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MIMETIC METAL®
IN-VITRO AND IN-VIVO RESEARCH SUMMARY

In-vitro and in-vivo results of two studies evaluating patented CoreLink Mimetic Metal titanium 3D printed technology

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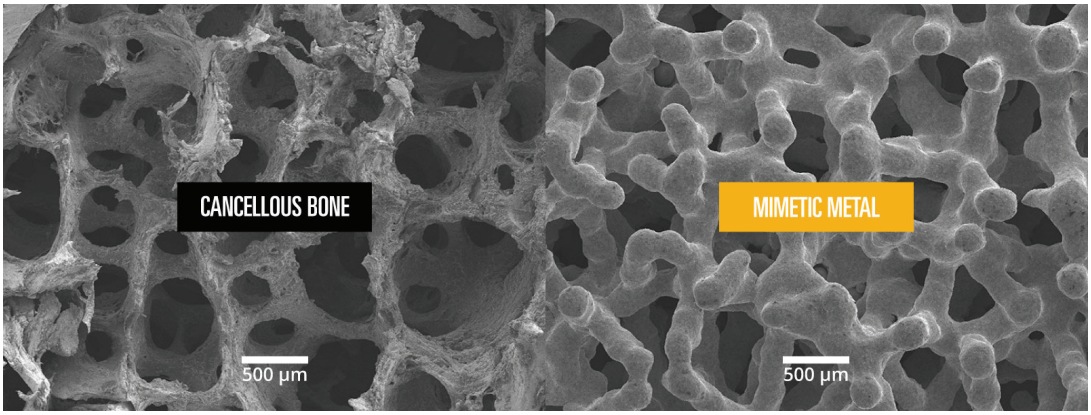
INTRODUCTION

Numerous types of materials (metals, polymers, titanium coated polymers, ceramics, etc.) have been used in designing interbody devices for clinical use[1]. Many of these materials are known to have inherent clinical limitations. Polyetheretherketone (PEEK) is a common interbody material known best for its radiographic imaging and elastic modulus properties, however it is bioinert, hydrophobic, and does not allow for bony ingrowth[2,3]. Due to these limitations, manufacturers have tried to enhance PEEK by adding bioactive hydroxyapatite (HA) within the material or titanium coatings to improve bony apposition. The major downfalls of HA-PEEK is that bony in-growth cannot occur and it only allows for improved bony attachment compared to regular PEEK, however fibrous tissue formation may still occur[4]. Additionally, solid, machined titanium is also frequently used in interbody cage implant design. Machined titanium is osteoconductive, though many surgeons prefer not to use it because it is radiopaque and can cause imaging scatter and artifact[5]. Machined titanium also is non-porous, similar to PEEK, so it does not allow for bony in-growth and is inherently an extremely stiff material which may cause subsidence of the implant[6, 7]. The application of 3D printing technology to spinal implant devices has made improvements to possible implant offerings. In general, porous 3D printed titanium has many advantages in comparison to other materials used for interbody devices.

CoreLink developed Mimetic Metal, a 3D printed porous interbody technology. Unlike other commonly used implants, Mimetic Metal implants are designed to generate osseointegration by replicating cortical and cancellous geometry features within the implant. **The technology is intended to address the clinical limitations of other interbody materials while enhancing the osteoconductive potential of the device, making bony in-growth and on-growth possible.**



OBJECTIVE

This technical report summarizes the in-vitro and in-vivo results of two separate studies evaluating patented CoreLink Mimetic Metal technology.

The in-vitro study evaluated osteoblast activity on Mimetic Metal porous titanium material compared to other commonly used interbody device materials[8].

The in-vivo study evaluated the osseointegration with Mimetic Metal as compared to machined and roughened titanium in an ovine model at 4 and 12 weeks[9]. The study evaluated bony in-growth and on-growth and quantified new bone formation over time in cortical and cancellous sites.

IN-VITRO OSTEOBLAST EXPERIMENTS

OVERVIEW

The following in-vitro cellular experiments evaluated various commonly used spinal implant materials and their abilities to elicit an osteoblast response.

MATERIALS

The following material test groups were evaluated:

Group A: CoreLink Mimetic Metal
Group B: Machined and roughened titanium alloy
Group C: PEEK
Group D: HA-PEEK
Group E: Tissue culture plastic (control)

METHODS AND RESULTS

Groups A – E were characterized for roughness (using Atomic Force Microscopy) and chemistry (using Energy Dispersive Spectrometry). Residuals were measured with Fourier Transform Infrared Spectroscopy and Inductively Coupled Plasma Mass Spectrometry. Surface energy was determined by standard sessile contact angles using three different liquids and following the modified Owens-Wendt surface energy calculation. These values were compared to the energy of critical proteins contained in serum that promotes osteoblast and decreases bacteria function. **Key proteins, such as fibronectin and vitronectin, promote osteoblast functions and have a surface energy close to 42.5 mN/m. The results of this comparison showed that Mimetic Metal has a surface energy closer to key endogenous proteins as compared to the other groups.** (Figure 1)

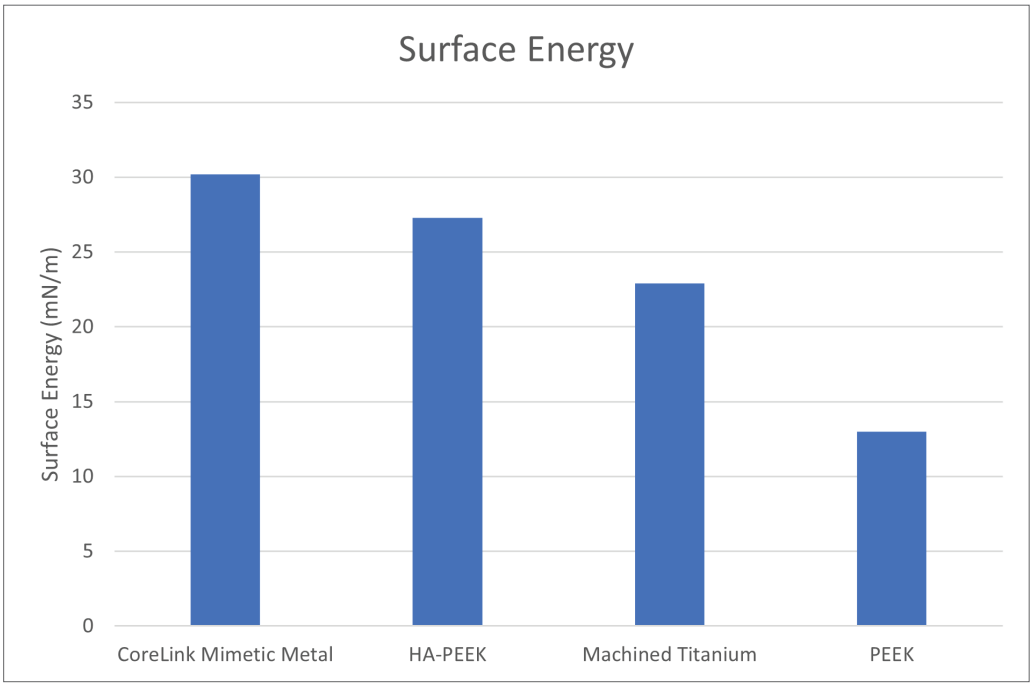


Figure 1: Increase in Surface Energy for the Mimetic Metal Group

CELL PREPARATION

To determine osteoblast activity on the proposed materials, human osteoblasts (ATCC 11372) were extracted from bone through collagenase digestions according to standard well-established procedures. These cells were cultured under sterile cell culture conditions (that is, a humidified, 37°C, 5%CO2/95% air environment) in DMEM supplemented with 10% FBS and 1% P/S and used at population numbers below 3.

All experiments were completed in triplicate and repeated at least three different times with differences between means assessed using ANOVA followed by Student's t-tests.

A. Osteoblast Adhesion Research Methods and Results

When confluent on the cell culture dishes, osteoblasts were suspended using trypsin in recommended media, separately seeded (at 3500 cells/mm^2) onto the various test group substrates, and allowed to adhere for 4 hours. After the prescribed time, non-adherent cells were removed by rinsing with phosphate-buffered saline. Adherent cells were fixed, fluorescently stained with rhodaminephalloidin (Sigma), and visualized in-situ using low and high magnification fluorescence microscopy. Cell density (cells per area) was determined by averaging the number of cells in ten random fields per substrate. **Results of this study show correlation between the increased surface roughness, the surface energy, and increased osteoblastic adhesion.** (Figure 2)

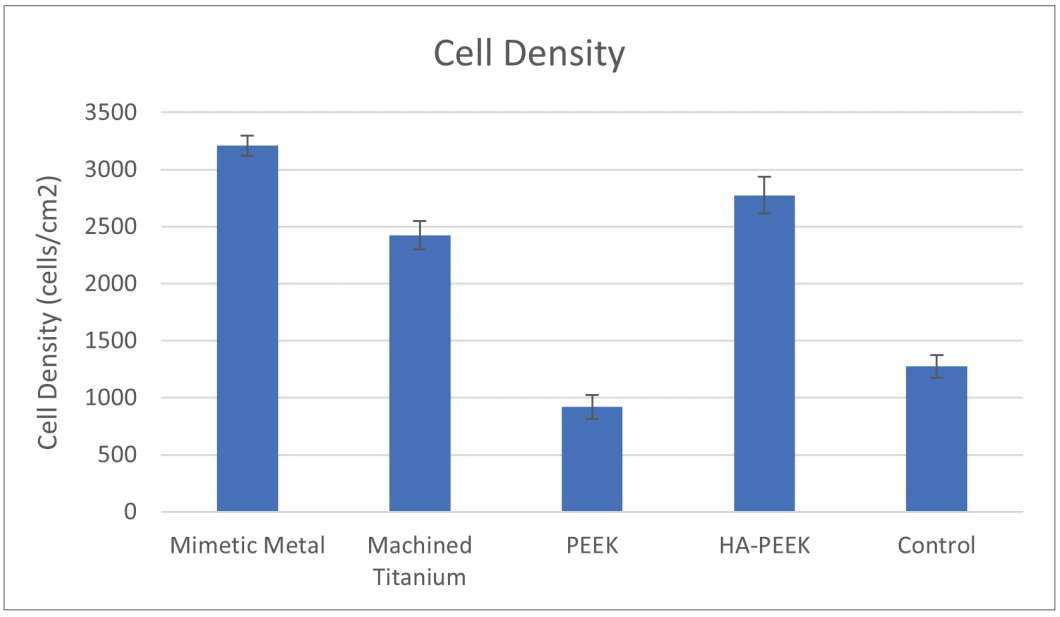


Figure 2: Greater Osteoblast Adhesion on the Mimetic Metal Group. All values were statistically significant (p<.1) from each other. Standard Error displayed. The sample size for each is 3.

B. Osteoblast Proliferation Research and Results

Osteoblasts were seeded (100,000 cell/cm^2) onto the various test group substrates and were cultured in complete DMEM (DMEM supplemented with 10% FBS, 1% P/S, 50 µg/ml ascorbate (Sigma), and 10 mM β-glycerophosphate (Sigma)) under standard cell culture conditions for 7, 14, and 21 days. The media was replaced every other day. Osteoblast proliferation was assessed by measuring the amount of DNA in papin-digests using Hoeschst 33258 dye (Sigma) and a fluorospectrophotometer (Milton Roy Company, Fluorospectronic) following methods reported in the literature. The number of cells in the experimental samples was determined from a standard curve correlating the amount of DNA per known number of cells (assay sensitive to approximately 1,000). Adhesion at these long-time periods was reported as cell density (cells per unit surface area). **Results of this study showed an increase in osteoblast proliferation in all groups especially the Mimetic Metal group.** (Figure 3)

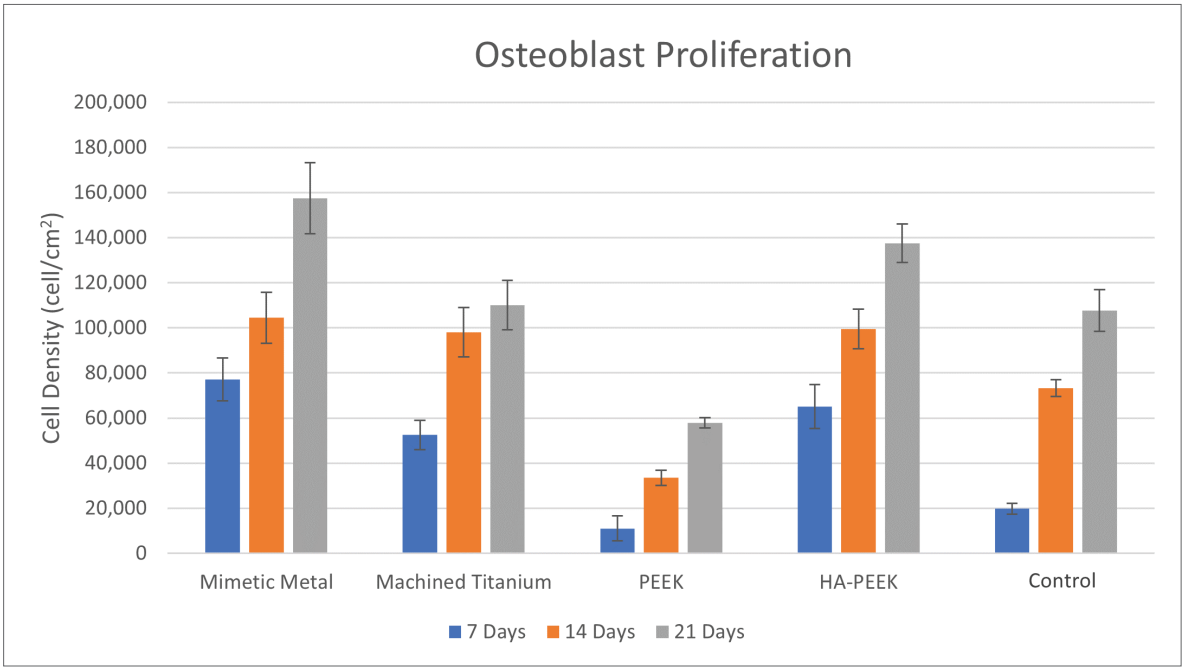


Figure 3: Osteoblast proliferation for each material group over 21 days. Greater osteoblast proliferation was observed on Mimetic Metal compared to all other groups at each respective time point. Osteoblast seeding density =100,000 cells/cm^2. All values were statistically significant (p<.1) from each other. Standard Error displayed. The sample size for each is 3.

C. Osteoblast Differentiation Research and Results

Total Intracellular Collagen Content

Collagen is a well-known protein contained in the extracellular matrix of bone. To determine these amounts, cell lysates were prepared by exposing the cells to three freeze-thaw cycles. 50 µl of osteoblast lysates were added per well of a 96-well plate (Corning). The collagen was allowed to dry on the plate through incubation at 37° C for 16 hours and will then incubated at 37° C for 24 hours in the presence of a desiccant (W.A. Hamond Drierite Company LTD.). Thereafter, the 96-well plate was rinsed three times with distilled water (1 min per wash and 200 µl per well). 100 µl of a 0.1% Sirius Red stain (Sirius Red powder in picric acid; Sigma) was dispensed into each well and allowed to sit for one hour at room temperature. After that, each well was washed 5 times with 200 µl of 0.01 M HCl (Sigma) for 10 seconds per wash. 200 µl of 0.1 M NaOH (Sigma) was added into each well and allowed to sit for 5 min. Then the solution in each well was mixed, transferred to a second plate, and absorbance read at 540 nm in a spectrophotometer (SpectroMAX; Molecular Devices). The total intracellular collagen synthesized by osteoblasts cultured on the substrates was reported in terms of intensity. **Greater collagen synthesis was observed in 21 days with Mimetic Metal compared to machined titanium, PEEK, and HA-PEEK.** (Figure 5)

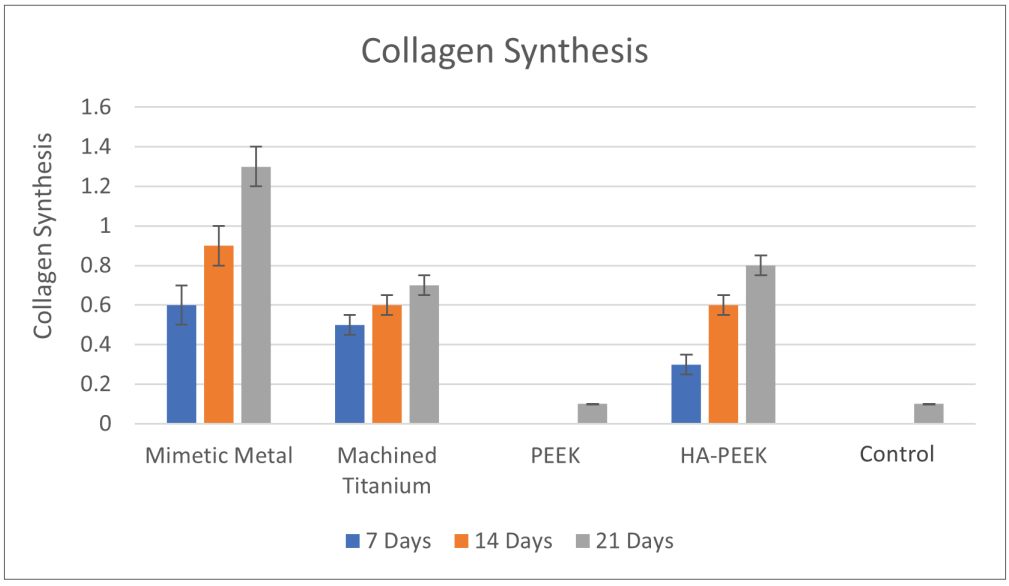


Figure 5: Greater Collagen Synthesis on the Mimetic Metal Group Over Time. (Arbitrary units) All values were statistically significant (p<.01) from each other. Standard Error displayed. The sample size for each is 3.

Alkaline Phosphatase Activity

Alkaline phosphatase is an enzyme whose synthesis indicates the differentiation of osteoblasts from non-calcium depositing to calcium depositing cells. To test this, cell lysates were prepared as previously described and a commercial Alkaline/Acid Phosphatase Assay Kit (Upstate) was used to determine the concentration of alkaline phosphatase in these cell lysates following manufacturer's instructions. Aliquots of the distilled water supernatants were first mixed and incubated with 40 mM NiCl2, 5 mg/ml BSA, 1 mM phosphopeptide solution, and Pnpp Ser/Thr Assay Buffer at 37° C for 10-15 min. Then, they were incubated with a Malachite Green solution for 15-20 min at room temperature. The optical absorbance values were measured by a spectrophotometer (SpectroMAX; Molecular Devices) at 650 nm. Alkaline phosphatase synthesized by osteoblasts cultured on the substrates were reported as intensity. **Greater AP synthesis was observed with 21 days on Mimetic Metal compared to other materials.** (Figure 6)

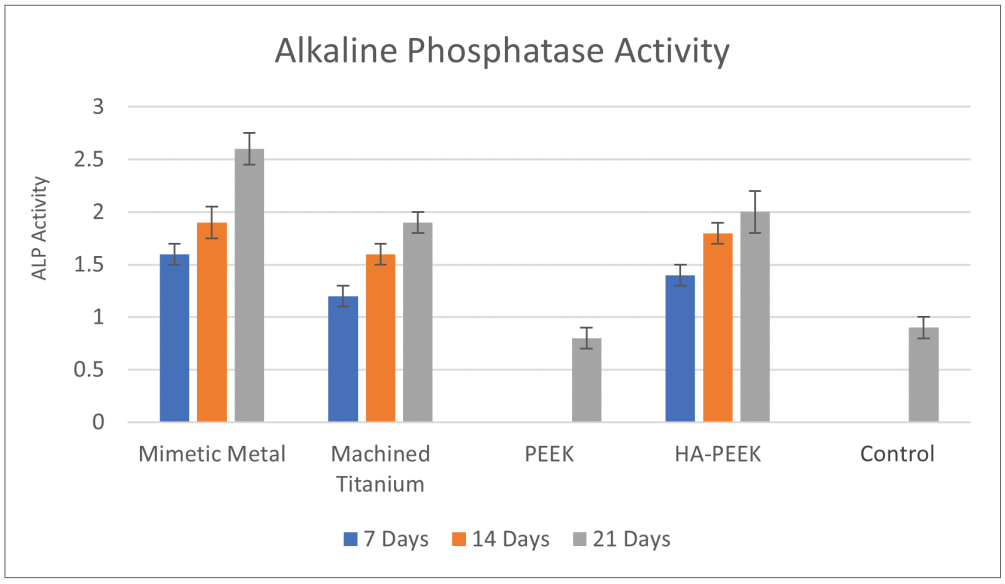


Figure 6: Greater Osteoblast Alkaline Phosphatase Synthesis on Mimetic Metal Group Over Time (Arbitrary units) All values were statistically significant (p<.01) from each other. Standard Error displayed. The sample size for each is 3.

D. Extracellular Calcium Quantification Research and Results

The ultimate indication of osteoblast differentiation is their ability to deposit calcium. To test this, after the cells were lysed as described above, the substrates (and remaining calcium deposits on them) were incubated with 0.6 N HCl (Sigma) at 37° C overnight. Samples were briefly sonicated prior to removing supernatant. The amount of calcium present in the acidic supernatant was quantified using a Calcium Quantification Kit (Sigma) following manufacturer's instructions; light absorbance of the samples will be measured using a spectrophotometer (SpectroMAX; Molecular Devices) at 575 nm. Total calcium will be reported as intensity. **Greater calcium deposition was observed within 21 days on Mimetic Metal compared to other material groups.** (Figure 7)

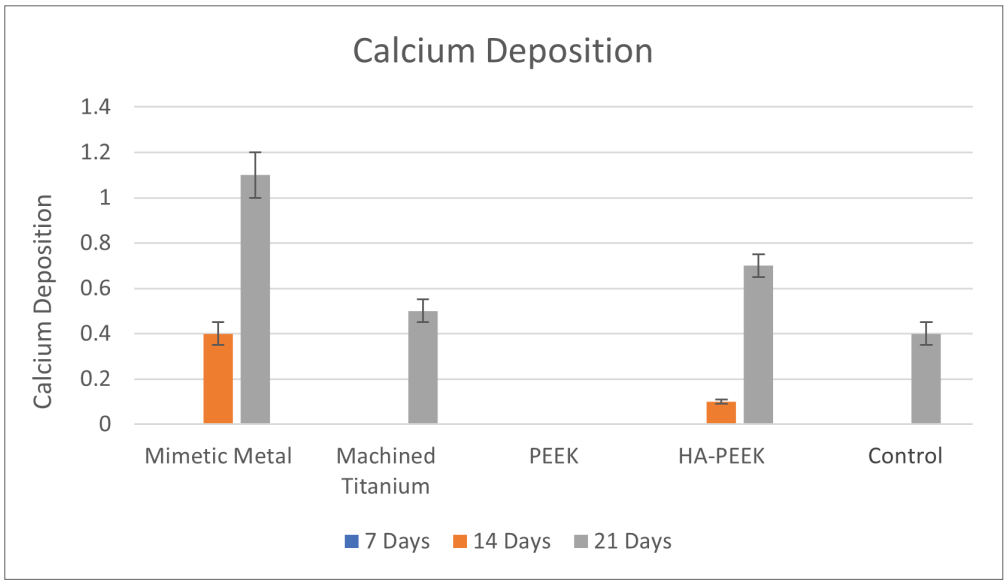


Figure 7: Greater Calcium Deposition on the Mimetic Metal Group Over Time. Intensity is an arbitrary unit. All values were statistically significant (p<.01) from each other. Standard Error displayed. The sample size for each is 3.

IN-VITRO STUDY CONCLUSION

In comparison to other commonly used interbody materials such as machined solid titanium, PEEK, and HA-PEEK, CoreLink Mimetic Metal consistently demonstrated increased osteoblast activity including adhesion, proliferation, and synthesis of calcified extracellular matrix as indicated by collagen, alkaline phosphatase, and calcium deposition in cell culture.

Although more tests are required to detail the mechanism of action, it is most likely due to a surface energy that is closer to optimal protein absorption that promoted osteoblast functions (42.5mN/M).

IN-VIVO OVINE SHEEP STUDY

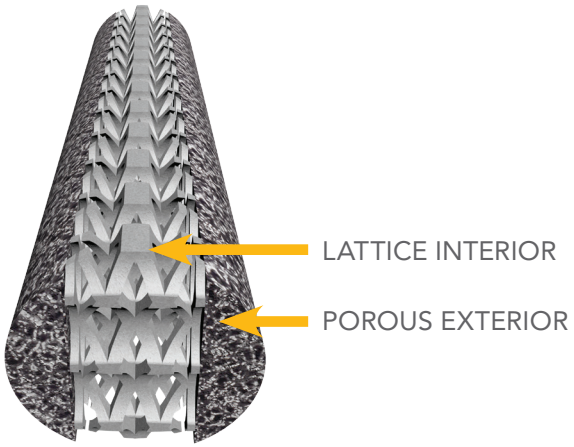
OVERVIEW

This in-vivo ovine sheep study aimed to compare Mimetic Metal to solid titanium implants regarding bone on-growth and in-growth at the bone-to-implant interface at 4 and 12 weeks post surgery.

MATERIALS

The following test groups were manufactured to be 25mm x 6mm implant dowels:

Group A: CoreLink Mimetic Metal Dowel



Group B: Solid titanium dowel

METHODS

The study followed the same protocol in previously published models[10-17]. Implants were implanted in the cancellous bone of the distal femur and proximal tibia in a press fit manner. Implants were placed in the cortical bone of the tibia in a line to line manner. The implant orientation was positioned so that the internal lattice framework was parallel to the length of the tibia and femur. This position ensures the implant's lattice is under an axial physiologic load to stimulate the adaptive response of bone (Wolff's Law).

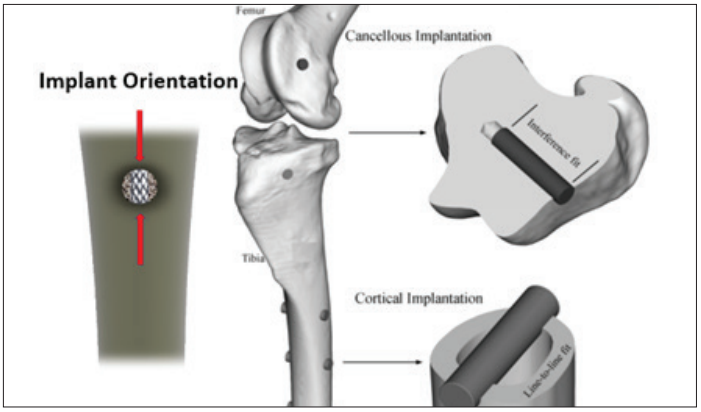


Figure 8-1: Implant Placing and Positioning

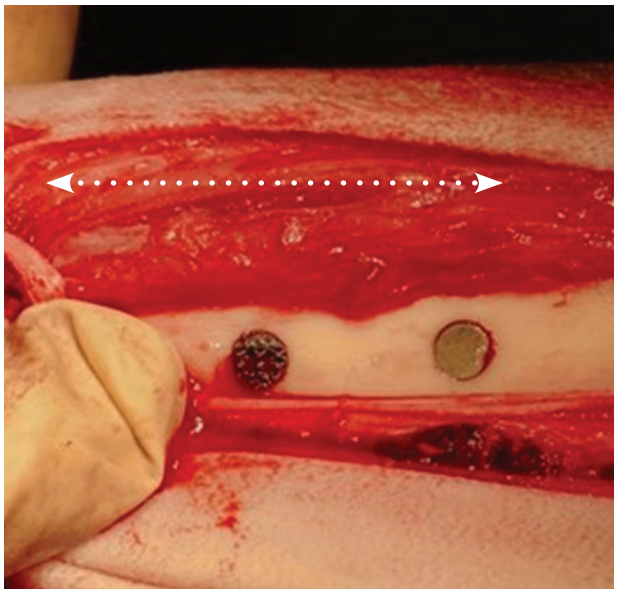


Figure 8-2: Implants in bi-cortical implantation of samples in the tibia demonstrating the alignment of the implant along the long axis of the bone. Long axis denoted by dotted arrow.

This osseointegration model incorporated six fully mature male sheep (of at least 18 months old). Randomized implantation was performed in the diaphysis of the tibia as cortical sites including n=4 bicortical implants (8 unicortical) per group for histology and histomorphometry.

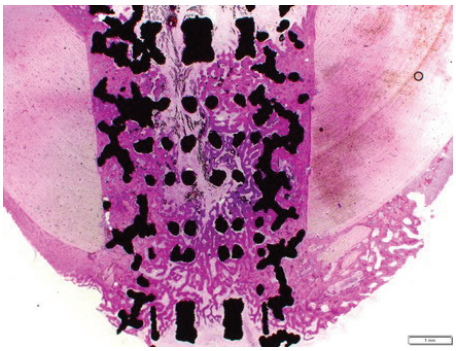
Implantation of each group was also performed in cancellous sites of the distal femur and proximal tibia with samples of n=4 for histology and histomorphology.

PMMA histology were completed at 4 weeks for the first three animals and then again at 12 weeks for the second group of three animals. Cortical and cancellous specimens were embedded, sectioned, and stained for PMMA histology. The staining results in bone staining pink and fibrous tissue blue/purple. PMMA images were taken at the bone implant interfaces for determination of bone in-growth and on-growth using MATLAB to determine the amount of substrate and new bone with histomorphology.

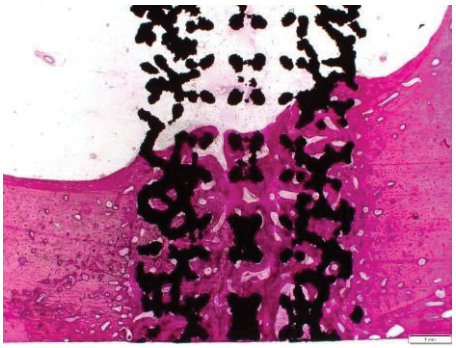
MECHANICAL TESTING:

Prior to histology and histomorphometry testing, implants were tested for implant-bone interface strength using a standard push-out test at 4 and 12 weeks. **Results showed that Mimetic Metal performed higher in Max Force, Shear Stress, and Stiffness in comparison to solid Titanium at both time periods. Mechanical testing results demonstrate that the Mimetic Metal lattice improves implant fixation strength.**

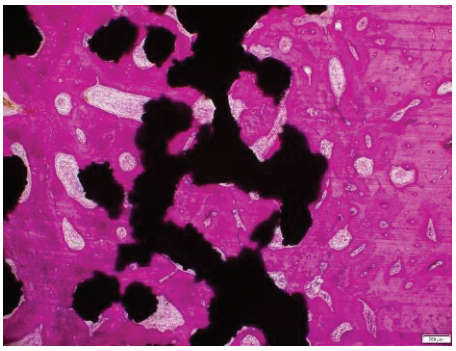
REPRESENTATIVE HISTOLOGY IN CORTICAL SITE:



Mimetic Metal at 4 weeks

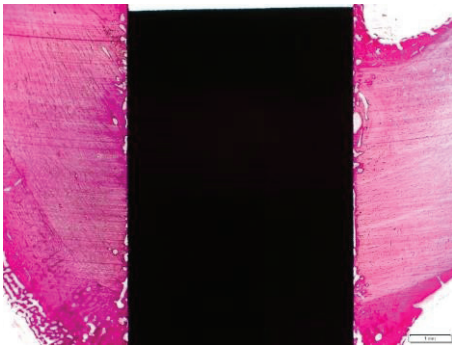


Mimetic Metal at 12 weeks

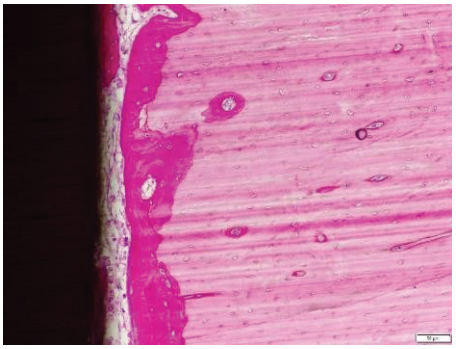


Mimetic Metal at 12 weeks (Close Up)

Solid Ti at 4 weeks

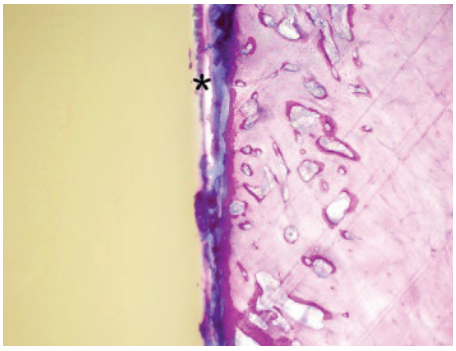


Solid Ti at 12 weeks

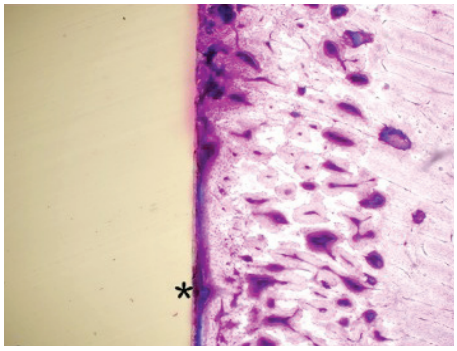


Solid Ti at 12 weeks (Close Up)

Cortical sites with Mimetic Metal demonstrated progressive bone in-growth with later timepoints demonstrating considerable amounts of bone. Solid Titanium demonstrated a mix of non-reactive fibrous tissue and bone at the host/implant interface.



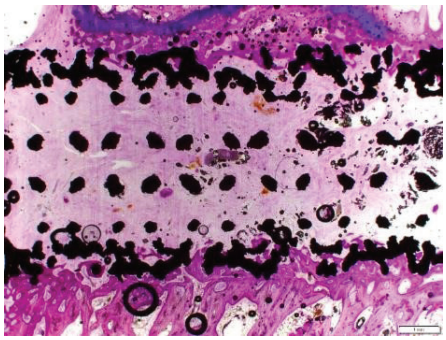
PEEK at 4 weeks[18]
**Example of fibrous tissue*



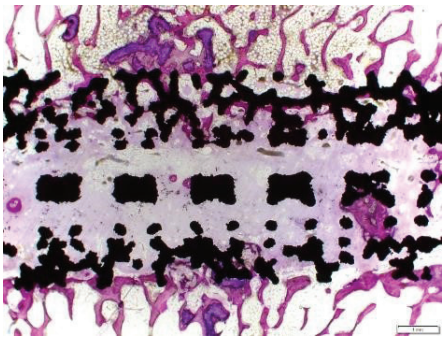
PEEK at 12 weeks[18]
**Example of fibrous tissue*

Walsh et. al. conducted a comparable animal model using PEEK, another commonly used spinal implant material. At 4 and 12 week time periods, the cortical sites demonstrated the presence of fibrous tissue.

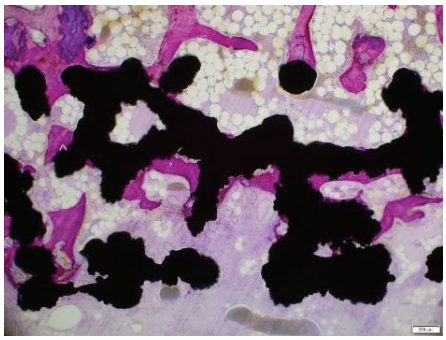
REPRESENTATIVE HISTOLOGY IN CANCELLOUS SITE:



Mimetic Metal at 4 weeks



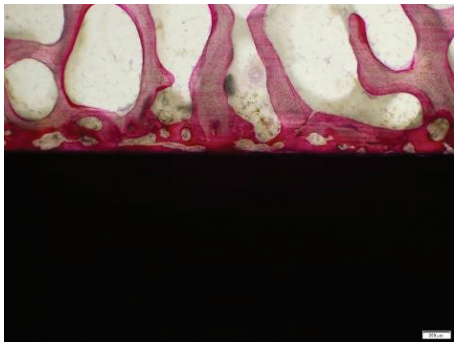
Mimetic Metal at 12 weeks



Mimetic Metal at 12 weeks (Close Up)

Solid Ti at 4 weeks

Solid Ti at 12 weeks

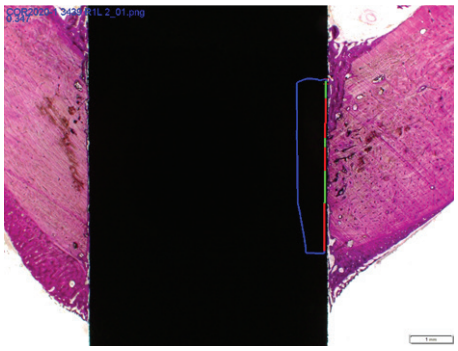


Solid Ti at 12 weeks (Close Up)

Mimetic Metal's performance in the cancellous model demonstrated bone on-growth into the outer regions of the porous material with some in-growth of bone in the interior of the implant. Typically, progressive bone on-growth is observed in the cancellous model due to the non-loading bearing cancellous environment. **Uniquely, Mimetic Metal demonstrated on-growth as well as some in-growth in the cancellous model. This observation could be because of Mimetic Metal's porous lattice features which allow area for in-growth as well as its inherent load sharing capabilities.** The solid, non-porous titanium group demonstrated only on-growth within the cancellous sites which in turn may reduce the load sharing ability of the implant.

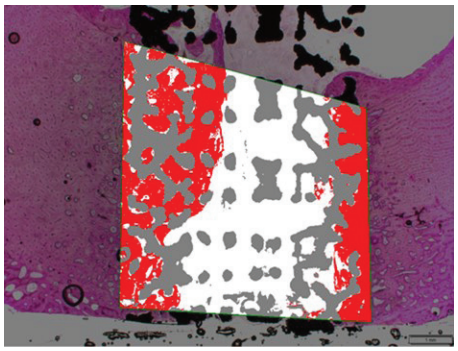
HISTOMORPHOMETRY

Histomorphometric analysis was performed using images from the processed PMMA histology. Samples were taken at the bone implant interfaces for determination of bone on-growth and in-growth. Solid Titanium has no potential for in-growth, therefore only on-growth was evaluated as contact ratio of available implant surface to have direct bony contact. The contact ratio of the Solid Titanium group in the cancellous sites was 33% and 42% at 4 weeks and 12 weeks, respectively. The contact ratio in the cortical sites was 42% and 43% in the cortical sites at 4 weeks and 12 weeks, respectively. This demonstrates that no further progression of bony attachment occurred in the Solid Titanium group from 4 to 12 weeks in this study.

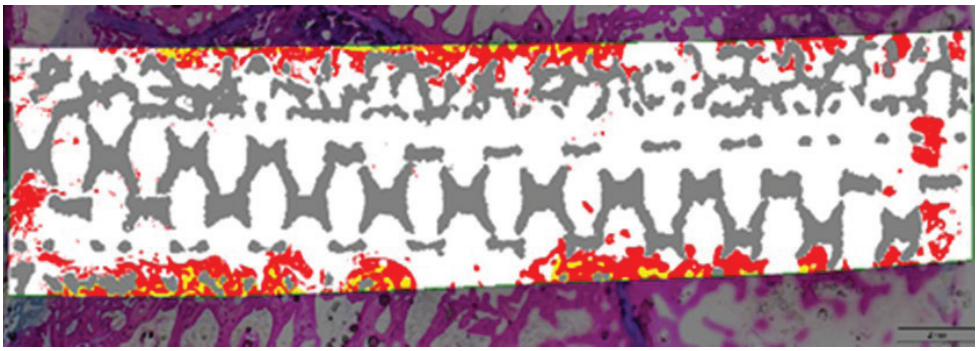


Solid titanium in cortical bone. Green areas show bone contact.

Bony in-growth in the Mimetic Metal group was calculated as the ratio of bone directly in contact with implants and in the available void spaces within the implant structure relative to the entire implant void created (BIAV). The progression of healing in the cortical sites demonstrated an increase from 30% to 80% at 4 and 12 weeks, respectively. In the cancellous sites, bone in the available void space increased from 12% to 16%.



Cortical implant



Cancellous implant

Red demonstrates area of bone, gray demonstrates implant, and yellow is marrow.

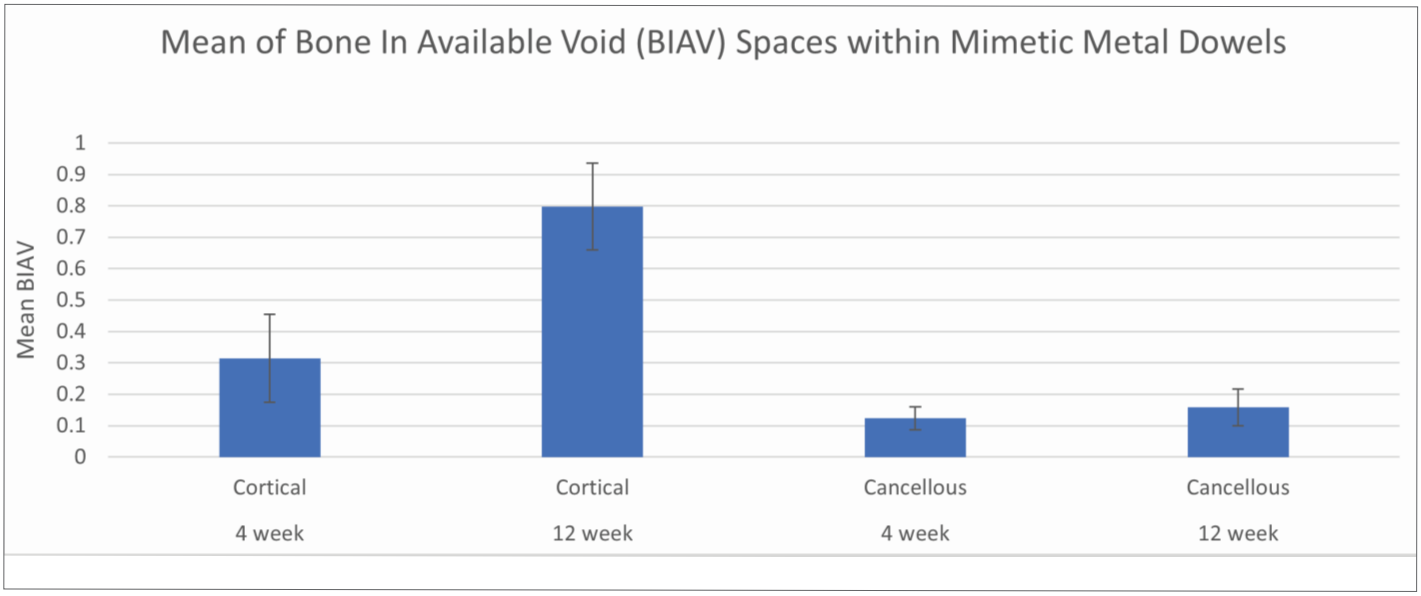


Figure 9: Histomorphometry demonstrated a trend of increased bone in-growth and on-growth with Mimetic Metal with time

IN-VIVO STUDY CONCLUSION

Histologic and mechanical testing results have shown promising results with greater bony on-growth and in-growth with the Mimetic Metal material compared to machined and roughened titanium shown in a sheep model at 4 and 12 weeks.

The sheep model also demonstrated that Mimetic Metal allows for direct bony attachment without formation of fibrotic tissue. In summary, this study demonstrated that Mimetic Metal's 3D structure supports bone in-growth and improves implant fixation during the bone healing process.

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For product information, including indications for use, contraindications, warnings, precautions, potential adverse effects and patient counseling information, see the product Instructions For Use (IFU) and information on the CoreLink website. To obtain a copy of the current Instructions for Use (IFU) for full prescribing and risk information, please call CoreLink Customer Service at (888) 349-7808 or visit corelinksurgical.com/ifu. CoreLink is part of CoreLink Holdings, LLC. U.S. Patent No. 10,512,545. Further data is held on file at CoreLink. The in-vitro test results may not be indicative of clinical performance.