

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

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**INTRODUCTION:** Implant associated infections are estimated to cost £7-11 million per year [1], and with the rise in antibiotic resistant bacteria is an important issue [2]. Once adhered many bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *S. epidermidis* form biofilms on the biomaterial surface, which can be difficult to clinically treat since the bacteria are protected from phagocytosis and antibiotics [3], hence the need to prevent initial bacterial adhesion. One approach is to either coat the surface with a PLL-g-PEG coating, which is protein resistant [4] and is known to inhibit cell and *S. aureus* adhesion [5-6]; or to coat with an RGD functionalised PLL-g-PEG coating, which also minimises protein and *S. aureus* adhesion [5-6] but allows cells to adhere [5]. This study describes the visualisation and quantification of different bacteria to PLL-g-PEG (PEG) and PLL-g-PEG/RGD (PEG/RGD) coatings on titanium surfaces.

**METHODS:** To visualize the adhesion of the chosen bacteria (Table 1), the bacteria were cultured on uncoated titanium (Ti), PEG and PEG/RGD coated Ti surfaces for 2h, 4h and 18h at 37°C.

Bacteria	Code
<i>Staphylococcus aureus</i>	SA
<i>Staphylococcus epidermidis</i>	SE
<i>Streptococcus mutans</i>	SM
<i>Pseudomonas aeruginosa</i>	PA

Table 1. List of bacteria used in study.

For SEM study, samples were fixed with 2.5% glutaraldehyde in PIPES buffer for 5 min, post-stained with 1% OsO<sub>4</sub> in PIPES for 1h, dehydrated, critical point dried, and coated with Au/Pd, and visualized with an SEM. To quantify the amount of bacteria adhering to the different surfaces, bacteria were cultured as before, then stained with fluorescent redox dye, 5-cyano,2-ditolyl tetrazolium chloride (CTC)5 for 1h, and visualized with a Zeiss Axioplan2 Epifluorescence microscope fitted with an Axiocam camera [6]. The density of adhering live bacteria observed in each image, were counted using KS400 software. Statistical analysis was performed using a one-way ANOVA with Tukey test.

**RESULTS:** SEM images showed all 4 bacteria strains adhering to the uncoated Ti surfaces, whilst significantly less bacteria were observed on the PEG and PEG/RGD coated surfaces ( $p \leq 0.05$ ) (Fig. 1). This observation was confirmed by the quantification of adherence (Fig. 2).

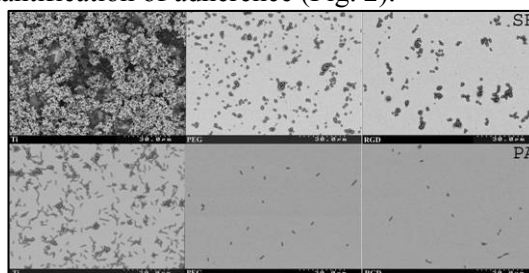
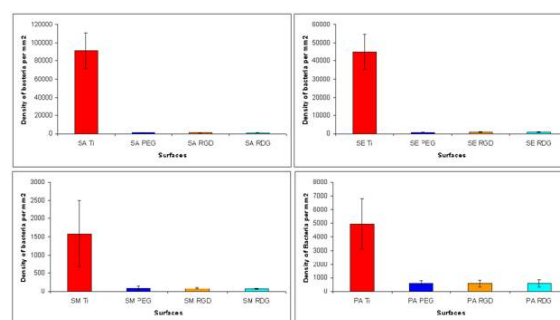


Fig. 1: SEM images of SE and PA on the different surfaces after 18h of culturing.

Fig. 2: Graphs showing the effect of PEG and



PEG/RGD coatings on SA, SE, SM and PA adhesion.

**DISCUSSION & CONCLUSIONS:** Coating a Ti surface with PEG or PEG/RGD coating, significantly decreased the adhesion of *S. aureus*, *S. epidermidis*, *Strep mutans*, and *P. aeruginosa* to the surfaces. It was also shown that all four bacteria do not to recognise the eukaryotic cell adhesion moiety RGD.

**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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