Calcium phosphate coated 3D printed porous titanium with nanoscale surface modification for orthopedic and dental applications

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HIGHLIGHTS

• Porous Ti implants were processed via LENS™, a commercial 3D printing technique with stiffness, similar to bone.
• A combination of nanoscale surface modification with TiO2 nanotubes and Sr-Si doped calcium phosphate coating was studied.
• Porous implants showed better bone tissue integration in vivo than the dense ones in a rat distal femur model.
• Calcium phosphate played a significant role to enhance early-stage bone tissue integration.

GRAPHICAL ABSTRACT

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ABSTRACT

This study aims to improve the interfacial bonding between the osseous host tissue and the implant surface through the application of doped calcium phosphate (CaP) coating on 3D printed porous titanium. Porous titanium (Ti) cylinders with 25% volume porosity were fabricated using Laser Engineered Net Shaping (LENS™), a commercial 3D printing technique. The surface of these 3D printed cylinders was modified by growing TiO2 nanotubes first, followed by a coating with Sr2+ and Si4+ doped bioactive CaP ceramic in simulated body fluid (SBF). Doped CaP coated implants were hypothesized to show enhanced early stage bone tissue integration. Biological properties of these implants were investigated in vivo using a rat distal femur model after 4 and 10 weeks. CaP coated porous Ti implants have enhanced tissue ingrowth as was evident from the CT scan analysis, push out test results, and the histological analysis compared to porous implants with or without surface modification via titania nanotubes. Increased osteoid-like new bone formation and accelerated mineralization were revealed inside the CaP coated porous implants. It is envisioned that such an approach of adding a bioactive doped CaP layer on porous Ti surface can reduce healing time by enhancing early stage osseointegration in vivo.

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1. Introduction

Titanium (Ti) is a widely used metallic biomaterial that is bio-inert [1]. Excellent fatigue and corrosion resistance [2] along with high strength-to-weight ratio make Ti an ideal metal for load-bearing implants [1]. In spite of commendable progress in the field of metallic orthopedic biomaterials, host bone and implant integration still needs improvement to minimize healing time and increase implant lifetime in vivo. Among others, mismatch of Young’s moduli, also known as stress shielding, between the bulk metallic materials like Ti (110 GPa), Co—Cr alloys (230 GPa) and bone (10–30 GPa) has been recognized as one of the reasons for implant loosening [3]. To enhance in vivo lifetime of implants, it is envisioned that modifying surface properties can...
improve tissue materials interactions and subsequent healing. Weber et al. reported usage of porous materials to enhance osseointegration in 1972 [4] and henceforth substantial research has been done in the development of porous materials [3,5–7] for biomedical usages.

To augment further cell adhesion and tissue ingrowth, research has also been devoted to nanoscale surface modification of the implants [8,9]. One of the common methods to perform nanoscale surface modification is by the growth of nanotubes on the surface of the metallic implants because of simplicity and flexibility of this process in terms of controlling the parameters [10–12]. Previous works have shown that excellent biocompatibility with higher surface to volume ratio and surface chemistry of the nanotubes has enhanced cell growth and proliferation due to osteoclast activity and intensifies the bone regeneration by augmenting osteoblast activity eventually leading to bone formation [30,31]. Strontium ion has also been used as an osteoporotic drug due to its stimulatory effect on bone remodeling [32]. Strontium induces osteogenesis by increased formation of ß-catenin [26]. Silicon ion, found frequently in connective tissue and bone of the body, structurally stabilizes collagen and influences early stage bone formation [27,33]. Reports have described the influence of silicon on bone formation by the dependence of bone mineral density on intake of silicon [34]. However, a knowledge gap still exists for combined effects of TiO2 with CaP, with or without dopants on early stage osteogenesis.

Additive manufacturing or 3D printing (3DP) comes with a variety of solutions to manufacturing limitations of conventional techniques. 3DP can be on-demand, site specific and allows better porosity control [35–37]. Laser based solid freeform technique, Laser Engineered Net Shaping (LENS™), has been utilized to develop the porous Ti cylinders used in this study. Since LENS™ is not a powder bed process, and the powder delivery happens through forced Ar gas, only metallurgically bonded particles bond together and loose powders are blown away. Nanotube films were grown by electrochemical anodization and metallic ion doped calcium coating was done by biomimetic coating technique. Nanoscale modification of the Ti implants does not interact with the physiological system, however, the mismatch of mechanical properties between the ceramic based coating and the metallic implant is minimized [38]. This plays an important role in improving the adhesion of the calcium phosphate coating on the metallic implants and prevents delamination of the coating in vivo. The objective of this study was to understand the influence of doped CaP coating on 3DP porous Ti implants with or without TiO2 layer towards the early stage osteogenesis. Addition of TNT on LENS™ processed porous implants with doped CaP is novel and the doped CaP coating on surface modified porous Ti implants

Fig. 1. Demonstrates the effects of combination of nanoscale surface modification with doped CaP coating on porous CpTi on in vivo osseointegration in a rat distal femur model.
was hypothesized to enhance osseous tissue ingrowth at the interface of
the implants in vivo. Male Sprague-Dawley rats were implanted for 4
and 10 weeks. Samples were characterized via mechanical push out
tests, histological imaging, histomorphometric analysis, computed to-
ography, and scanning electron microscope imaging to measure the
influence of surface modification on early stage osseointegration.

2. Materials and methods

2.1. Fabrication of porous samples using LENS™

Porous Ti rods of 3.00 mm average diameter were fabricated for
in vivo analysis using CAD software, referred as LENS porous Ti hence-
forth. The software was utilized to design cylinders of 75 mm in length
and average diameter of 2.3 mm. The diameter of the cylinders in the
developed CAD design was too small to incorporate any kind of de-
signated porosity, therefore, the powder was partially melted using low
power of the laser to create the porosity in the cylinders. The developed
CAD design was uploaded to the LENS™ motion control software and
further translated into tool path files for processing. Fig. 2 shows a sche-
matic design of the LENS™ [10]. Commercially pure titanium powder
(ATI Powder Metals, Pittsburg, PA, USA), 99.99% pure, having spherical
particles of average diameter of 44 μm to 149 μm was used for the fab-
rication of the cylinders. Similar 99.99% commercially pure 3 mm thick
titanium plate (President Titanium, Hanson, MA USA) was used as the
substrate for the building of the cylinders. Fabrication of the cylinders
had been achieved using the LENS™ system (Optomec Inc., Albuquer-
que, NM USA) armed with continuous wave Nd:YAG laser capable of
generating a maximum power of 500 W. Laser was focused on the sub-
strate to melt and create a melt-pool where metal powders were added
to increase the volume of the melt pool. The substrate was moved in X
and Y direction to write with the molten metal. After each layer, laser
moved up in the Z-direction and continue the build on top of the previ-
sous layer. After each batch was processed, the porosity was measured
using Archimedes’ method. The processing parameters for fabrication
were further altered based on the measured porosity of the cylinders.
Several such endeavors led to a success in producing the porous Ti rods
with a laser power of 280 W and raster scanning speed of
60 cm/min to 80 cm/min. During the fabrication process, the oxygen
flowrate of growing nanotubes, voltage was continuously maintained at 20 V
for 60 mins by DC power supply from Hewlett Packard
(0–60 V/0–50 A, 1000 W). The cylinders were rinsed thoroughly with
deionized (DI) water before further treatment.

2.3. Calcium phosphate coating

Biomimetic coating method was utilized to coat the surface modified
porous scaffolds because of its inherent advantage of not being a line of
sight technique. Porous LENS™ processed Ti-NT cylindrical rods were
kept immersed into 10 mL of 10× simulated body fluid (SBF) for 72 h
in static condition at 37 °C. SBF solutions were changed every 24 h. Spec-
imens were taken out from SBF after 72 h followed by three times gentle
washing in 2 mL DI water. Specimens were then air dried and coating
morphologies were observed under SEM. These samples are hence re-
ferred as LENS™ porous Ti-NT-CaP. To prepare Sr²⁺ and Si⁴⁺ doped
CaP coating, 2.1 atomic wt% Sr²⁺ (equivalent to 1 wt% SrO and 0.5 wt
% SiO₂ doped in HA) and 0.006 wt% Si⁴⁺ (equivalent to 1 wt% SrO and
0.5 wt% SiO₂ doped in HA) were added in the form of SrCl₂·6H₂O and
Na₂SiO₃ in 10× SBF [39]. After that, a similar procedure was followed
as described above for CaP coating and the fabricated cylinders are al-
lowed as LENS™ porous Ti-NT-CaP-Sr-Si.

2.4. In vivo study

2.4.1. Surgery and implantation procedure

All surgical and experimental procedures have been performed in
accordance to protocols approved by the Institutional Animal Care and
Use Committee (IACUC) of Washington State University. Male Sprague
Dawley rats (Simonsen Laboratories, Gilroy, CA, USA) each weighing
around 280–300 g underwent bilateral surgery for the entire set of the
study. The rats were kept in properly labelled individual cages and accli-
matized in humidity and temperature controlled rooms with alternate
12-h cycles of light and darkness. The rats were anesthetized with

![Fig. 2. Schematic representation of the LENS™ process [24].](image-url)
Fig. 3. (a) Schematic of push-out test set-up. (b) Picture of push-out test set-up connected with the Instron. (c) Top view of the push-out test set-up with a sample attached.

Fig. 4. (a–c): SEM and optical image [24] of the porous surface nature of LENS™ processed porous sample and porous Ti implant with fabrication of nanotubes with diameter 105 ± 30 nm and length 375 ± 35 nm using anodization method. (d): CaP and Sr-Si-CaP coating morphology on cylindrical LENS™ Porous Ti-NT samples at different magnifications showing the Sr and Si crystals on the cylinders. (e): CaP and Sr-Si-CaP coating thickness on cylindrical LENS™ Porous Ti-NT samples showing 120 nm and 170 nm thickness respectively.
sition and time point were used for the push out test. Fig. 3 shows the compression, 3-point bending, or peel tests. 2 implants at each composition for the samples that can avoid any translational motion along X and Y direction when the load is applied from the Z-direction. Interfacial shear modulus values were calculated from the linear region of the stress vs. strain plot generated from the load-displacement data.

2.4.2. CT scan analysis and interfacial strength by push out test
Radiographic images were acquired from X-ray energy source on the IVIS® Spectrum CT and further scanned by 40 μm voxel size and 150 μm resolution to create a three-dimensional volume. The obtained 3D scans are further fed into Living Image® Software 4.4 to generate the three-dimensional images. 2 samples of each composition after 10 weeks of implantation were used for the CT Scan.

Push out tests were done with the aim of calculating the shear modulus between the growing tissue along the implant surface and the implant itself. An idea of the shear modulus will help determine the bonding strength between the host tissue and the implant surface. Shear moduli were calculated from the slope of the shear stress vs. shear strain plots. Push out tests were performed using a 136.07 kg load cell with a servo-hydraulic controlled Instron 3343 load frame at a 0.33 mm/s cross-head speed. This test frame is capable of 1 kN tensile, shear, and CaP coating have been processed porous Ti implants, nanotubes and CaP coating have been described in Fig. 4. The volume porosity of the fabricated implant was 31.6%. Harvested bone samples were wrapped in a gauze pre-wet with saline and brought to the test location immediately for push-out test.

2.4.3. Histology, histomorphometry and SEM characterization
Post-surgery, the clipped bone-implant composites were fixed immediately in 10% formalin solution to allow tissue preservation. After 72 h of immersion, they were dehydrated in a consecutive series of ethanol drying using 70%, 95%, and 100%, 1:1 acetone-ethanol solution, and finally 100% acetone. The samples were then embedded into Spurr’s resin and thin slices were cut out using a diamond blade. The sections were rinsed with water and mounted on glass slides before being stained with a modified Masson Goldner’s trichrome staining method [24]. The stained tissue implant interface was observed under light microscope (Olympus BH-2, Olympus America Inc., USA). At least 5 sections from different rats were used for the histology images and follow-up histomorphometric analysis. Histophotometric analysis had been performed as a quantitative analysis of the histology images from ImageJ to study total osteoid formation and total bone formation at the interface of the implant using 3 images [200 μm by 200 μm] from each section, i.e., 15 images for each datum presented here. A region of 80 μm in radius has been selected around the implant to study the bone interlocking and effects of the compositions on the osteoid formation in the region surrounding the bone from three different images. The data has been normalized over the diameter of the implant. The percentage of the osteoid formation and total volume of bone formation were plotted against the different compositions at the two-time points. For further clarity, the interface of all the cut sections described above was observed under FESEM (FEI Quanta 200, FEI Inc., OR, USA), at low voltage of 10 kV and low vacuum.

3. Results
3.1. Surface morphology of LENS™ processed porous Ti implants, nanotubes and CaP coating
SEM images of LENS™ processed porous Ti rods along with grown nanotubes film before and after calcium phosphate coating have been described in Fig. 4. The volume porosity of the fabricated implant was 31.6%. Harvested bone samples were wrapped in a gauze pre-wet with saline and brought to the test location immediately for push-out test.

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approximately 25%. A macro image of the LENS processed Ti implant has been shown in Fig. 4(a) [24]. Pore sizes were measured from the scanning electron microscopy (SEM) image shown in Fig. 4(b) and was found to be in the range of 200–300 μm, in accordance to our previous studies [7,24]. Previously, we have also analyzed the Young’s modulus of LENS™ processed porous Ti structures and observed them in the range of 2–44 GPa, which is similar to that of human cortical bone, 7–30 GPa [7]. Also, the mechanical strength for these porous structures tend to vary from 24 to 463 MPa [7]. Porosity of the processed cylinders have been measured to be 25% by Archimedes’ method. After anodization, the length of the nanotubes has been measured to be 375 ± 35 nm and the diameter around 105 ± 30 nm as evident from Fig. 4c.

Fig. 4(d) and (e) show morphology and thickness of the of Sr2+ and Si4+ doped CaP coating. CaP coating thickness measurement was done on planar Ti substrate under similar conditions for CaP coating on LENS™ Porous Ti-NT. The thickness of the CaP coating has been observed to be around 120 nm and that of the doped CaP coating as 170 nm. Since the CaP and doped CaP coatings were done using biomimetic process, uniformity in the dopant concentration was achieved. Moreover, the CaP coating strongly adhered to the nanotubes.

3.2. Histological evaluation

Biocompatibility, new bone formation, and mineralization were analyzed by histological evaluations to study the effects of the nanotube film and CaP coating on porous Ti cylinders after 4 and 10 weeks, seen in Fig. 5. Osteoid–like new bone formation has been observed even at the 4 weeks’ time point. The orange-reddish color around the implant at the early time point indicates osteoid formation without any cytotoxicity around the area of implantation. Mineralized bone has been observed by the greenish color and nuclei have been stained with bluish black spots. More osteoid formation has been noticed after 10 weeks of implantation on the CaP coated porous Ti rods. No visible gaps of osteoid formation have been seen around the area of implantation. Sr2+ and Si4+ doped CaP coated Ti rods have shown more osteoid formation after 10 weeks of implantation compared to Ti rods with nanotube film. Early stage osteogenesis was noticed proving that there was no cytotoxic effect due to the implantation. From Fig. 6(a) and (b), almost 100% bone formation and higher osteoid formation have been noticed after 10 weeks in undoped and doped CaP coated samples compared to around 60% bone formation in control samples. The effects of compositions in the interfacial bone formation have been more pronounced in the early time points, compared to the later time points. Thus, by the combinations of titania nanotubes with doped CaP coating, we have been able to achieve accelerated bone formation in 4 weeks, which can be compared to the bone formation in control samples in 10 weeks. This further indicates towards the strong bone interlocking and confirms presence of no gaps at the implant interface in the CaP coated implants as early as 4 weeks.

3.3. CT scan and push out test analysis

To detect any visible gaps or defects at the interface of the bone tissue and implant, CT scan analysis was executed on few samples, shown in Fig. 7. Proper bone lodging into the implant was seen in the cylinders with metallic ion doped CaP coating. Images showed the effect of the porosity on the osseointegration but were limited by the machine related resolution issues. However, no signs of defects or cytotoxicity were noted from the CT scan analysis which was an indication of good osteogenesis.

Interfacial shear modulus values were calculated from the push out tests to analyze the interfacial bonding strength between the implant and bone tissue. After 4 weeks of implantation, the shear modulus in CaP coated porous Ti rods with and without dopants was almost 4 times (~80 MPa) compared to porous Ti with or without nanotubes (~26 MPa). Increased shear modulus values after 10 weeks of implantation compared to 4 weeks prove more osseointegration gradually with time. Shear modulus values (~110 MPa for all compositions) after 10 weeks of implantation may not be an appropriate reflection of the actual mechanical interlocking because the bone broke while performing the push out tests in all cases.

3.4. SEM characterization

SEM images of porous Ti with and without nanotube film are shown in Fig. 8(A) and (B). Some notable gaps are seen at the interface of the bone tissue and implant for both samples 4 weeks after implantation. These gaps have been seen to heal over time and notably less gaps are seen after 10 weeks of implantation. SEM micrographs of CaP coated porous Ti rods with nanotube film are shown in Fig. 8(C). Osteoid formation has been seen even after 4 weeks of implantation shown by the...
color contrast. Notable gaps were not seen at the interface proving good osseointegration. Fig. 8(D) shows Sr$^{2+}$ and Si$^{4+}$ doped CaP coated Ti rods with nanotube film. From the comparison of the SEM images at the interface of the host tissue and the implant, it is noted that visible gaps at the interface considerably decrease from the 4 weeks to 10 weeks’ time point. Cylinders with doped metallic ions show strong bonding at the interface with no noticeable gaps after 10 weeks of implantation. This also signifies much improved bonding of the host tissue with the implant with the usage of Sr$^{2+}$ and Si$^{4+}$ doped CaP coated Ti rods compared to porous Ti rods with nanotube films.

4. Discussion

Often, metallic implant failures have been attributed to the lack of early stage osteogenesis [40]. Interconnected porosity in scaffolds influences tissue ingrowth and enhances lifetime of the implant by improving the biological properties. Connected pores are also helpful in transporting metabolic wastes and exchanging of nutrients and blood, which is crucial for vascularization [28]. LENS™ has been categorized under directed energy based additive manufacturing technique by ASTM Standards for Terminologies for Additive Manufacturing, F2792-12a [41]. It is a directed energy based deposition technique and had been used to fabricate primarily metallic parts or metallic composite parts from powdered materials. The desired part is designed in a CAD software and fed into the machine to be converted into motion control designs which influences the actual build design [35,37]. The multi-hopper system incorporated in the LENS™ allows usage of multi-material and control of the process parameters, and laser power allow the fabrication of various complex geometry. Partial melting of the powder and usage of low laser power also allow fabrication of in situ porosity.

The experimental procedures optimized in the present study have enabled fabrication of titanium implants with random porosity to mimic the structure of the natural bone. The primary objective of the study was to induce early stage osteogenesis and tissue ingrowth at the interface of the implant and host bone tissue in the presence of a combination of nanotubes and doped CaP coating on porous metallic scaffolds.

Previous reports have demonstrated that an optimal porosity >20% is required for effective osseous tissue integration [42,43]. This study had been able to optimize the process parameters of the LENS™ system and was able to fabricate porous Ti rods with porosity of around 25% keeping up with the requirement of porous implants [24]. Scaffolds fabricated with 25–42% porosity can be modified to possess mechanical properties like compressive strength and Young’s modulus similar to cortical bone [44] and improve the implant life in vivo. Pores >200 μm allow the migration of osteoprogenitor cells into the scaffolds and lower the overall scaffold density. This is crucial in reducing the mismatch of stiffness between the metallic implant and surrounding osseous tissue [17,45].

Rough surfaces facilitate enhanced osteoblast cell attachment and differentiation compared to an untreated surface [17,44]. Nanoscale surface modification by the growth of titanium nanotube film has been one of the most commonly used methods to promote early stage osseointegration [8]. The higher surface to volume ratio also helps in making the surface hydrophilic which probes as a perfect environment for osteoblast cell attachment. CaP coating has also been done on the surface modified titanium implants to improve the bioactivity of the implants [45]. In many cases, CaP coated implants suffer from lower adhesive strength and delaminate in vivo within a short period of implantation. The presence of the nanoscale modification by the titanium nanotubes is crucial in reducing the mechanical mismatch between the ceramic coating and metallic implants [24]. This improves the coating quality and prevents the coating delamination in vivo. This study aims to address the knowledge gap, should the doped CaP coating in the presence of nanotubes on porous scaffolds enhance the early stage osseointegration.

Fig. 7. CT scan images of implants after 10 weeks showing tissue ingrowth into the implants with no notable defects or gaps along the interface in the doped coating. (n = 2).
The increased bone formation by the presence of the dopants has been demonstrated in the histology analysis presented in Figs. 5 and 6. Push-out tests were performed to validate our histological data in terms of bone tissue adherence and related mechanical strength of implants. Push-out tests are more relevant in rabbit or other large animal models where larger cross-section of bone is available to interact with the implant. However, our aim was to have some preliminary information to see if push-out data can correlate with the histology, and
Fig. 8. (A): SEM images of stained porous Ti samples after 4 (a, b) and 10 (c, d) weeks showing the interfacial bonding between the implant and the tissue. (B): SEM images of stained porous Ti-NT samples after 4 (a, b) and 10 (c, d) weeks showing the interfacial bonding between the implant and the tissue. (C): SEM images of stained porous Ti-NT-CaP samples after 4 (a, b) and 10 (c, d) weeks showing the interfacial bonding between the implant and the tissue. (D): SEM images of stained porous Ti-NT-CaP-Sr-Si samples after 4 (a, b) and 10 (c, d) weeks showing the interfacial bonding between the implant and the tissue.
Porous metallic implants have been fabricated through laser processed 3D printing. These results show that strontium oxide and silicon oxide doped calcium phosphate coated porous Ti rods with nanotube film enhance the early stage osseointegration and improves mechanical interlocking between the host bone and implant compared to pure porous Ti rods. Hence the study could successfully lead to accelerated healing by enhancing osteogenesis in a bone defect repair model in a rat distal femur.

5. Conclusions

Commercially pure Ti rods, with interconnected random porosity of around 25\% vol with and without nanoscale surface modification, have been 3D printed using LENS™ to analyze the effects of surface modification on the bonding strength at the interface between the implant and the osseous tissue, and early stage osseointegration in vivo. The porous surface modified Ti was further coated with doped calcium phosphate to enhance osseointegration, and then implanted into a rat distal femur model for 4 and 10 weeks. Shear modulus values, computed from the push out tests, revealed high values of 80.09 MPa for LENS porous Ti-NT-CaP compared to the 25.85 MPa for porous Ti after 4 weeks of implantation divulging evidences of enhanced early stage osteogenesis. After 10 weeks of implantation, evidence of fully integrated implants was demonstrated due to bone fracture during push out tests. Histological micrographs, CT scan analysis and SEM images also confirmed that CaP coated Ti cylinders showed enhanced osteogenesis and strong interfacial bonding at the interface of the bone tissue. Porous Ti implants with TiO\textsubscript{2} nanotubes induced early stage osteogenesis while the addition of calcium phosphate coating further enhanced defect healing and mechanical interlocking at the interface.

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Data availability

The raw data required to reproduce these findings are incorporated in this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.matdes.2018.04.049.

References
