Is it a hardware associated infection, aseptic loosening, or just colonization? In vivo spine hardware microbial influenced corrosion

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INTRODUCTION

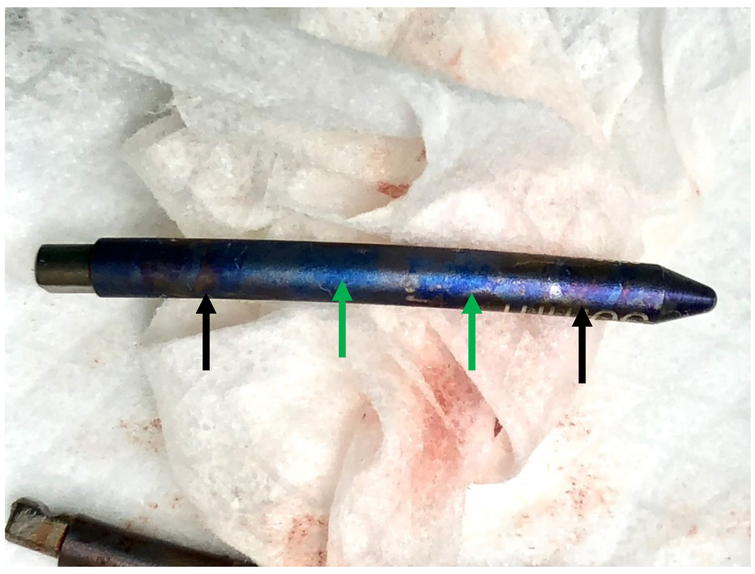


Figure 1: Geen arrows show the original anodized surface while the black arrows show the in vivo chemically altered surfaces.

Approximately 275,000 to 400,000+ spinal fusion procedures are performed annually in the United States.

The metals used are vulnerable to microbial colonization which leaves patients susceptible to Microbial Influenced Corrosion (MIC) which leads to metal leaching and device breakage.

There is the risk of these biofilms harboring pathogens that are commonly associated with hardware associated infections.

Figure 1 shows a spine rod where the deep blue surface oxide has been chemically altered in the patient. This chemical alteration is not possible in the patient tissue only environment. Environmental electrochemical causes resulting in discoloration are a highly acidic (<3 pH) or electrically charged surface which are unlikely in a human.

<u>Another possibility is endemic microbes utilizing the surface oxygen and metal ions for metabolic functions.</u>

RESULTS

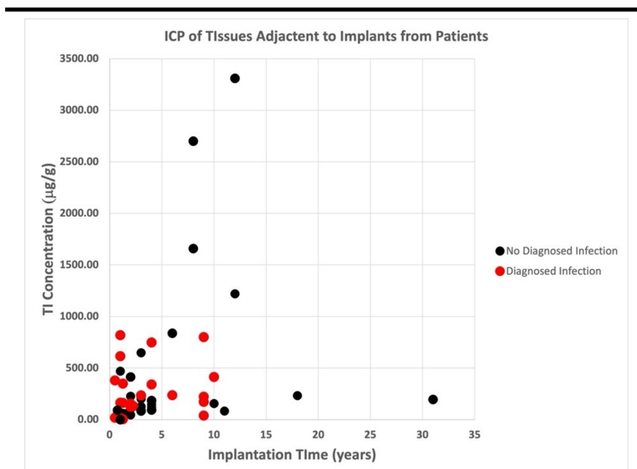


Figure 2: ICP of muscle tissue surrounding retrieved spine rods.

Inductively Coupled Plasma– Mass Spectroscopy (ICP) enabled metal ion tissue concentration measurements to the parts per billion level.

There is a small amount of Ti in tissues, approx. 13.12 ± 11.43 µg/g, based on our surgically naïve control of patients who had no orthopedic instrumentation of any kind (N=6) as the result from exposure to cosmetics, sunscreen, and food processing. This is sigificantly less than patients with instrumentation.

<u>Figure 2:</u> 54 patients who had tissue tested for significant levels of Ti ions (>100µg/g), 18 (33.3%) were diagnosed with an infection; the highest concentration of tissue Ti was from non-infected patients.

<u>No significant difference between patients with a clinically diagnosed infection and otherwise healthy patients is noted, suggesting metal loss from instrumentation is not the result of a clinically determined pathogen.</u>

Time of Flight – Secondary Ion Mass Spectroscopy (ToF-SIMS) is a surface analysis method that allows chemical characterization of a surface down to a single atom in an 8nm^2 area. This enabled us to physically observe the microbial metabolites that are actively incorporated into the implant surface during its lifespan. Explanted rods were ultrasonically cleaned followed by an ammonium formate rinse and then sputtered with an Argon Gas Cluster source to remove any molecules that may not be chemically bound to the surface, such as, adventitious carbon, hydrocarbons, aldehydes, amines, and oxidation.

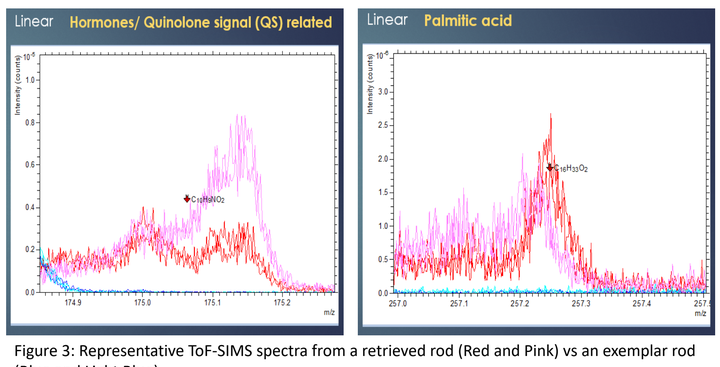


Figure 3: Representative ToF-SIMS spectra from a retrieved rod (Red and Pink) vs an exemplar rod (Blue and Light Blue).

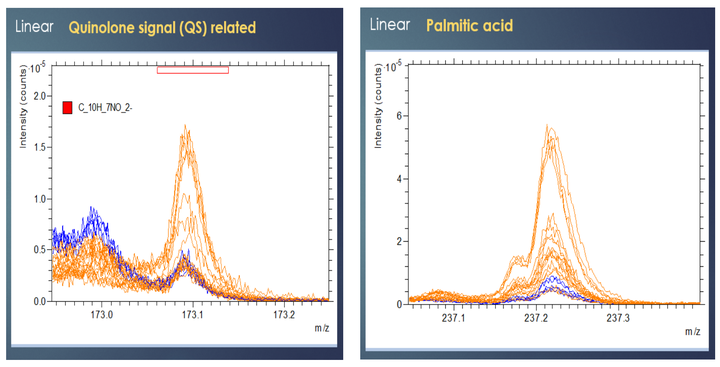


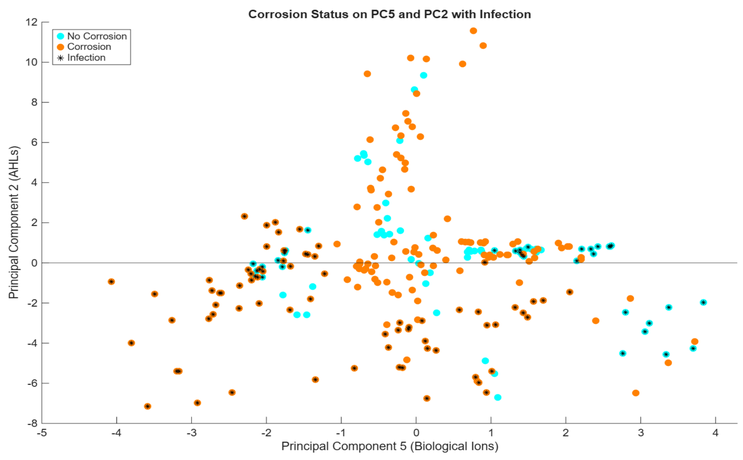
Figure 4: Representative ToF-SIMS spectra from a retrieved corroded region (Orange) vs the same rod in an uncorroded region (Blue)

<u>Figure 3: All rods, regardless of patient infection status showed the presence of microbial molecules such as quinolones (a bacterial specific communication and electron exchange molecule not produced in humans) and palmitic acid (bacterial structural fatty acid and metabolite) collocated on corroded surfaces as compared to unimplanted rods.</u>

<u>Figure 4: The quinolones and palmitic acids were colocated to only corroded regions on all rods</u>. Corrosion was verified using optical and Scanning Electron Microscopy.

The combination of quinolones and palmitic acid are strong indicators that a microbial biofilm was present. The presence of these molecules post cleaning shows that a biofilm was active and modifying the surface, integrating these molecules into the implant.

RESULTS CONTINUED



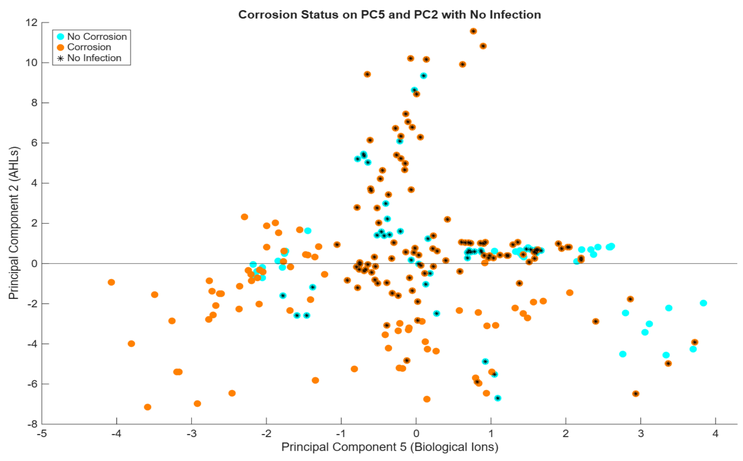


Figure 5: PCA of showing a comparison of corrosion regions (orange dots), uncorroded regions (blue dots), and patient infection status (*) with PC2 (N-homoserine lactones and quinolones) and PC5 (surface metal elements, Ti, V, Al)

<u>Figure 5:</u> Principle Component Analysis (PCA) of the ToF-SIMS data shows two separate functions occurring during the implant in vivo lifespan. Each point is one region of a ToF-SIMS scan on retrieved rods (256 total regions). 14 patients total are included (10 with no infection and 4 with infection).

Top PCA shows patients with infection are not associated with the presence of surface quinolones and homoserine lactones (AHLs) nor the presence of exposed metal ions and oxides.

<u>This suggests other mechanisms are active during hardware associated infection; not from the hardware itself.</u>

Bottom PCA shows that patients with no diagnosed infection are associated with the presence of microbial metabolic molecules.

There is no difference concerning the corrosion occurring with it being positively associated with both PCs.

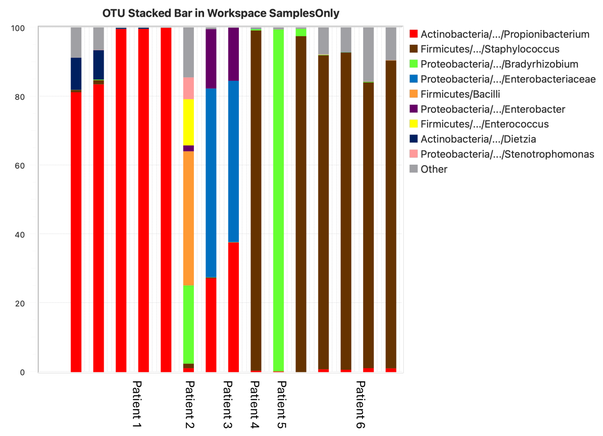
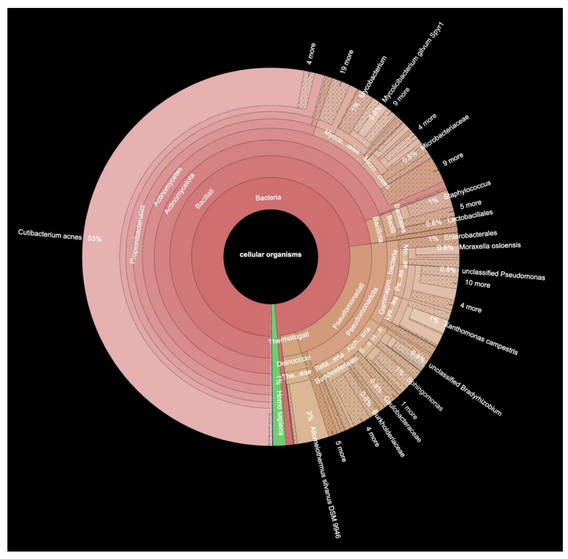


Figure 6: 16s metattaxanomic analysis of 6 patients post sonication prior to ToF-SIMS.

16s and shotgun metagenomic sequencing was used to to determine what microbes were present that would produce quinolones as well as actively modify the titanium rod surfaces. While this data is preliminary with deeper sequencing upcoming, it provides hints as to what the make up of the biofilm is and can explain the physical evidence seen on the explants.

<u>Figure 6:</u> 16s sequencing shows the presence of proteobacteria (pseudomonadota) was present on the rods retrieved from these patients.

Patients 1 and 6 had C. acnes, and Staph. a, cultured in clinic. Patient 3 had only C. acnes. Patients 2 and 4 had no diagnosed clinical infection.



Shotgun metagenomic sequencing (N=17patients; 10 uninfected and 7 infected) produced no definitive identification due to low patient numbers and insufficient microbial DNA mass.

However, Figure 7 shows <u>possible</u> microbes present based on the data obtained.

What is important to note is the possible presence of microbes in the pseudomonadota phylum as they produce alkyl quinolones which explains the physical ToF-SIMS evidence observed.

DISCUSSION

1) This is the start of a much larger study that needs to be performed. We can't reliably explain all data, but the presence of a pseudomonas non-auregenosa is undeniable, e.g Pseudomonas fluoroscens.[1,2]

2) The spine is a different clinical space than joints but is similar to trauma. It is hardware in direct contact with muscle, not in a joint space. While joint devices have contact to bone, the spine has several surrounding tissues that may be part of the explanation.

3) Given the direction our understanding of the microbial biome, our data may suggest the presence of the bacteria, but in a manner that does not fit current infection diagnoses. That would suggest that all patients are exposed, but not all patients have sequelae. For this reason, we must push further to understand the host tissue microbiome.

REFERENCES

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