

# Assessment of Cell-Material Interactions on 3D Nanostructured Titania-Polymer Surfaces Toward the Improvement of Osseointegration of Orthopedic and Dental Implants

Jason Howard, Shraddha Patel, Jeffery Fontenot, Stephen McMullen, Austin Thompson,  
Jillann Walker, David K. Mills, and Scott A. Gold

Institute for Micromanufacturing, Louisiana Tech University, 911 Hergot Ave., Ruston,  
LA, 71272-0043

## Introduction

As the life expectancy of the general population continues to rise, so has the number of people experiencing varying types of physical decline. The number of individuals experiencing degeneration or loss of musculoskeletal function is increasing among the older adults in society. This increase has facilitated a need for a means of managing such a loss of functioning. One area of current focus is in the incorporation of bone regenerative properties into implantable biomaterials.

Titanium is widely used for orthopedic and dental implants for its good biocompatibility, corrosion resistance, and excellent mechanical properties. Titanium, however, is bioinert, which means that upon implantation into the body it cannot directly bond to the bone. The process of implant integration with the bone following implantation involves the encapsulation of the titanium implant by a fibrous tissue that isolates the implant from the surrounding bone [1]. One way to overcome this problem has been through various types of surface modifications of the titanium to promote osseointegration (or attachment to the bone) of the implant. It is well known that the adhesion of cells to the surface of a biomaterial is vitally important for *in vivo* integration [2].

The current struggle lies in the ability of titanium to attach to the bone *in situ* and the quality of said attachment at its insertion. The current average functional lifetime of bone implants range from 10 to 15 years [3]. In the US over 3 million musculoskeletal procedures are conducted each year with the majority being fracture repair and total joint replacements [4]. While many of the procedures result in success, implant loosening and failure still remain significant problems. One of the established causes of these implant failures is the result of implant degradation and osteolysis, which is bone tissue death [5]. As the bone tissue becomes less viable at the biomaterial interface, the implant becomes less fixed to the bone, eventually requiring surgery to correct. Recent studies have reported that 25% of hip replacement surgeries were corrections to

previously failed implants [6], while up to 29% of patients that received hip implants died in the year post-surgery [7]. In order to overcome this problem, bone implant materials must be designed to activate rapid bone growth to accommodate any implant loosening or degradation to ensure a secure attachment to the bone. To achieve this goal, the surface of the implant material needs to be able to attract osteoblasts and provide a viable environment for osteogenesis of the cells. In order to overcome this problem, bone implant materials must be designed to activate rapid bone growth to accommodate any implant loosening or degradation to ensure a secure attachment to the bone. To achieve this goal, the surface of the implant material needs to be able to attract osteoblasts and provide a viable environment for osteogenesis of the cells. Thus, there is a growing interest in modifying the surface at the biomaterial interface to allow the growth of healthy osteoblasts and to promote their long-term survival, such that the revision surgeries can be circumvented.

In this current work, we utilize porous titania, formed by electrochemical anodization of titanium as a template for the fabrication of polymer nanostructures on a surface. Osteoblasts are then grown on this modified surface and their viability compared to that on a flat and unmodified porous titanium surfaces using MTT and PicoGreen assays. This surface modification approach shows much promise for improving osseointegration of titanium implants.

## **Methods**

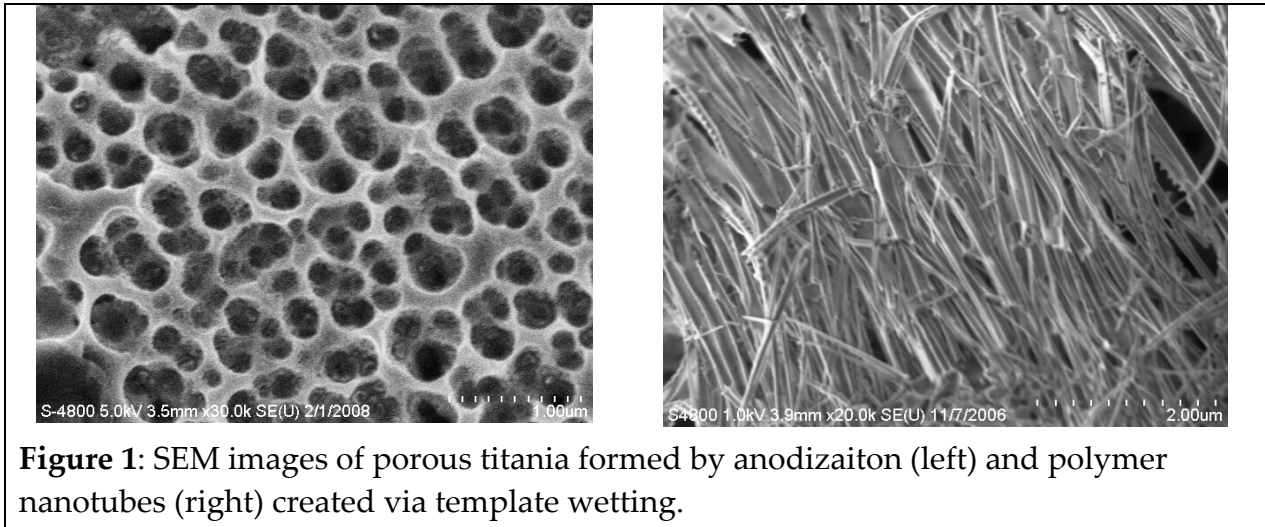
### ***Anodization***

Anodization is a widely used surface modification technique for many metals. By varying the current density and the anodization time, different pore diameters and depths can be fabricated [8]. In this study, this surface modification process was performed using a hydrofluoric acid (HF) solution to produce nanoporous titanium (shown in Figure 1), which then served as the template for the polymer wetting resulting in a polymeric nanostructured surface for cell seeding experiments. All anodization experiments were performed using an electrolyte consisting of 1 wt.% HF + 50 vol% methanol. The current density was varied from 10 to 30 mA cm<sup>-2</sup> for an anodization time of 30 minutes.

### ***Template Wetting***

Template wetting nanofabrication process utilizes wetting phenomena to create uniform coatings of low surface energy materials on high surface energy porous surfaces. This technique has been successfully used to produce polymer nanotubes (Figure 1) and nanowires for several polymers, including PMMA, and PVDF [9]. The polymer nanotubes were fabricated using the template wetting process on the anodized titanium templates by wetting with the polymer poly(vinylidene fluoride) (PVDF) solution. PVDF is biocompatible, and thus has been used widely as a surface

modification agent of biomaterials. Following evaporation of the solvent, reactive ion etching (RIE) was used to remove any residual polymer solvent. The titania was chemically etched to exposed the polymer nanotubes.



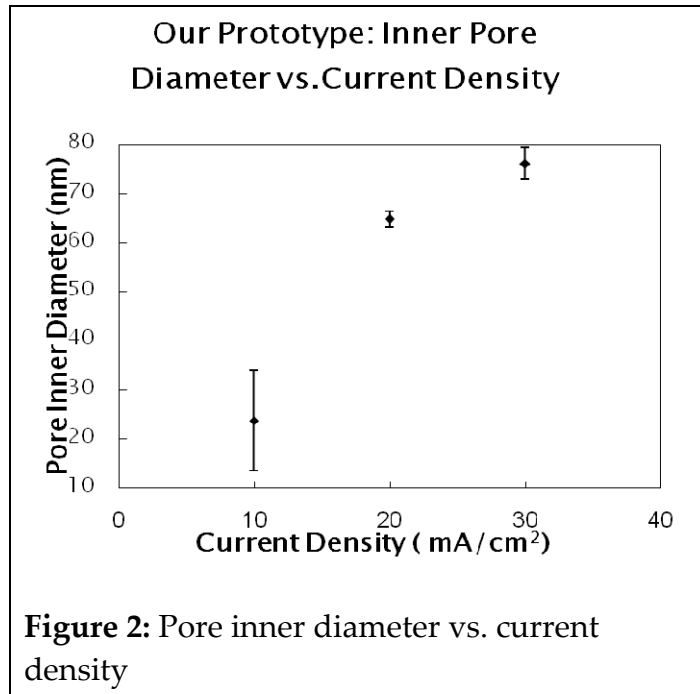
### *Cell Characterization*

*MTT assay (cell viability):* The MTT assay is used as a test of cell viability through the quantification of adherent cells. This assay utilizes the formation of purple formazan crystals from the mitochondrial dehydrogenases of viable cells cleaving the tetrazolium ring. This purple solution is then measured at 570 nm using a spectrophotometer. The amount of formazan formed can be correlated to the resulting viability or cytotoxicity of the cells on the surface.

*PicoGreen assay (DNA quantification):* The PicoGreen assay provides another measure of cell viability. This assay uses the fluorochrome PicoGreen which selectively binds to dsDNA. Bound PicoGreen is then measured at 260 nm using a spectrophotometer. The amount of bound PicoGreen can be correlated to the resulting cell viability.

### **Results and Discussion**

ImagePro Plus software was used to analyze the SEM images of the anodized titanium samples to determine inner pore diameter. The trend between the inner pore diameter and current density observed in Figure 2 is similar to the trend found in the literature [10], however the inner pore diameters achieved in this study were not as large as the reference diameters for the given current densities. These results confirm that a consistent pore size can be achieved.



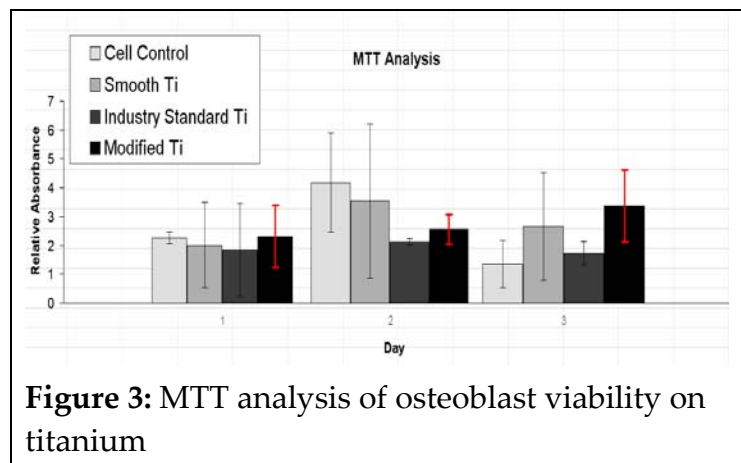
Following anodization, the titanium samples were then coated with a PVDF polymer solution. Energy-dispersive x-ray spectroscopy (EDS) confirmed the presence of polymer on the surface of the wetted titanium samples.

The results of the MTT assay (Figure 3) show that the polymer-coated modified titanium demonstrated better osteoblast viability than the smooth titanium and the micro rough industry standard titanium. Figure 3 shows the 3 day MTT assay results with a 95% confidence interval. All values in Figure 3 have been normalized using a baseline control

for each day. These initial results are promising for the future of the nanoporous structures that will be created with the process.

### Conclusion

Titanium oxide nanoporous films were grown on titanium surfaces via anodization and wetted with a PVDF polymer coating on the surface of the modified titanium. Cell proliferation using MTT assays showed that the best proliferation was on modified polymer-coated titanium compared with the industry standard roughened titanium and smooth titanium.



## References

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