

## Bacterial adhesion to PLL-g-PEG modified surfaces

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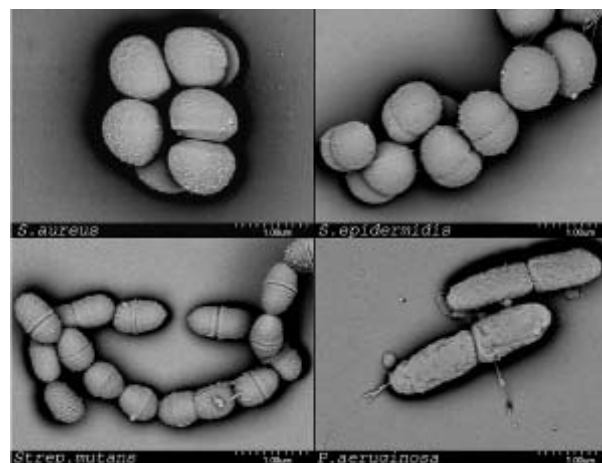
**INTRODUCTION:** Infections associated with implant are estimated to cost £7-11 million per year<sup>[1]</sup>. With the rise in antibiotic resistant bacteria this is an important issue<sup>[2]</sup>. Once adhered many bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* form biofilms on the implant surface. These can be difficult to clinically treat since the bacteria are protected from phagocytosis and antibiotics within the biofilm complex<sup>[3]</sup>, hence the need to prevent initial bacterial adhesion.

One approach to prevent bacterial adhesion is to coat the surface with a protein resistant coating, such as PLL-g-PEG<sup>[4]</sup>. PLL-g-PEG is known to inhibit both eukaryotic cells and *S. aureus* adhesion<sup>[5-6]</sup>. The approach can be modified by functionalising the coating with an adhesion moiety (specific peptide sequence) e.g. RGD (Arg-Gly-Asp), functionalised PLL-g-PEG coating, which also minimises protein and *S. aureus* adhesion<sup>[5-6]</sup> but allows eukaryotic cells to adhere<sup>[5]</sup>. This study describes the visualisation and quantification of various common bacteria to PLL-g-PEG (PEG) and PLL-g-PEG/RGD (PEG/RGD) and PLL-g-PEG/RDG (PEG/RDG) control coatings on titanium surfaces, to see if they recognise the RGD adhesion moiety present in ECM proteins, that eukaryotic cells adhere to via their integrin-receptor mechanism.

**METHODS:** To visualise the adhesion of the various bacteria (Fig 1), the bacteria were cultured on uncoated titanium (Ti), PEG and PEG/RGD coated Ti surfaces for 2h, 4h and 18h at 37°C in brain heart infusion broth. For scanning electron microscopy (SEM) imaging, samples were fixed with 2.5% glutaraldehyde in 0.1M PIPES buffer for 5 min at pH7.4, post-fixed with 1% OsO<sub>4</sub> in PIPES at pH6.8 for 1h, dehydrated in an ethanol series, critical point dried, coated with 10nm Au/Pd and visualised with a Hitachi S4100 SEM. To quantify the amount of living bacteria adhering to the different surfaces, bacteria were cultured as before, then stained with fluorescent redox dye, 5-cyano,2-ditoyl tetrazolium chloride (CTC) for 1h, and imaged with a Zeiss Axioplan2 Epifluorescence microscope fitted with an Axiocam camera<sup>[6]</sup>. The density of adhering live bacteria observed in each image, were counted using KS400 software. The experiment was

repeated three times and statistical analysis was performed using a one-way ANOVA with Tukey pair wise posthoc test.

Fig. 1: SEM images of the four different bacteria strains used in the study. Note their different shapes and cell wall topography.



wall topography.

**RESULTS:** SEM images showed all 4 bacteria strains adhering to the uncoated Ti surfaces and over time the amount adhering increased due to the colonisation of the surface by the bacteria. With all four bacteria, less bacteria were observed on the PEG, PEG-RGD and PEG-RDG coated surfaces (Fig. 2). The observations made with the SEM were confirmed by quantification of adherence using the fluorescence microscope (Fig. 3).

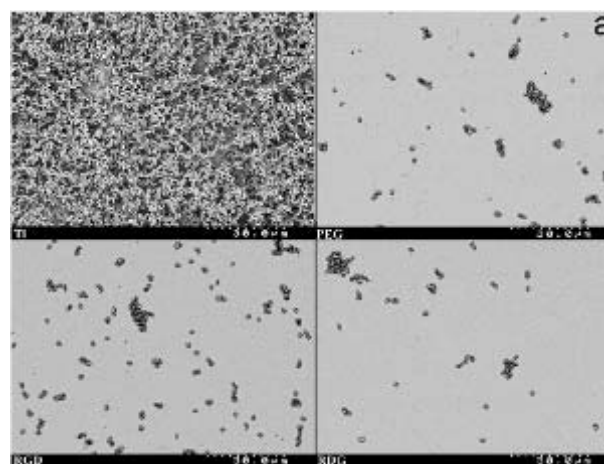


Fig. 2 SEM images of *S. aureus* on the different surfaces after 4h of culturing. There are less bacteria on the PEG, PEG-RGD and PEG-RDG coated surfaces than the uncoated Ti.

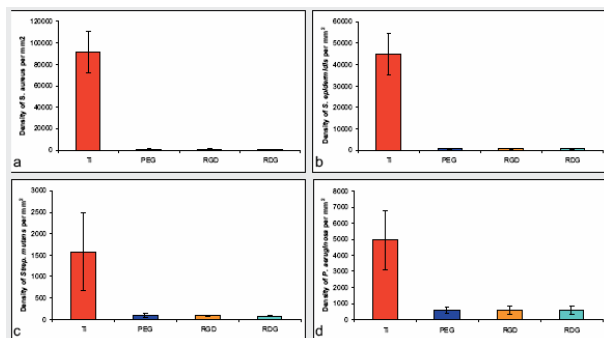


Fig.3: Graphs showing the effect of PLL-g-PEG coating on the density of a) *S. aureus*; b) *S. epidermidis*; c) *Strep. mutans*; and d) *P. aeruginosa* adhering to the different surfaces.

Statistical analysis showed that the difference between Ti and PEG coated surfaces as significant with all four bacteria strains ( $p > 0.05$ ), but not between the PEG, PEG-RGD and PEG-RDG coated surfaces ( $p > 0.05$ ).

**DISCUSSION & CONCLUSIONS:** PEG minimises non-specific protein adsorption, Eukaryotic cells do not attach to PEG or PEG/RDG (functionalised with a scrambled adhesion moiety), but do attach to PEG/RGD [5]. RDG (Arg-Asp-Gly) has no specific activity towards integrin receptors, therefore serves as a control for the presence/absence of specific interactions in bacteria-surface studies. The results from this study indicate that a PEG or PEG-RGD coating inhibits the adherence of *S. aureus*, *S. epidermidis*, *Strep. mutans* and *P. aeruginosa*. All four bacteria do not appear to recognise the eukaryotic cell adhesion moiety RGD.

Hence, PLL-g-PEG and PEGRGD coatings have potential as coatings on medical devices as they inhibit initial bacterial adhesion, and depending on the polymer used either encourage or discourage host cell adhesion.

**References:** <sup>1</sup>Flock JI (1999) Mol. Med. Today 5:532-537; <sup>2</sup>Lowy FD (1998) New Eng J Med 339:520-532; <sup>3</sup>Hoyle BD, Costerton JW (1991) Prog Drug Res 37:91-105; <sup>4</sup>Huang NP, Michel R, Voros J, Textor M, Hofer R, Rossi A, Elbert DL, Hubbell JA, Spencer ND (2001) Langmuir 17:489-498; <sup>5</sup>Tosatti S (2003) PhD thesis No. 15095 ETH Zurich; <sup>6</sup>Harris LG, Tosatti S, Wieland M, Textor M, Richards RG (2004) Biomaterials 25:4135-4148.

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