

Characterization of Osteoblast Function on Cold Spray Deposited Bio-Composite Coating for Titanium Bone Implants

Joana Villanueva (Humboldt State University)

Faculty Advisors: Dr. Michael West, Dr. Grant Crawford, Dr. Alfred Boysen

Introduction/Background

- Titanium implants offer low density, good mechanical properties, biocompatibility and corrosion resistance.[1]
- The leading cause of implant failure is loosening of the implant due to poor early stage osseointegration.
- Hydroxyapatite (HA) is believed to improve osseointegration due to its chemical similarities to bone.
- HA is commonly deposited on Ti bone implants via thermal spray techniques (e.g. Plasma Spray). However, this high temperature process often results in a phase transformation of HA resulting in reduced biological performance (e.g. biodegradation).
- Using high-pressure cold spray deposition (~300°-600°C) allows HA to retain its chemical structure and bioactive properties.

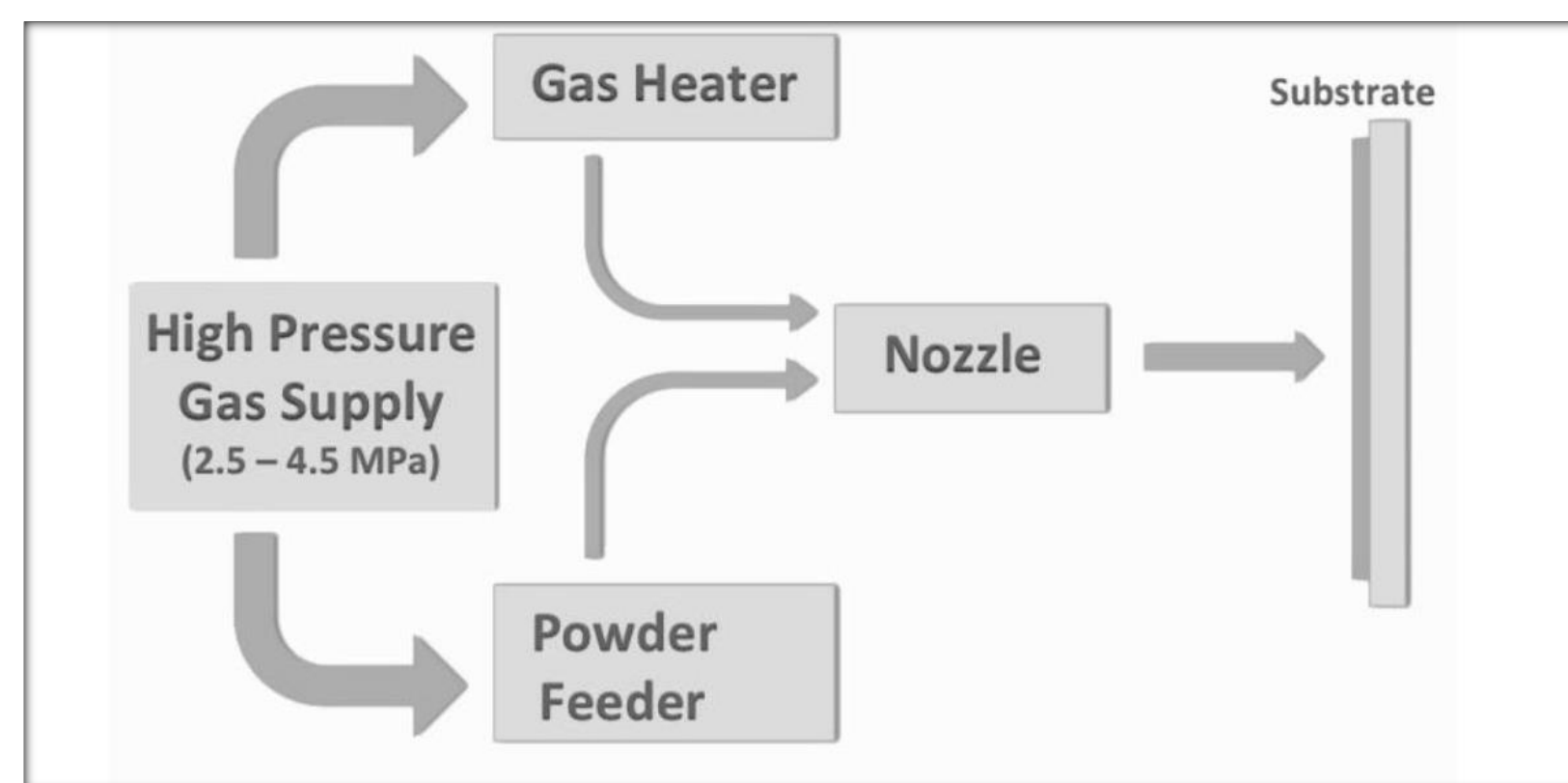


Figure 1: Schematic of a high-pressure cold spray system. (Image from VRC Metal Systems Technologies)

- Bone cell function depends on 4 factors: Surface energy, surface roughness, surface topography, and chemical composition. [3]

Objective

The purpose of this research is to characterize osteoblast function on cold spray deposited bio-composite coating for titanium load bearing implants. This research will specifically focus on:

- Characterizing mineral deposition,
- Understanding the relationship between different concentrations of bio composite coatings [HA/Ti] and the mineral deposition,
- Investigate the impact of cold spray deposition on the biological response (bone mineralization) of cp-Ti.

Experimental Process

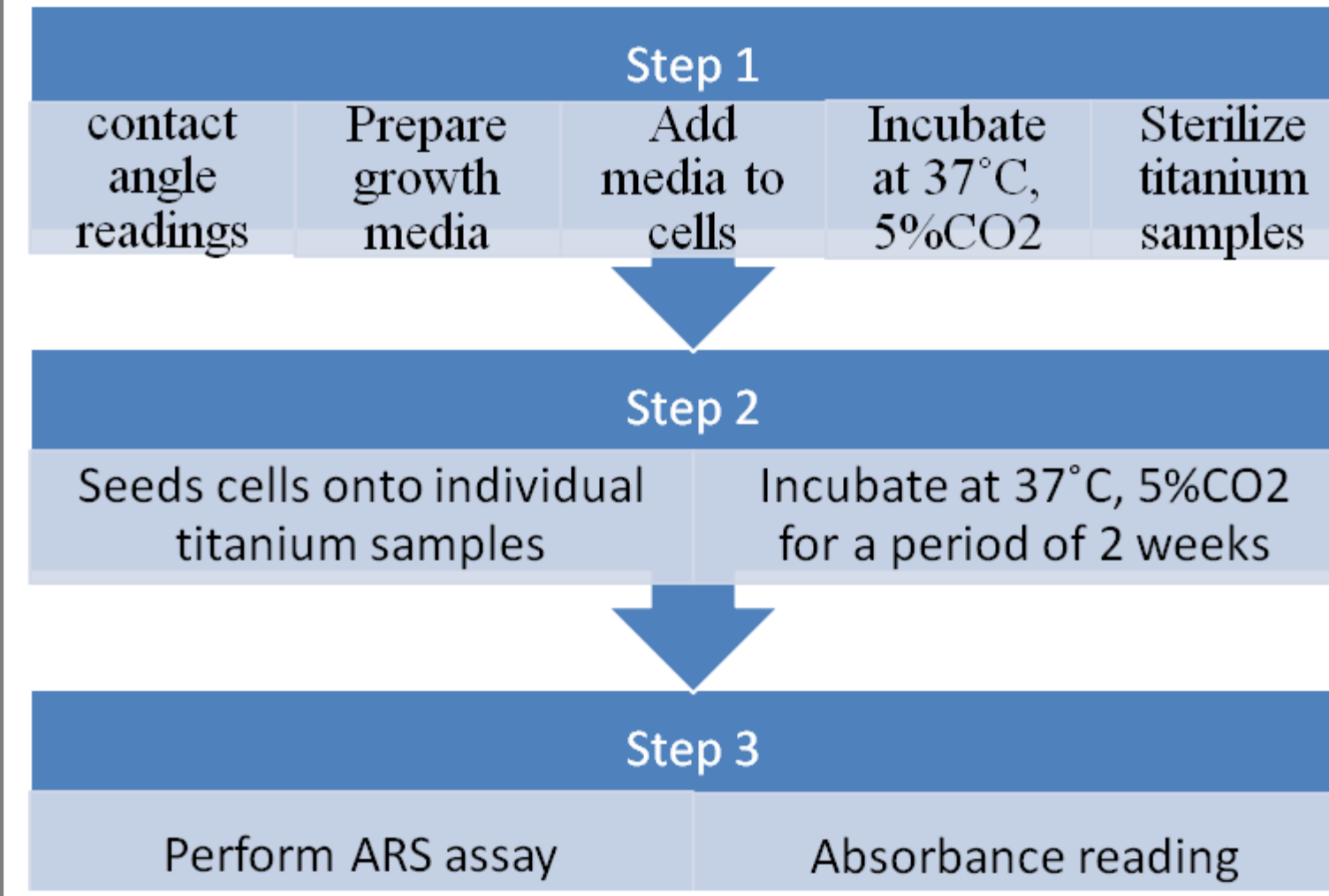
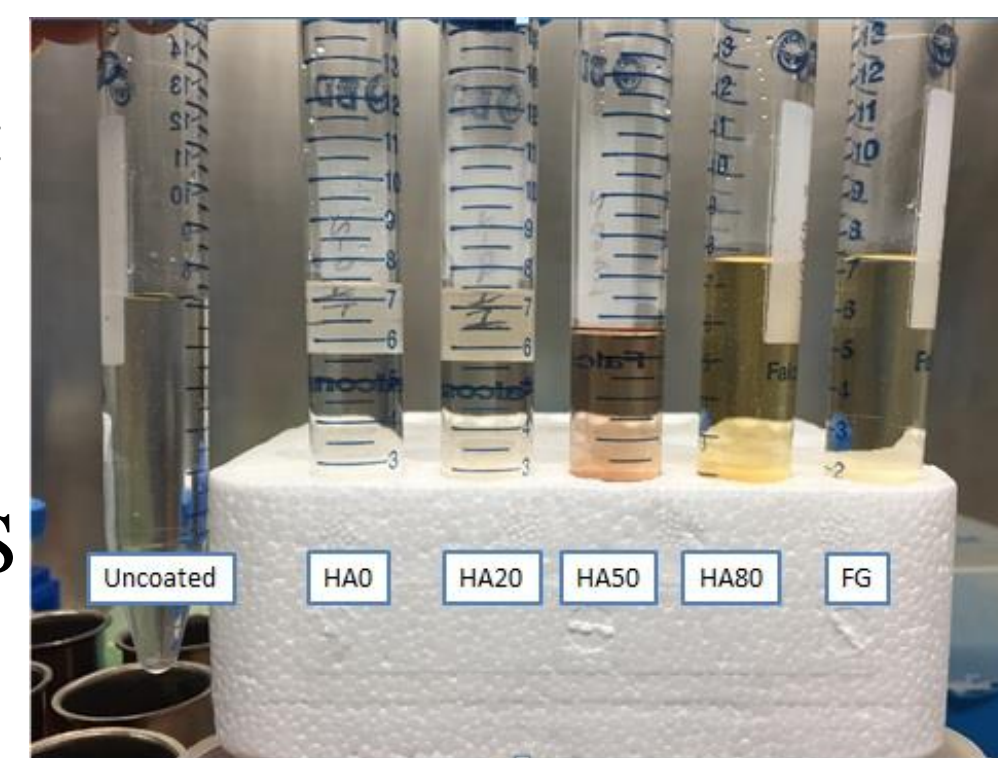


Figure 2: Generalized overview of the experimental procedure.

Figure 3: Stained titanium samples after ARS assay.



Experimental Overview

- Contact angle
- Cell culturing
- Cell seeding (2 weeks)
- Alizarin Red S assay (ARS)
- Quantification of mineral deposition

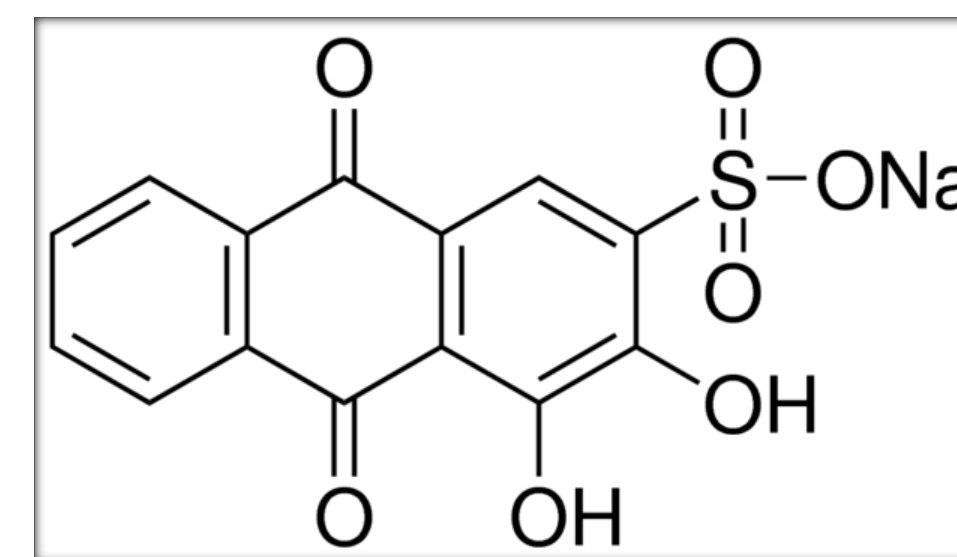


Figure 4: Alizarin Red S, used to determine the presence of calcific deposition by cells of an osteogenic lineage. (Image from Sigma-Aldrich)

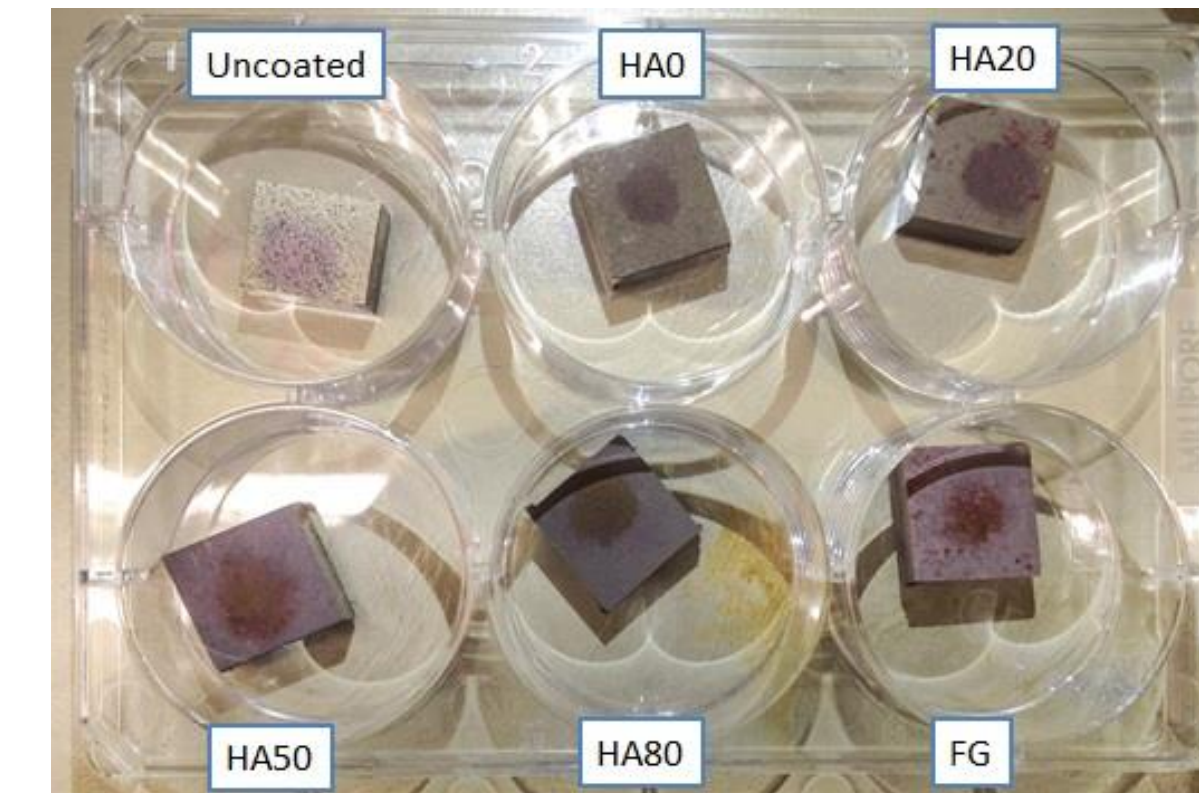


Figure 5: Samples of ARS extract before absorbance readings.

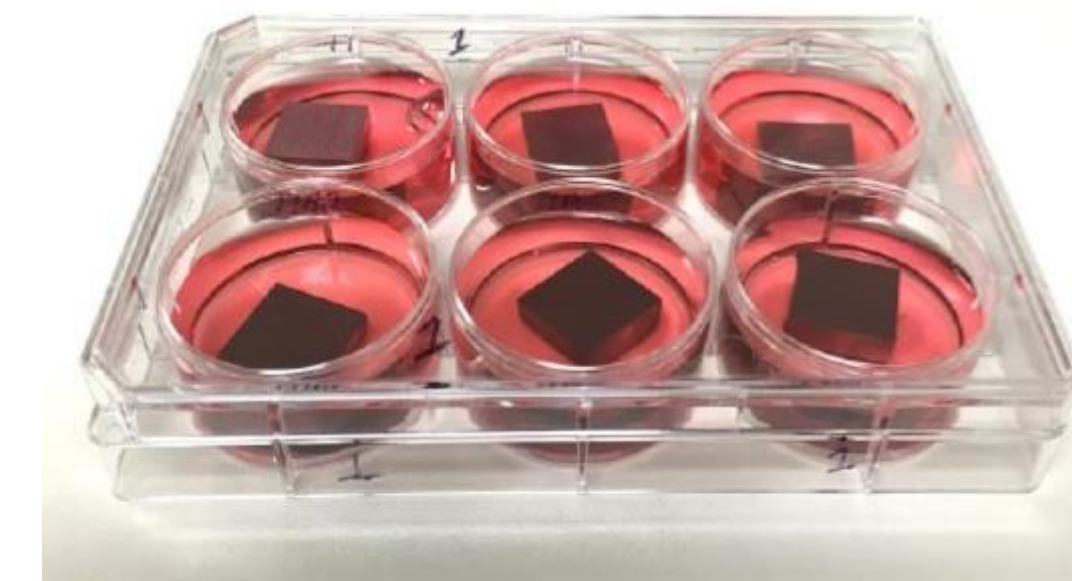


Figure 6: Titanium samples in 6-well culture plate submerged in media.

Experimental Results

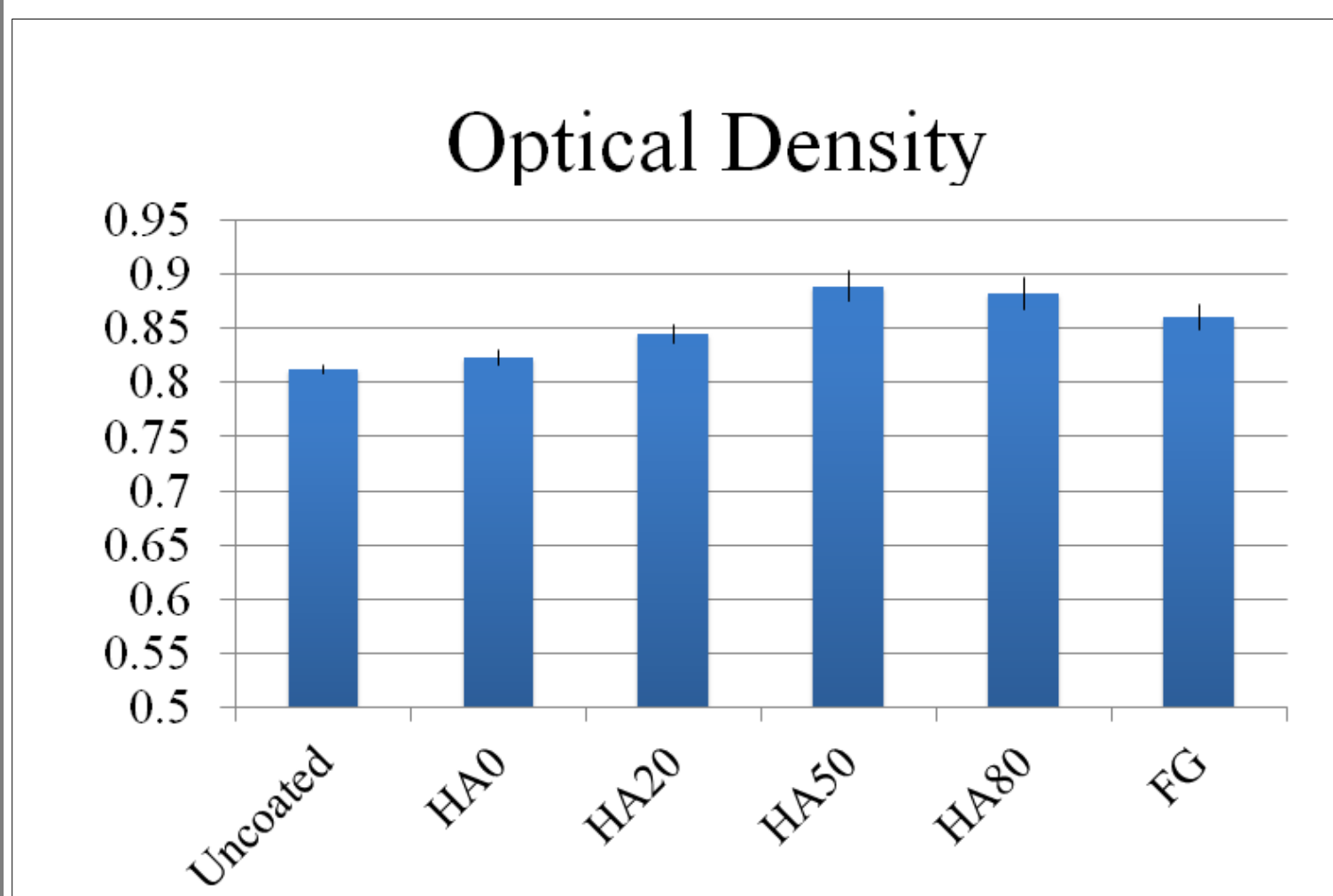


Figure 8: SEM-EDS images for area fraction analysis at coating surface. The red area represents titanium region and the green area represents Hydroxyapatite a.) HA20 coating, b.) HA50 coating, c.) HA80 coating, d.) Functional Grade coating. [4]

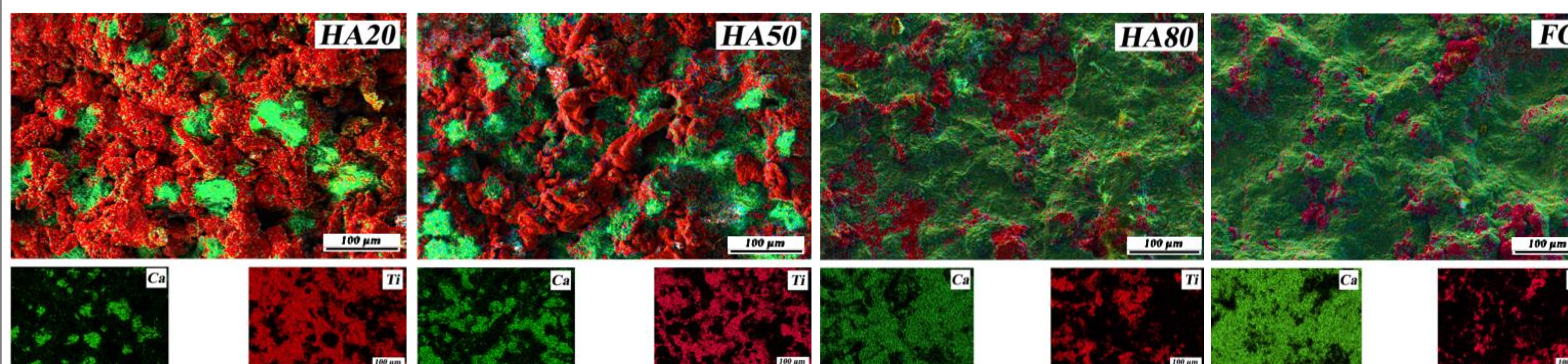


Figure 7: Absorbance readings taken from ARS staining tests. Readings were taken in triplicates. Sample with coating HA50 shows to have a greater OD reading than uncoated titanium sample.

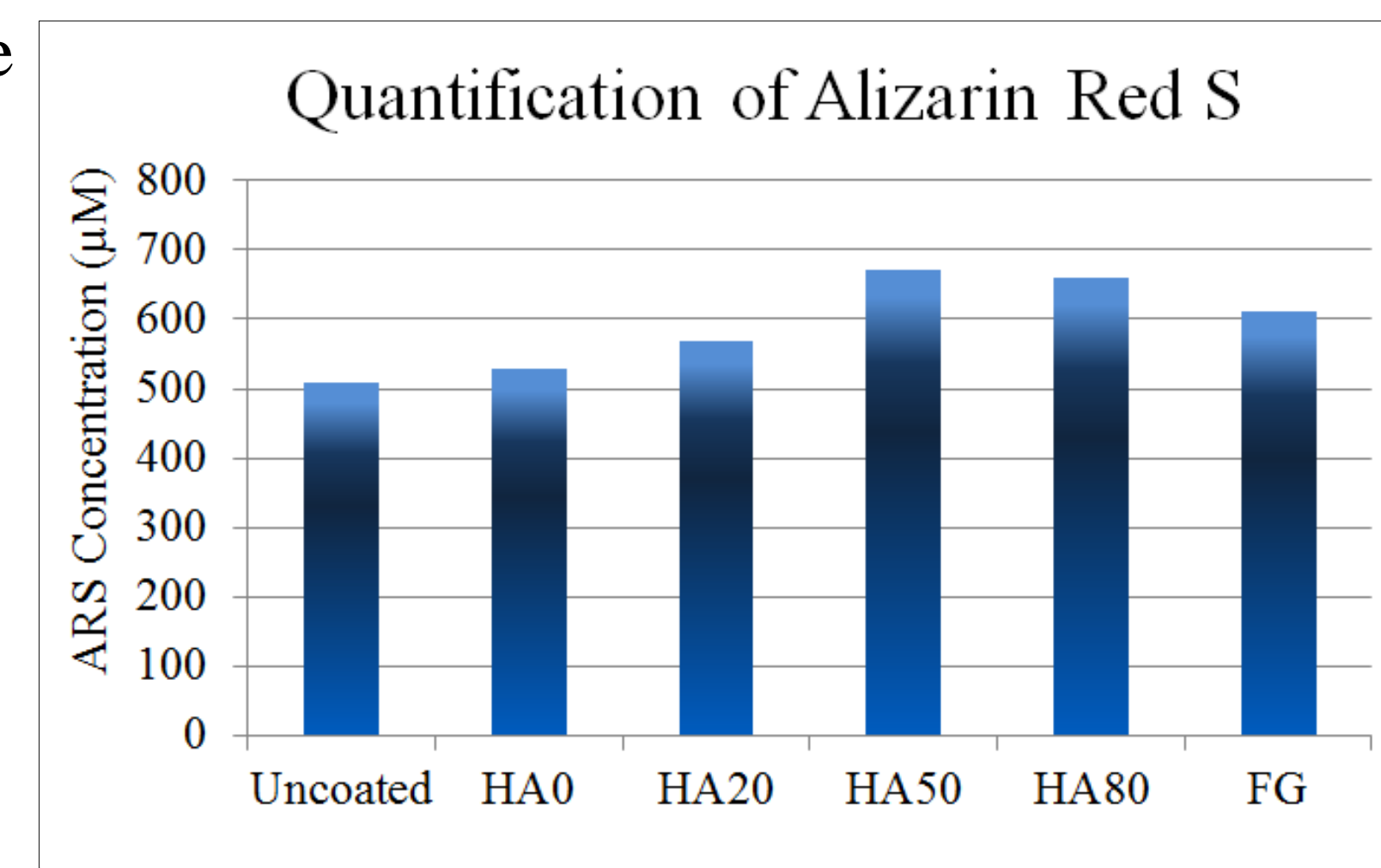


Figure 9: ARS concentration. ARS stain present in samples is relative to the amount of differentiation (i.e. mineral deposition).

Conclusion

- Tissue formation on surface of bio-composite coatings were confirmed.
- All samples showed tissue formation, however, HA50 had the greatest calcium deposition.
- All coated samples (HA20, HA50, HA80, and FG) showed greater tissue formation than uncoated titanium samples
- Surface energy should be investigated.
- HA shows potential to improve osseointegration and eventually improve the longevity of titanium load bearing implants. [5]

Future Work

- Long term ARS testing to explore cell proliferation over extended periods of time.
- Tests on different cell types, i.e. tissue cells or human osteoblasts,
- The influence of contact angles and surface energy needs further investigation.
- Type 1 Collagen test to compare.

References

- [1] Jamesh, M., Narayanan, T., Chu, P., Park, I., & Lee, M. (2013). Effect of surface mechanical attrition treatment of titanium using alumina balls: Surface roughness, contact angle and apatite forming ability. *Frontiers of Materials Science Front. Mater. Sci.*, 285-294.
- [2] ©1995-2015 American Academy of Orthopedic Surgeons.
- [3] Schwartz, Z., & Boyan, B. (1994). Underlying mechanisms at the bone-biomaterial interface. *J. Cell. Biochem. Journal of Cellular Biochemistry*, 340-347.
- [4] Bhatta, Eden. (2015). Processing, Microstructure Characterization and Biological Response of Cold Sprayed Biocomposite Coating
- [5] © Direct Healthcare International Limited 2010

Acknowledgements

This work was made possible by the National Science Foundation REU Back to the Future Site DMR-1460912. A special thank you to Dr. Grant Crawford and Dr. Michael West for all of their guidance throughout this research, and Dr. Alfred Boysen for his help in putting all of my research together. Also, a special thank you to Eden Bhatta for all of his help and guidance and allowing me to work with him on his research.