Interaction of Osteoblast-Like Cells with the Micro/Nano-Structure of Laser Additively Manufactured 316L Stainless Steel and its Implications for Biomedical Applications

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Abstract

Commercially available 316L stainless steel (316L SS) is used for orthopedic implants because of its corrosion resistant properties which promote biocompatibility. These implants are bonded to the bone with grout. However, implants fail after approximately 10 years, because the grout deteriorates and there is poor implant-tissue integration. Researchers have been investigating methods to modify the surface of 316L SS to aid substrate-tissue integration. However, these current methods can be expensive and time intensive. Three-dimensional (3-D) printed 316L SS has been observed to have a modified micro/nano surface structure when chemically etched. The increased surface roughness of etched 3-D printed 316L SS could be a more time and cost-effective alternative to improve substrate-tissue integration and prolong operational life time of the implant. Our investigation presents the chemical characterization and biomedical evaluation of 3-D printed 316L SS. Our results concur with previous work that the bulk chemistry composition of both polished and etched 3-D printed 316L SS is similar to commercial 316L SS. However, the 3-D printed 316L SS surface chemistry elemental distribution is heterogeneous, which leads to the modified micro/nano-structure after etching. Here we compared bone cell interaction with various pre-treated 3-D printed 316L SS and commercial 316L SS. The pre-osteoblast-like cells were able to attach to all of the substrate surfaces. However, there are differences in cell density and morphology within a single sample as well as between varying samples. Furthermore, differentiated osteoblast-like cells on an etched sample exhibited the ability to make deposits containing high levels of phosphorus and calcium. In conclusion, further studies are needed to determine if cellular effects are due to surface chemistry or topography.

Introduction

- The Center for Disease Control and Prevention reported that 310,800 hip replacements were performed in the United States in 2010 alone.[1]
- Given that current implants only last about 10 years, there is a need to decrease their failure rate, which is dictated by degradation of the glue that holds it in place and poor integration with surrounding tissue. [2]
- Commercial 316L stainless steel is used for orthopedic implants. 3-D printed 316L steel is now available and has been observed to have a modified micro/nano-structure when chemically etched. [3]
- Bone cells are known to prefer a rougher substrate topography. [4]
- As opposed to classic implants used now that are smooth, creating a micro/nano-structure on the surface of 316L steel through chemical etching could promote cellular focal adhesions and therefore improve bone cell attachment. Ultimately this may improve longevity of bone implants.

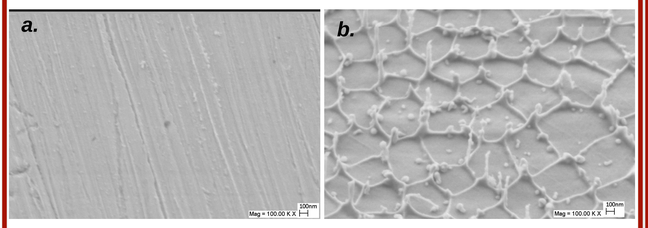
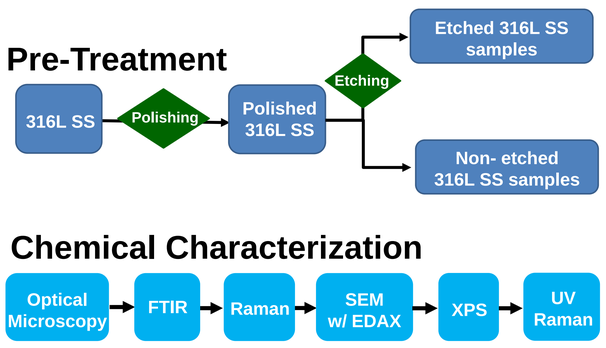
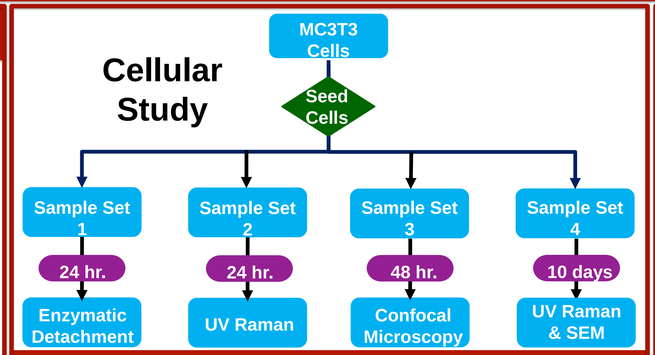


Figure 1 a. SEM image at 100.00 K x magnification of 3-D printed 316L SS sample K, which was polished but non-etched. Figure 1 b. SEM image at 100.00 K x magnification of 3-D printed 316L SS sample D, which was polished and then chemically etched using Vilella's Reagent.

Methods





Optical Microscopy

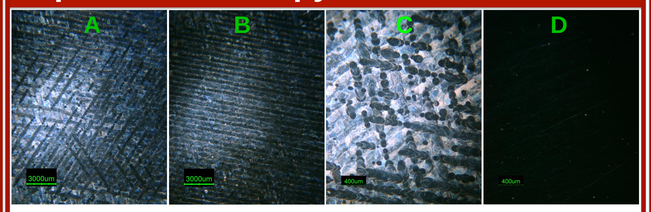


Figure 2: (A) is a dark field image of Sample A post etch at 4x magnification. (B) is a dark field image of Sample A post etch at a different position at 4x magnification. (C) is a dark field image of Sample A post etch at 10x magnification. (D) is a dark field image of a non-etched sample at 10x magnification.

FTIR

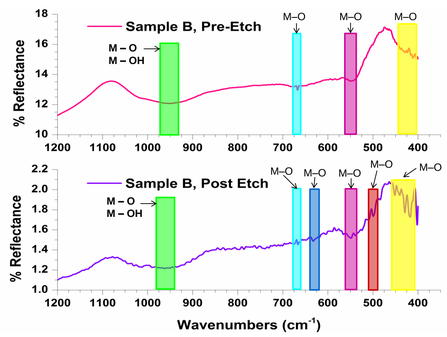


Figure 3: Fourier Transform Infrared Spectroscopy of Sample B pre and post etching. Metal oxides and hydroxides vibrations are apparent in both spectra. M stands for transition metal, here it is most likely either chromium, molybdenum, iron, or manganese. Percent reflectance is lower in the post etch and M-O vibrations are more apparent in the yellow 400 cm^{-1} range.

UV Raman

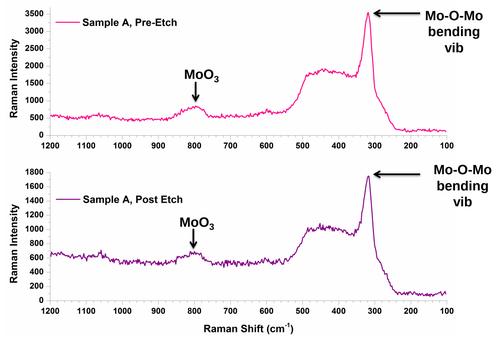


Figure 4: Ultraviolet Raman Spectroscopy of Sample A pre and post etching. Molybdenum dioxide and molybdenum-oxygen-molybdenum bending vibrations are apparent in both spectra.

XPS

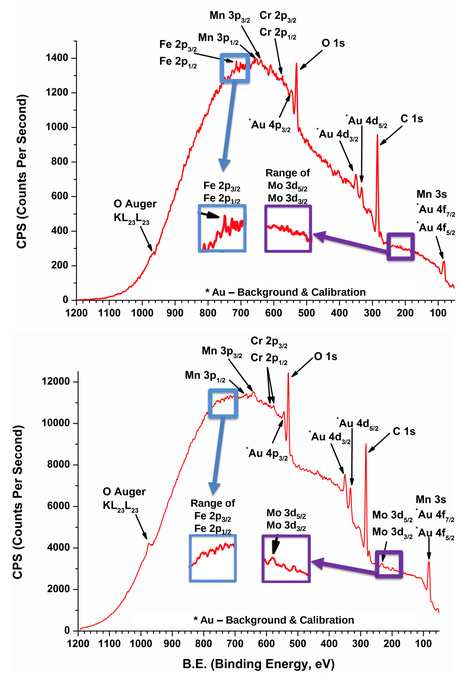


Figure 5: A is X-ray Photoelectron Spectroscopy (XPS) spectrum of a non-etched sample at a takeoff angle of 65 degrees. B is XPS spectrum of an etched sample at a takeoff angle of 90 degrees. In the non-etched spectrum an iron peak was apparent, and no molybdenum peak was apparent. In the post etching spectrum a molybdenum peak was apparent, and no iron peak was apparent.

Confocal Imaging with Cells, 48 Hours

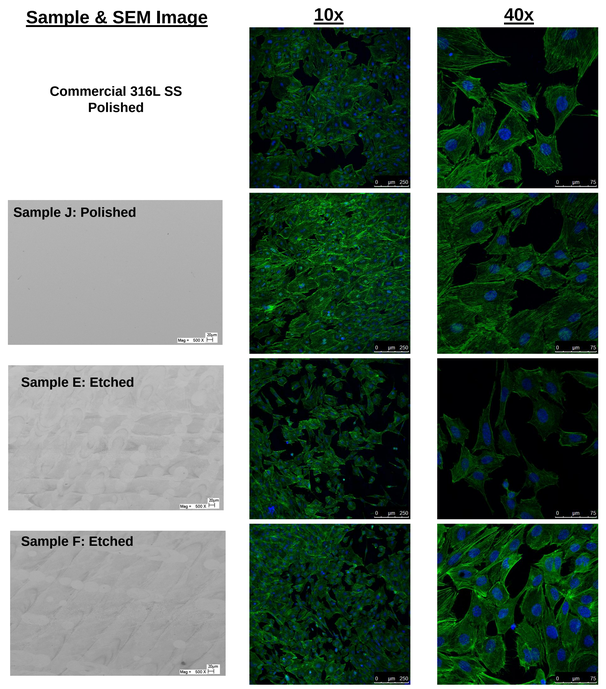


Figure 6: Shows a sample's corresponding Scanning Electron Microscope image taken at 500x magnification, and Confocal Microscope images, with MC3T3 pre-osteoblast-like cells 48 hour culture, taken at both 10x magnification and 40x magnification. The green actin stain is Alexa Fluor 488 and the blue nuclei stain is DAPI 358.

SEM & EDS with Cells 10 Days

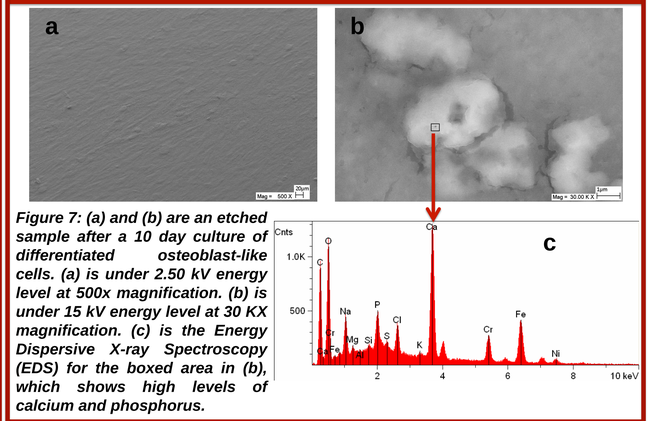


Figure 7: (a) and (b) are an etched sample after a 10 day culture of differentiated osteoblast-like cells. (a) is under 2.50 kV energy level at 500x magnification. (b) is under 15 kV energy level at 30 KX magnification. (c) is the Energy Dispersive X-ray Spectroscopy (EDS) for the boxed area in (b), which shows high levels of calcium and phosphorus.

Conclusions

- <u>Chemical Characterization:</u>
  - Substrate surface chemical characterization via XPS confirms that the surface chemistry changes slightly from pre-etching to post etching. Especially with regard to loss of Fe and gain of Mo upon etching.
  - FTIR and UV Raman confirm that substrate bulk chemistry composition does not drastically change upon etching.
- <u>Cellular Study:</u>
  - Confocal Microscope images demonstrated that the pre-osteoblast-like cells were able to attach to all of the substrate surfaces after a 48 hour culture (Figure 6).
  - There are differences in cell density and morphology within a single sample as well as between varying samples.
  - On the polished samples the cells were slightly larger in area, compared to the etched samples (Figure 6).
  - The etched samples appeared to have an increased amount of protruding lamellipodia, indicative of cellular migration (Figure 6).
  - After a 10 day culture with differentiated osteoblast-like cells on an etched sample there were small deposits containing high levels of Phosphorus and Calcium, indicative of calcium-phosphate deposits and therefore bone growth.
  - The results from the cellular study indicate that the changes in surface chemistry and surface micro/nano-topography had an effect on the cells.

Future Work

- Further study needed to differentiate the effects of surface chemistry and topography on osteoblast cell adhesion, morphology and migration.
  - Live cell imaging to confirm if cell morphology changes are due to cell migration.
  - Need to map surface chemistry and topography to spatially correlate data from different techniques at the same location on the sample.
  - Change printing parameters to determine how topography pattern from laser rastering affects osteoblast adhesion and morphology.

References

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