Inhibitory Role of a Polymer Brush-Coating in Bacterial Adhesion to Silicone Rubber

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Statement of Purpose: Rate of biomaterial associated infection (BAI) for the first time insertion of implant varies from 1 to 30 % with the mortality risk of up to 25% depending on the type of medical device. This great impact illustrates the necessity of developing coatings that prevent bacterial adhesion as the first step in a BAI.

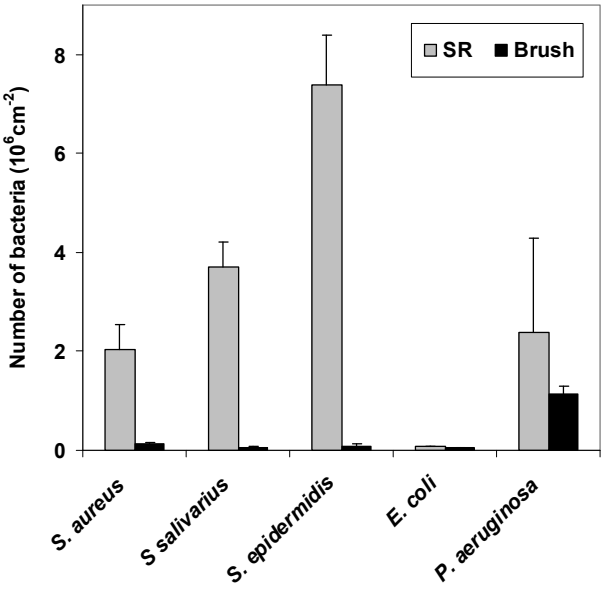
Polymer brush coatings are currently considered as the most promising non-fouling coatings, as these surfaces reduce the adhesion of bacteria by orders of magnitude. Physisorption of triblock copolymers of polyethylene oxide (PEO) and polypropylene oxide (PPO) with the structure of PEO_n-PPO_m-PEO_n at hydrophobic surfaces constitutes a brush coating that inhibits protein adsorption. Being easy to make and exhibiting a high level of biocompatibility, this system yields advantages for several applications, however, it is an open question whether such a brush coating can resist adhesion of various bacterial strains. In this study we investigated adhesion of five bacterial strains to pristine and polymer brush coated silicone rubber under different surface shear conditions.

Methods: Implant grade silicone rubber sheets (Medin, Groningen, The Netherlands) were rinsed thoroughly with a detergent, ethanol and demineralized water. They were fixed on the bottom plate of a parallel plate flow chamber (PPFC) and exposed to a solution of 0.5 g/l Pluronic F-127 ($PEO_{99}PPO_{65}PEO_{99}$, Sigma-Aldrich, USA) in phosphate buffered saline (PBS: 10 mM potassium phosphate, 150 mM NaCl, pH 6.8) for 20 min. Nonattached polymer was removed from the chamber by flow of an excess amount of PBS.

Five bacterial strains (*Staphylococcus epidermidis* HBH 276, *Staphylococcus aureus* ATCC 12600, *Streptococcus salivarius* GB 24/9, *Escherichia coli* O2K2 and *Pseudomonas aeruginosa* #3) were used in this study. All strains were first grown aerobically overnight at 37°C on blood agar plates. One colony was used to make a pre-culture in 10 ml tryptone soya broth (TSB, OXOID, Basingstoke, England) which was incubated at 37°C for 24 h and used to inoculate a second culture of 200 ml incubated for 16 h. The culture was harvested by centrifugation and washed twice with demineralized water. To break up bacterial aggregates, bacteria were sonicated intermittently while cooling in an ice/water bath. Finally, bacteria were suspended in 200 ml of PBS to a concentration of 3×10^8 per ml for all experiments.

The PPFC allows flow of a bacterial suspension with desired flow rate. Microscopy images were taken from pristine silicone rubber and silicone rubber coated with a PEO brush, affixed to the bottom plate of the chamber. In our protocol the flow was started applying a wall shear rate of 300 s^{-1} for 1 h, and then the shear rate was decreased to 200, 100 and 10 s^{-1}, flowing for 1 h at each shear rate. All experiments were done in triplicate.

Results: Adhesion of all five strains was considerably reduced by coating the silicone rubber with a PEO brush coating (see Figure). This reduction exceeded 95% for two staphylococci and the streptococcus strains. Given the fact that two third of BAIs are caused by staphylococci the presented method can have a great influence on infection rate. Adhesion of Pseudomonas was only 40% reduced which is in line with other findings that this strain has the ability to adhere to a brush coating.



Number of adhering bacteria to pristine (**SR**) and brush coated (**Brush**) silicone rubber after flow of bacteria for 4h.

Rate of adhesion seems to be higher at lower shear rates and for every shear it decreases in time due to saturation of the surface. For the motile Pseudomonas however shear has less effect on the rate of adhesion.

The physisorbed brush coating maintains its non-adhesive effects during the experiment indicating that the adsorbed layer is stable against the relatively high shear rate of 300 s^{-1} and for four hours.

These in vitro results perfectly support our recent finding that these coatings reduce the risk of BAI in a revision surgery in mice by inhibiting adhesion of bacteria to implanted silicone rubber disks.

Conclusions: Adhesion of infectious bacteria to silicone rubber is significantly suppressed by a physisorbed PEO brush coating. Being stable against shear stress, this coating can potentially be used to reduce the risk of BAI in a variety of applications.

References:

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