Nano SUS (Ultra Fine Grained Stainless-Steel) for Orthopedic Implants

Shaunak Kelkar [1, 2], Bahram Saleh[1, 2], Caterina Bartomeu-Garcia[1, 2], Fumie Yusa[3], Takafumi Komatsu[3], and Thomas. J. Webster[1, 2]
[1] Department of Chemical Engineering, Northeastern University, Boston, MA 02115
[2] Nanomedicine Science and Technology Center, Northeastern University, Boston, MA 02115
[3] Komatsuseiki Kosakusho Co., Ltd., Production Department, Japan

Statement of Purpose: Orthopedic implants are important therapeutic devices for the management of a wide range of orthopedic conditions. However, bacterial infections of orthopedic implants remain a major problem, and not an uncommon one, leading to an increased rate of osteomyelitis, sepsis, implant failure and dysfunction, etc. Treating these infections is more challenging as the causative organism protects itself by the production of a biofilm over the implant's surface (*1*). Infections start by the adhesion and colonization of pathogenic bacteria such as *Staphylococcus aureus* (SA), *Staphylococcus epidermidis* (SE), *Escherichia coli* (*E. coli*), Methicillin-Resistant *Staphylococcus aureus* (MRSA), and Multi-Drug Resistant *Escherichia coli* (MDR *E. coli*) on the implant's surfaces. Specifically, *Staphylococcus* comprises up to two-thirds of all pathogens involved in orthopedic implant infections (*2*). However, bacterial surface adhesion is a complex process influenced by several factors such as chemical composition, hydrophobicity, magnetization, surface charge, and surface roughness of the implant (*3*). Considering the intimate association between bacteria and the implant surface, we measured the effect of stainless-steel surface properties on bacterial surface attachment and subsequent formation of biofilms controlling above mentioned factors.

Method: The prominent bacteria responsible for orthopedic implant infections (SA, SE, *E. coli*, MRSA, and MDR *E. coli*) were used in this study. We were able to control the grain size of medical grade 304 and 316L stainless steel without altering their chemical composition (grain size range= 20µm-200nm) (*4*). Grain size control affected the nano-topography of the material surfaces which was measured by an Atomic Force Microscope (AFM). Grain sizes, such as 0.2, 0.5, 1, 2, 3, 9, and 10 µm, were used both polished and non-polished. All the stainless-steel samples were cleaned by treating with acetone and ethanol under sonication. Triplicates of all polished and non-polished samples with different grain sizes were subjected to magnetization of DM, 0.1T, 0.5T, and 1T, before seeding them with the bacteria. Controls were used in the form of untreated samples. Bacterial were grown in Tryptic Soy Broth (TSB). An actively growing bacterial suspension was seeded onto the stainless-steel discs into 24-well micro-titer plates and kept for incubation. After 24 hours of incubation, the stainless-steel discs were washed with Phosphate Buffer Saline (PBS) to remove the plankton bacteria and allow the sessile bacteria in the biofilm to remain. The degree of development of the bacterial biofilms on the stainless-steel discs were measured

using spectrophotometric analysis. For this, the bacterial biofilm was removed from the stainless steel by sonication. The formation of biofilms was also determined by performing a biofilm staining method using Safranin.

Results: AFM results revealed a slight decrease in roughness by decreasing the grain size of the material. Moreover, the samples were segregated into two categories of polished and non-polished samples, in which polishing decreased roughness significantly. After careful analysis we found out that polished surfaces showed a higher degree for biofilm formation in comparison to the non-polished ones. We also observed that bacteria showed a higher rate for biofilm formation for the demagnetized samples, whereas 0.5T magnetization showed the least amount of biofilm formation. After 0.5T, there was no significant change in the rate of biofilm formation on the stainless-steel samples. Altogether, stainless steel samples containing 0.5 µm and less grainsize, and magnetized with 0.5 tesla and stronger magnets demonstrated the least degree of biofilm formation.

Conclusion: In summary, the results demonstrate that controlling the grain size of medical grade stainless steel can control and mitigate bacterial responses on, and thus possibly infections of, orthopedic implants or other implantable devices. The research was funded by Komatsuseiki Kosakusho Co., Ltd (KSJ: Japan)

References:

1. M. Ribeiro, F. J. Monteiro, M. P. Ferraz, Infection of orthopedic implants with emphasis on bacterial adhesion process and techniques used in studying bacterial-material interactions. *Biomatter* **2**, 176-194 (2012).
2. M. Wang, T. Tang, Surface treatment strategies to combat implant-related infection from the beginning. *Journal of orthopaedic translation* **17**, 42-54 (2019).
3. I.-S. Yeo, H.-Y. Kim, K. S. Lim, J.-S. Han, Implant surface factors and bacterial adhesion: a review of the literature. *The International journal of artificial organs* **35**, 762-772 (2012).
4. S. Torizuka, E. Muramatsu, T. Komatsu, S. Nagayama, in *Nanostructured Metals and Alloys*. (Elsevier, 2011), pp. 715-746.