Bacterial Reactions to Modified Biomaterial Surfaces

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INTRODUCTION: Infections are serious complications associated with osteosynthesis implants [1-3]. Prolonged hospitalisation with systemic antibiotic therapy, several revision procedures, possible amputation, or even death may occur with implant associated infections. *Staphylococcus aureus* and *S. epidermidis* are both associated with implant infections, and have increasing resistance to various antibiotics [4-5]. The need to minimize their initial adhesion is great. A possible solution may be to modify the implant surface topography and/or chemistry, or by using an antimicrobial or protein resistant coating. This study describes the visualization and quantification of *S. aureus* and *S. epidermidis* adhering to a variety of different treated/coated titanium surfaces, including polymer coatings impregnated with an antiseptic.

MATERIALS AND METHODS: To visualize *S. aureus* and *S. epidermidis* adherence on the different surfaces, the bacteria were cultured on the surfaces in brain heart infusion broth (BHI) for 1h at 37°C. For SEM study, samples were fixed with 2.5% glutaraldehyde in PIPES buffer for 5 min, post-stained with 1% OsO_4 in PIPES for 1h, dehydrated, critical point dried, and coated with Au/Pd, and visualized with an SEM. To quantify the amount of bacterial adherence on surfaces, bacteria were cultured as before, then stained with fluorescent redox dye, 5-cyano,2-ditolyl tetrazolium chloride (CTC) for 1h, and visualized with a Zeiss Axioplan 2 Epifluorescence microscope fitted with a Axiocam camera [6]. The density of live bacteria adhering to the surfaces were counted using KS400 software. On surfaces that autofluoresce, adherent bacteria were detached by sonication in Tween 80, then stained with a live/dead assay (Molecular Probes). The amount of bacteria present were counted using a Partec PAS flow cytometer. Statistical analysis was performed using a one-way ANOVA with Tukey test.

RESULTS: SEM showed *S. aureus* adhering to all standard metal osteosynthesis surfaces, and significantly less to the hydrophilic coatings, PLL-*g*-PEG and hyaluronic acid (Fig. 1). Fluorescence microscopy confirmed the SEM results (Fig. 2). Electropolishing the TAN surface also had a significant effect on *S. aureus* adhesion compared to the standard TAN surface. Coatings impregnated with an antiseptic also decreased bacterial adhesion (Fig 2), but the antiseptic was found to be cytotoxic to host cells.

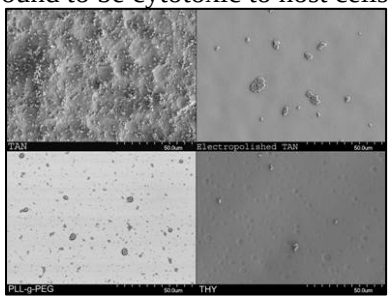
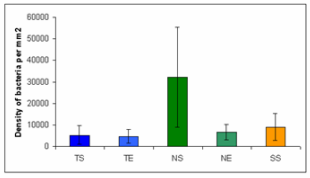
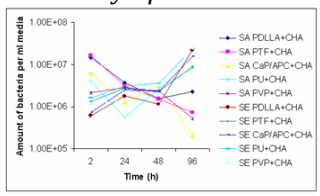


Figure 1: SEM images of S. aureus on different surfaces.

Figure 2: Graphs showing the density of S. aureus adhering to standard surfaces(left), and S. aureus (SA) and *S. epidermidis (SE) on different coated surfaces impregnated with antiseptic (right).*





DISCUSSION & CONCLUSIONS: *S. aureus* adhered to all standard biomaterials, although electropolishing TAN had a significant effect in decreasing adhesion compared to standard TAN. Hyaluronic acid & PLL-*g*-PEG coated titanium surfaces also significantly decreased the density of *S. aureus* & *S. epidermidis*, and hence have potential use as coatings for orthopaedic implants. The polymeric coatings impregnated with antiseptic studied have potential to be used as drug-delivery systems in association with implantable biomaterials, however using an antiseptic is not recommended due to host cell cytotoxicity.

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