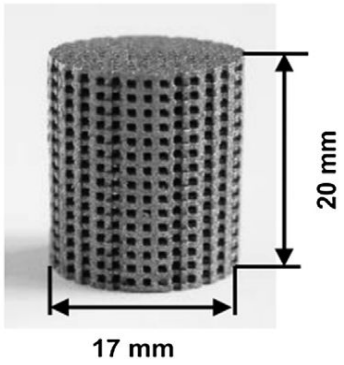


Figure 2 B ii: AM volumetric porous SLM fabricated Ti6Al4V scaffold. Reproduced from Wieding, J. et al. Biomechanical stability of novel mechanically adapted open-porous titanium scaffolds in metatarsal bone defects of sheep. Biomaterials. (2015). https://doi.org/10.1016/j.biomaterials.2014.12.010

Porosity and Pore Size

AM orthopedic devices have been designed to have prescribed properties, and to imitate the structure of bone in order to achieve load sharing. Bone ingrowth into porous metallic Implants is influenced by the implant's porosity, pore size and shape, and interconnectivity. The ranges of porosity and pore size reported across all studies were 18%-92% and 100-1500 µm respectively (Figure 2C).

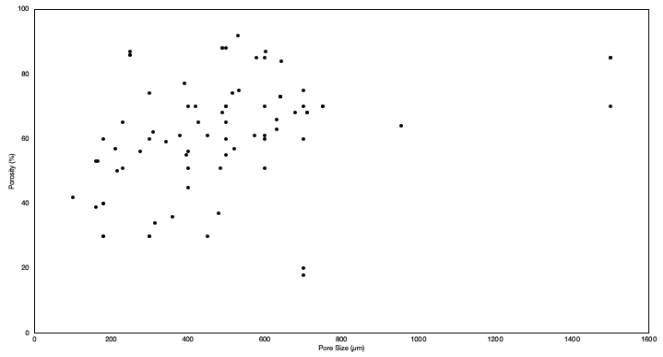
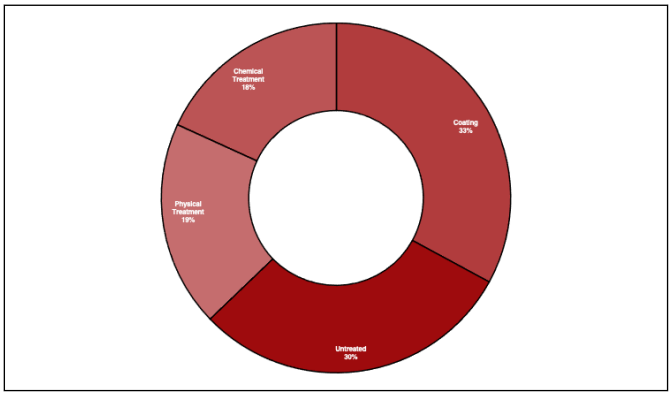


Figure 2C: Scatterplot of material porosity and pore size of porous titanium implants.

The mean porosity and pore size across all studies was 61% and 493 µm. No strong relationship was seen between the fabricated implant's porosity and pore size. In fact, in many studies researchers chose to independently vary these design parameters to determine their effect on in vivo performance. For example, Prananingrum et al. studied porous implants with a fixed porosity in the range of 38.48-43.07% and increasing pore sizes of 60 µm, 103 µm, 203 µm, and 611 µm in rabbit calvaria and found that bone regeneration in these scaffolds is pore-size dependent. Conversely, Bandyopadhyay et al. varied the porosities between 2.8%, 10.7%, and 25% by increasing the hatch distance, thereby increasing the mean pore diameter of the implants. Evaluation in a rat femoral defect model demonstrated that the implants with the higher porosity had a faster rate of tissue generation and integration than those with lower porosity. This evidence of modulation of different design parameters demonstrates that there is no universally optimal value for porosity or pore size, and design considerations for the site, loading conditions, and bone quality are important factors.

Functionalization of Porous Metal Implants for Osseointegration

An advantage of porous implants is the increased surface area which can promote osteoconduction, allowing for direct bone-implant contact, and avoidance of fibrous encapsulation. Both the chemical and physical surface properties of metallic implants have been altered in preclinical studies by methods such as the addition of coatings (30% non-AM, 33% AM), alteration of the surface with chemical treatment (17% non-AM, 18% AM), or physical surface treatments (28% non-AM, 19% AM) (Figure 3A, Figure 3B).



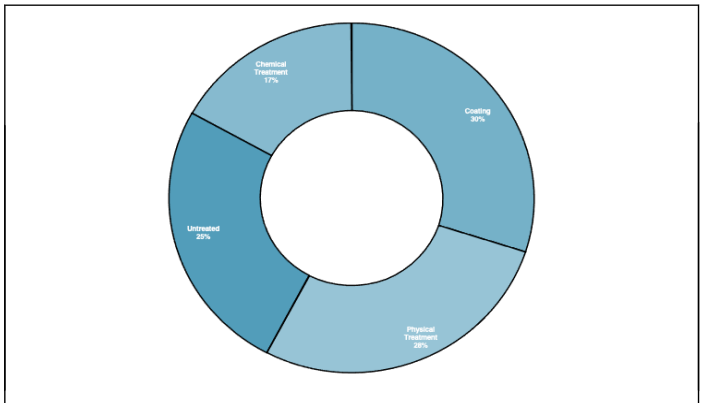


Figure 3 A,B : Concentric pie charts of surface alteration (chemical treatment, physical treatment, or coating) or untreated surface, and manufacturing process of implants. A, AM. B, Non-AM.

In studies where surface coatings were used, comparison to the bare surface was common to elucidate the role of the treatment on in vivo performance. The most prevalent surface coatings investigated in the surveyed literature included hydroxyapatite and calcium phosphate, to increase the osteoconductive nature of the surface. Other coatings have also shown promise in increasing bone regeneration, such as the magnesium-coated EBM Ti6Al4V scaffolds studied by Gao et al., which had significantly increased new bone formation compared to bare scaffolds implanted in a rabbit model. Coatings on titanium surfaces have also been investigated to improve bacterial resistance and mitigate the formation of robust biofilms in cases of infection. Williams et al. showed local protection of the implant surface from bacterial colonization by adding an antibiotic loaded coating to the surface of a CPTi porous-coated cylindrical titanium plugs manufactured by machining (Figure 2Bi).

In other studies, chemical surface treatments, such as an anodization were used to alter the surface chemistry namely the titanium oxide layer at the surface to improve bone formation. Chemical etching, as well as physical surface treatments, can also be used to alter the surface topography. Particularly in AM implants produced by PBF where the process results in a rough surface, which can be reduced with such post-processing treatments. For example, Xu et al. analyzed the bioactivity of anodization and alkali-heat treatment of selective laser melted (SLM) CPTi compared to untreated and acid etched implants in rabbits and found that the addition of nano-porous and roughened surface improved the bioactivity and bone regeneration performance . Similarly, de Wild et al. examined osteoconductivity in untreated, sandblasted, and sandblasted and acid-etched SLM titanium implants in rabbits and demonstrated that scaffolds with both physical and chemical treatments resulted in an increase in defect bridging.

Additional enhancement of performance can be achieved through incorporation of other materials which can be composited into the void volume. Such “graft” materials include both cellular (allograft, autograft), and synthetic materials. In studies using a composite of scaffold and graft, the use of synthetic graft was higher than cellular (Figure 4).

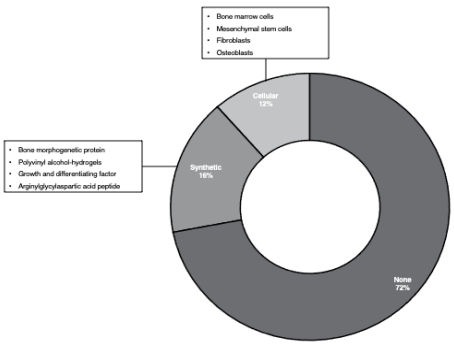


Figure 4: Concentric pie chart of type of bone graft in porous titanium implants.

The greater use of synthetic material in preclinical models may be attributed to an inability to harvest autogenic graft in some models or concerns about allogenic immune response. However, more than two thirds of studies investigated “empty” scaffolds with no graft material.

Preclinical Animal Models

The preclinical models used for evaluation of porous titanium implants were highly varied in animal, timepoints, defect site and type (Figure 5).

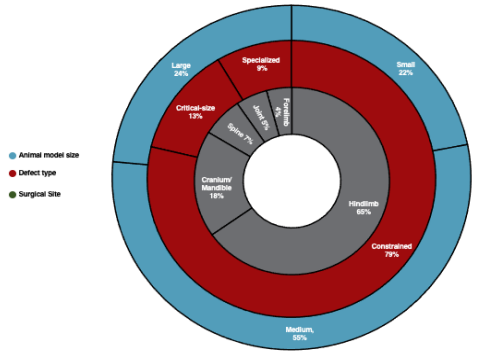


Figure 5 : Concentric pie chart shows the in vivo animal model sizes, bony defect type, and surgical site used for titanium porous implants published from 1979 to June 2020. Small, medium, large; infant, adolescent, adult; constrained, critical-size, specialized; hindlimb, cranium/mandible, spine, joint, forelimb.

Most commonly, medium-sized animals were used, and adult animals were almost always used. Notably, the single study that utilized a non-adult animal model by Warnke et al., studied the implantation of a human-sized titanium mandible in the latissimus dorsi muscles of infant miniature pigs and evaluated their mechanical integrity. As indicated by the author, this study was specifically designed for a young growing organism and was eventually used to subsequently validate the in vivo engineering of a mandible replacement in an adult human.

The hindlimbs (tibia and femur) were the most common sites for bone defect models, including treatment of critical-size defects made in the diaphysis . Pobloth et al. also investigated the ability to translate the critical-size bone defect regeneration via a Ti-mesh scaffold in a large animal model for clinical use. In the study, they included a direct

application of these implants in humans: a 61-year-old woman with a nonunion of the femur and a 45-year-old man with a fractured femur; and analyzed the formation of bone bridging and growth.

In general, specialty defect models had longer timepoints (average of 16 weeks) and were larger animals (88%), compared to those of constrained or critical-size defects (Figure 6A).

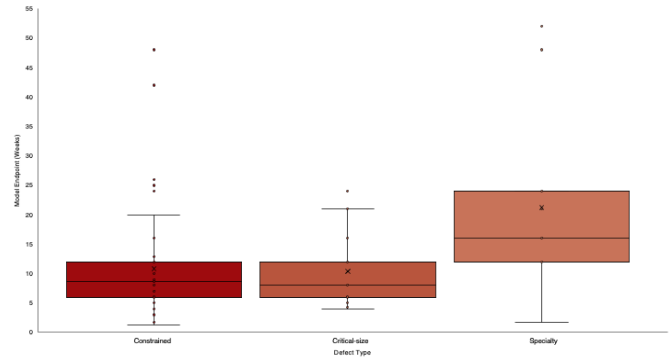


Figure 6 A: Box and whisker plot of length in weeks of in vivo experiments for constrained, critical-size, and specialty bony defect types of porous titanium implants.

With increasing animal size, the endpoints of the studies increased, where on average the maximum timepoints were of 6, 8 and 12 weeks for small, medium, and large animal models, respectively (Figure 6B, 6Ci,6Cii,6Ciii).

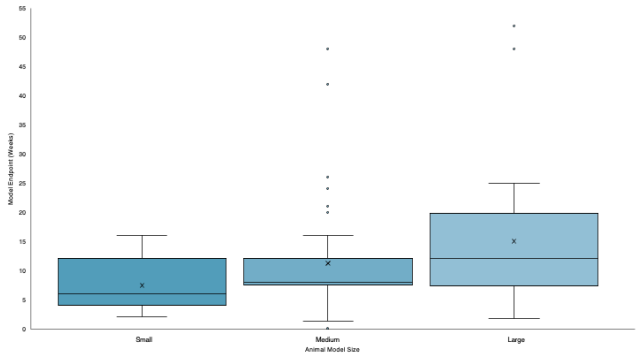


Figure 6B: Box and whisker plot of length in weeks of in vivo experiments for small, medium, and large animal models of porous titanium implants. C, Ti6Al4V AM porous implants for in vivo experiments in different defect types.

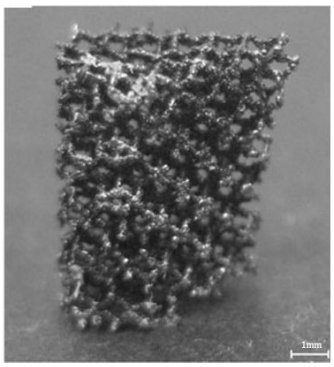


Figure 6B i: SLM fabricated porous titanium scaffold for critical-sized defect. Reproduced from Van der Stok, J. et al. Enhanced bone regeneration of cortical segmental bone defects using porous titanium scaffolds incorporated with colloidal gelatin gels for time- and dose-controlled delivery of dual growth factors. Tissue Eng Part A. (2013). https://doi.org/10.1089/ten.TEA.2013.0181[19]

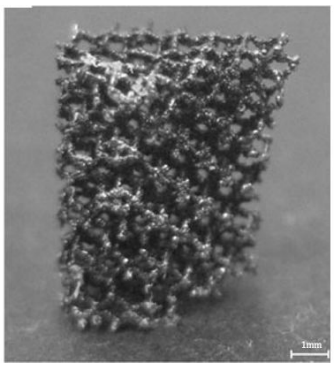


Figure 6B ii: EBM fabricated titanium alloy porous cage for specialty defect. Reproduced from Li, P. et al. A novel 3D printed cage with microporous structure and in vivo fusion function. J. Biomed Mater Res. (2019).

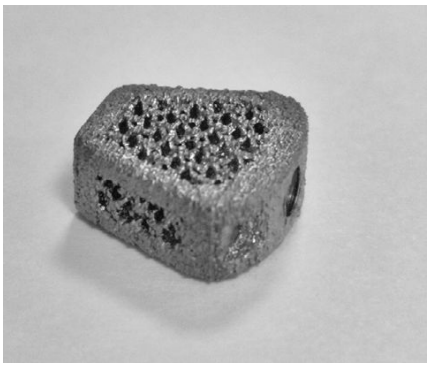


Figure 6B iii: In vivo porous Ti6Al4V scaffold fabricated using EBM for constrained defect. Reproduced from Feng, L. et al. A Comparison of 1- and 3.2-MHz Low-Intensity Pulsed Ultrasound on Osteogenesis on Porous Titanium Alloy Scaffolds: An In Vitro and In Vivo Study. J Ultrasound Med. (2019). https://doi.org/10.1002/jum. 14683

Overall, medium-sized, adult, hindlimb models with constrained defects were the most common (Figure 5), and larger animal models and specialty defect models had the longest experimental timepoints. Moreover, studies with multiple time points were common (57% with 2, 32% with 3, and 12% with 4 or more), allowing for longitudinal assessment and comparison of osseointegration over time.

Evaluation of In Vivo Performance

The evaluation of implants through non-destructive and destructive testing methods are critical to assessment of their in vivo performance in a given model. Often, a combination of radiological, histological, and biomechanical evaluations is employed.

Radiological Evaluations

While a majority of studies employed radiological evaluation post-sacrifice, only a few used in vivo imaging via computed tomography to assess bone growth over the course of the study. For example, van der Stok et al. carried out both in vivo and ex vivo longitudinal cross section miro-computed tomography images of rat femur uncoated and osteostatin-coated implants. Challenges in using in vivo as well as ex vivo computed tomography methods are caused by the attenuation of the titanium, leading to artifact. The metal artifact must be distinguished from bone through image segmentation and can lead to varied values for bone ingrowth volume and bone-to-implant contact area depending on the methods used.

Histological Evaluations

Assessment of bone growth inside the porous scaffolds using histological evaluation is critical in assessment of in vivo performance. With metal implants, polymethyl methacrylate histology is typically used. Various stains were employed to assess the tissues, including hematoxylin and eosin, as well as more specialized stains for bone or connective tissue. An exemplary histological results using Van-Gieson staining (Figure 7A) versus methylene and basic fuchsin (Figure 7B) to visualize tissue ingrowth into SLM fabricated Ti6Al4V porous implants.

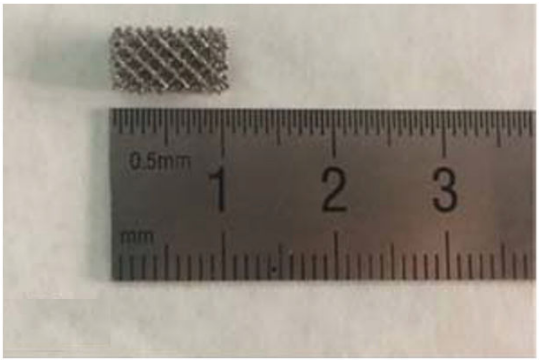


Figure 7 A: Van-Gieson staining of histological sections of 3D printed Ti6Al4V disk at 4 weeks after implantation. Reproduced from Wang, S. et al. Fabrication of bioactive 3D printed porous titanium implants with Sr ion-incorporated zeolite coatings for bone ingrowth. J Mater Chem B. (2018). https://doi.org/10.1039/c8tb00328a [45]

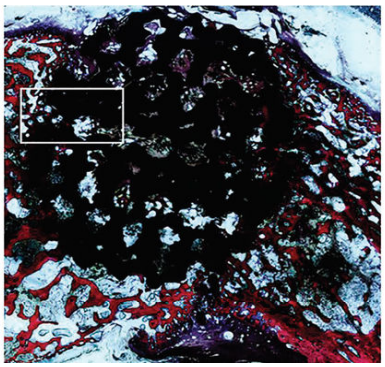


Figure 7B: Histology quantification of new bone ingrowth into SLM fabricated Ti6Al4V porous implants in cortical and cancellous sites as well as the porous walls of the interbody cages at 12 weeks. Reproduced from Walsh, WR. et al. Does implantation site influence bone ingrowth into 3D-printed porous implants? Spine J. (2019). https://doi.org/10.1016/j.spinee.2019.06.020 [46].

. In the former, the stain allows visualization of collagen and connective tissues, while in the latter is used for visualization of bone. While histological evaluation is typically qualitative or semi-quantitative, employment of histomorphometry allows researchers to quantify bone architecture, giving valuable insights into cellular activity. While less than a quarter (23%) of the surveyed studies reported histomorphometric evaluation, many of these were more recent studies, and likely the use of these methods will continue to grow. Of those studies employing such methods, evaluation of the structure of bone within the porous network was most common, with quantification of metrics such as bone ingrowth, bone volume (BV), and bone-to-implant contact (BIC). For example, Van der Stok et al. determined a 16% and 20% increase in BV measured within the pore volume of SLM titanium scaffolds with porosities of 88% and 68%, respectively, implanted in a critical femoral bone defect rat model. In porous cylindrical dowels implanted into the cortical and cancellous bone of sheep femur and tibia, Walsh et al. observed an increase of 74% of bone in the available void of cortical sites and 15% in cancellous sites. Moreover, in SLM Ti6Al4V interbody cages in a sheep spine model, Walsh et al. observed a 21% increase of bone in the available void of the walls at 12 weeks when filled with autograft.

Biomechanical Evaluations

One-third of surveyed studies conducted biomechanical tests, and of those that did, the majority were destructive (Figure 8A).

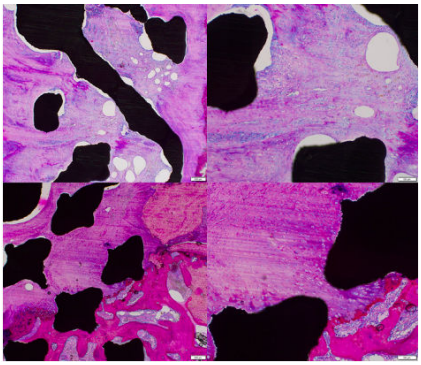


Figure 8 A: Concentric pie chart of biomechanical tests conducted for porous titanium implants.

For instance, van der Stok et al. carried out torsional testing of SLM Ti6Al4V implants loaded with BMP-2 containing fibrin gels in critical-size femoral bone defect rat model using a static mechanical testing machine to measure torsional strength and determined the the porous titanium implants improved bone regeneration.

The destructive biomechanical tests were primarily push-out tests (49%), with fewer instances of pull-out (21%), compression (11%), torsion (11%), and tension or shear (8%) (Figure 8B, Figure 8Ci).

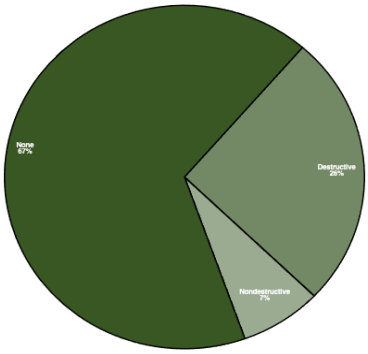


Figure 8 B: Concentric pie chart of type of biomechanical destructive test conducted for porous titanium implants.

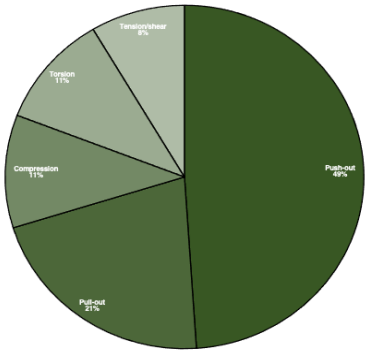


Figure 8 Ci: Biomechanical testing. i, Destructive push-out test using a universal mechanical testing machine. Reproduced from Li, L. et al. Early osteointegration evaluation of porous Ti6Al4V scaffolds designed based on triply periodic minimal surface models. J Orthop Translat. (2019). https://doi.org/10.1016/j.jot.2019.03.003 [48].

For specialty defects, a larger proportion of studies reported nondestructive testing than destructive testing, such as the six-degree-of-freedom spine simulator utilized by Cunningham et al. to carry out multidirectional flexibility testing of total disc replacements in baboons (Figure 8Cii).

Figure 8Cii: Nondestructive multidirectional flexibility test using a six-degree-of-freedom spine simulator. Reproduced from Cunningham, BW. et al. General principles of total disc replacement arthroplasty: seventeen cases in a nonhuman primate model. Spine (Phila Pa 1976). (2003). https://doi.org/10.1097/00007632-200310151-00005.

Another example is Walsh et al., who performed a range-of-motion test (nondestructive flexion-extension, lateral bending, and axial rotation using a 6° of freedom musculoskeletal simulator) to measure spinal stability of a SLM-fabricated Ti6Al4V interbody fusion sheep model and found that the stability was not significantly different with autograft or super-critical fluid-treated allograft in the cage opening. The study showed that the porous ingrowth at the bone-metal interface was higher for total disc replacement relative to the cementless total joint components due to the sustained compression in the lumbar spine. For both critical-size and constrained defects there was largely no biomechanical testing reported (75% and 69%, respectively) (Figure 9).

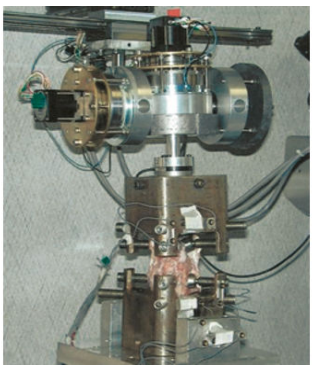


Figure 9: Stacked bar chart of bony defect type and biomechanical tests conducted for porous titanium implants.

Comparisons in Preclinical Studies

Comparison of methods and results across the surveyed studies is valuable in extracting trends in preclinical models and determining the efficacy of porous titanium scaffolds for promoting osseointegration. As discussed above, methods for such models widely vary, limiting the ability for direct comparisons in many cases. However, comparisons can be made for studies which report quantitative design parameters and results, enabling deduction of insightful information about the performance of porous titanium in varied preclinical models.

While the definition of the geometry and topology of the porous implants varies amongst studies, many report the overall porosity of the implant, as well as the average pore size. Similarly, while various biomechanical test methods were described, a comparable metric of osseointegration is the shear strength of the bone-implant interface. Given that shear strength is a property of the interface failure force normalized by the area, this allows comparison of different implant types, and across models that may vary in defect site, or animal size. Shear strength can also be reported for varying destructive biomechanical tests (i.e., torsion, tension, pull-out, push-out), although direct comparison between shear modes must be considered carefully.

For studies which reported a shear strength resulting from a destructive biomechanical test, no strong relationship between porosity and interfacial strength between bone and implant was observed (Figure 10A).

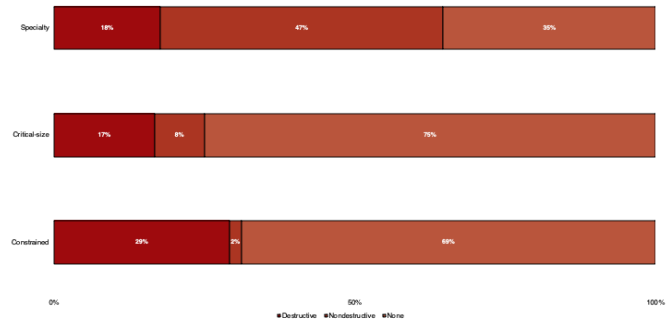


Figure 10 A: Scatterplot of shear stress and porosity of porous titanium implants with both surface and volumetric porosity that underwent destructive biomechanical testing. PCD: Porous coating delamination. FE: Fibrotic tissue encapsulation.

Similarly, there was also no strong correlation between pore size and shear strength when considering all models (Figure 10B).

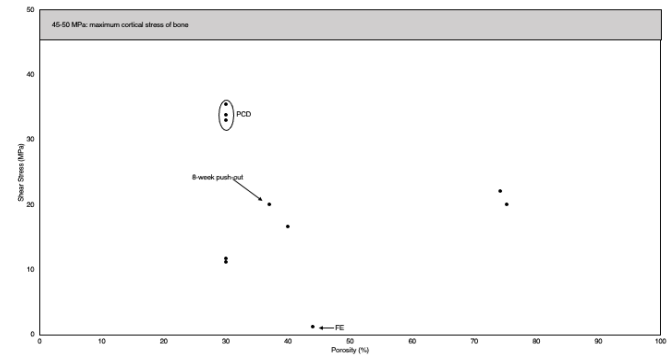


Figure 10 B: Scatterplot of shear stress and pore size of porous titanium implants both surface and volumetric porosity that underwent destructive biomechanical testing.

In at least one study, reports of fibrous encapsulation (FE in Figure 10A, Figure 10B) significantly reduced shear strength at bone-implant interface. Conversely, three data points with shear stresses greater than 30 MPa for their relatively low porosity (30%) were representative of a failure in the implant, where the authors discussed observation of porous coating delamination (PCD in Figure 10A) between the plasma-sprayed hydroxyapatite or anodic spark deposition coating and the core of the implant. Some reported values from ex vivo biomechanical tests neared the shear stress of human cortical bone (40 - 50 MPa), which represents an upper bound of the interfacial bone-implant shear stress .

It was found that for a shear strength had a positive relationship with endpoint length in all studies with two or more biomechanical endpoints (Figure 10D). Li et al., for example, demonstrated that the BIC, BV, and shear strength of both SLM and sintered Ti6Al4V scaffolds in a rabbit model continually increased between the 2, 6, and 12 weeks post-implantation. Similarly, Vasconcellos et al. showed an increase of 40% in the shear strength from 4 to 8 weeks for porous implants assessed by pushout. Reported shear stresses ranged from less than 10 MPa up to 35 MPa, while the bone ingrowth evaluated from ex vivo imaging varied between 42% up to 70.3% (Figure 10D).

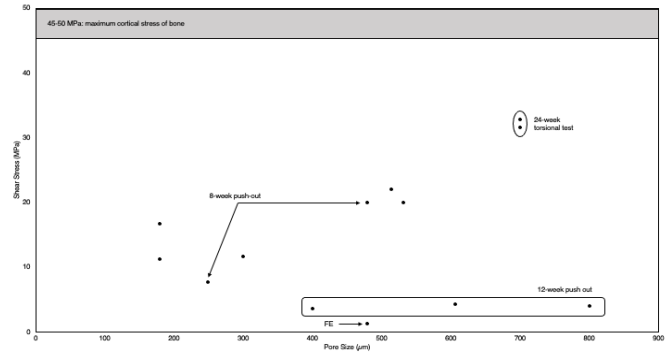


Figure 10 D: Scatterplot of biomechanical testing from studies with multiple endpoints.

As shown in Figure 10C, similar shear stresses are observed across a wide range of reported bone ingrowth. While it would be expected to see an increasing shear stress with increasing bone ingrowth, there was no strong positive correlation. This may be attributed to the various methods used to assess bone ingrowth and shear strength, leading to difficulty in such overarching comparisons (Figure 10C).

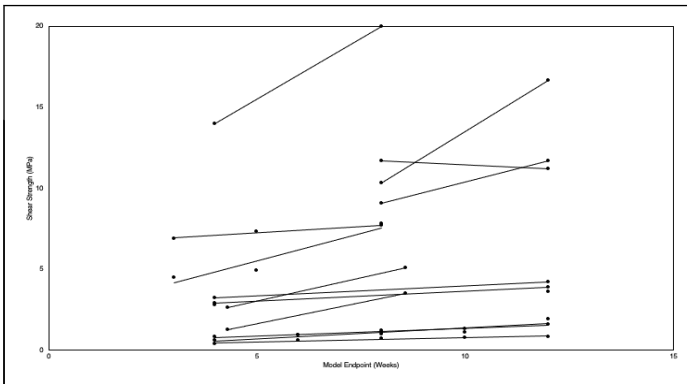


Figure 10 C: Scatterplot of shear stress and bone ingrowth of porous titanium implants with both surface and volumetric porosity that underwent destructive biomechanical testing.

Given the small percentage of studies with histomorphometry (23%) and biomechanical testing (34%), an increase in quantitative evaluations would improve the ability to compare between groups within a study, as well as to other preclinical studies in similar models.

For instance, van der Stok et al. examined the bone regeneration, bone quality, and mechanical strength of porous SLM Ti6Al4V femur implant in critical-sized bone defects in rats by using an intact control for biomechanical torsion test, thus being able to compare between groups within the study, and found that the combination of porosity and fibrin gels loaded with BMP-2 improved the bone regeneration performance in load-bearing critical-size defects. Moreover, Walsh et al. evaluated the performance of porous SLM Ti6Al4V implants in a cortical and cancellous sheep model via comparison of biomechanical testing, histology, histomorphometry, and micro-computed tomography results of an interbody fusion model using the same implants, and determined that anatomical site, surgical preparation, biomechanical loading, and graft material does indeed substantially influence in vivo response.

Clinical Translation

While the use of animal models is extremely valuable for translation of clinical use, the associated studies were infrequently reported together. Out of the 181 papers surveyed, only two directly mentioned the clinical use of titanium implants in conjunction with the preclinical research. Warnke et al., studied the implantation of a human-sized titanium mandible in the latissimus dorsi muscles of miniature pigs to later validate the mandible replacement in an adult human. Pobloth et al. examined the critical-size bone defect regeneration of a Ti-mesh scaffold in a sheep model for subsequent application in two human patients. Although limited, these results highlight the translational potential for such models to improve clinical care.

Challenges and Opportunities

The use of porous titanium for reconstruction of bone defects is not novel, but there is still great opportunity for use of preclinical animal models to assess the relative efficacy of porous scaffolds for bone repair. AM has revolutionized the capabilities for fabrication of complex porous scaffolds for use in orthopedics, and questions around scaffold architecture and its influence on osseointegration are unanswered.

Gaps in current understanding could be addressed by wider adoption of standardized methods for evaluation bone ingrowth in these models. While direct comparison between preclinical animal studies is often obscured by differences in animals, surgical technique, and other factors, standardization of methods where possible would improve the overall understanding of the field. Further, the ability to more directly compare previous studies may result in a more informed study approach in the future, or the ability to reduce the number of animal studies needed. Current techniques for evaluation of bone ingrowth via in vivo or ex vivo computed tomography vary greatly, including a maximum voxel size, treatment of metal artifact, and quantitative evaluation of BIC or neo-BV. While some standard techniques do exist, their reference was limited in the studies surveyed. Additionally, use of biomechanical testing was low, and standardized techniques were lacking. Design of preclinical studies with biomechanical tests normalized to intact controls could greatly improve the ability to compare interstudy results in the future.

A final major challenge to be addressed in coming years is the relative throughput of preclinical models for assessment of performance. While this has been true in the past, it is exacerbated by the vast topological design space unlocked by advanced computational aided design tools, and the relatively fast speed of AM to produce such implants. Additionally, as the materials science and AM community continue to develop new alloys and printed structures, the resulting innovative implants will need to be tested in preclinical models. One such example is the development of titanium niobium (Ti-Nb) alloys which were developed to have a lower modulus than traditional titanium alloy but remains to be proven in vivo. Weng et al. tested Ti-25Nb alloys prepared via powder metallurgy with various porosities as femoral stem prostheses in a rabbit model and determined that the titanium alloy implants had proficient biocompatibility and biological fixation between the bone and the implant One potential solution is the development of robust computational models to accelerate the generation of preclinical data. While in depth discussion of such computational models is outside the scope of this review, there exists great opportunity in the development of computational models for bone ingrowth which can be used for screening of novel implants.

Conclusion

Although bone has a natural ability to spontaneously regenerate itself, the repair of bone defects, especially critical-size defects, still proves to be clinically challenging. Therefore, surgical reconstruction using metallic implants is often necessary to repair these defects. Accordingly, preclinical models are imperative to answer questions around implant design and manufacturing prior to being translated to the clinic. From the survey, preclinical studies focused on evaluation of porous titanium implants for bone growth has increased in recent years. Moreover, trends in research show that previous technologies for manufacturing porous titanium implants have been increasingly superseded with AM technologies over the past decade. This trend in the preclinical research literature is in alignment with an increased adoption of this technology in the orthopedic medical device industry.

The variety in preclinical research of porous titanium implants speaks to the breadth of technologies to be evaluated. In addition to investigation of the effects of porosity, pore size, and other design-related factors, researchers have also investigated the effect of the surface on osseointegration. The evaluation of coatings, such as hydroxyapatite on titanium substrates, has been the most widely investigated surface modulation in bone defect models. Promising results have been shown in some preclinical studies of porous scaffolds composited with synthetic grafts to impart additional functionality to the treatment construct. Models themselves vary in type and timepoint, as well as the bone defect site and type. Evaluation methods in the surveyed models were widely varied. In general, imaging and histology were used for assessment. Biomechanical testing and histomorphometry were less widely used, despite the increased quantitative nature of such evaluations. Given the many experimental design parameters researchers in the field may investigate, future work should focus on development of standardized ex vivo evaluation methods, such as biomechanical testing using intact controls for normalization, which would increase the ability to compare between research studies. Overall, the ability to further improve clinical outcomes through the use of preclinical bone defect models is high and will continue to be important in translational research.

Acknowledgements

All authors have read the journal's authorship statement and policy on disclosure of potential conflicts of interest. CNK and KG have

stock ownership in restor3d, but did not receive funding for this study from restor3d.

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