Polymer brush-coatings to prevent biomaterials associated infection

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Bacterial colonization of polymer brush-coated and pristine silicone rubber implanted in infected pockets in mice

Abstract

Curing biomaterial-associated infection (BAI) frequently includes antibiotic treatment, implant removal and re-implantation. However, revision implants are at a greater risk of infection as they may attract bacteria from their infected surrounding. Polymer brush-coatings attract low numbers of bacteria, but the virtue of polymer brush-coatings in vivo has seldom been investigated. Here we determine the possible benefits of polymer brush-coated versus pristine silicone rubber in revision surgery, using a murine model. BAI was induced in 26 mice by subcutaneous implantation of silicone rubber disks with a biofilm of Staphylococcus aureus Xen29. During development of BAI, half of the mice received treatment of rifampicin/vancomycin. After 5 days, the infected disks were removed from all mice, and either a polymer brush-coated or pristine silicone rubber disk was re-implanted. Revision disks were explanted after 5 days and the number of colony-forming units (cfu’s) cultured from the disks and surrounding tissue was determined. None of the polymer brush-coated disks after antibiotic treatment appeared colonized by staphylococci, whereas 83% of the pristine silicone rubber disks were re-infected. Polymer brush-coated disks also showed reduced colonization rates in the absence of antibiotic treatment as compared with pristine silicone rubber disks. Tissue surrounding the disks was culture positive in all cases. We conclude that Polymer brush-coatings are less prone to re-infection than pristine silicone rubber when used in revision surgery, i.e. when implanted in a subcutaneous pocket infected by a staphylococcal BAI. Antibiotic pre-treatment during the development of BAI hardly had any effect in preventing colonization of pristine silicone rubber.
Introduction

Along with the rapid expansion in use of biomaterial implants for restoration of function after trauma, oncological surgery or wear, the number of patients suffering from biomaterials-associated infection (BAI) increases continuously [1]. BAI results from the colonization of a biomaterials implant surface by bacteria excreting slime and the subsequent formation of an antibiotic resistant complex, called “biofilm”. BAI is often initially treated with antibiotics, but most frequently with little success, and ultimately revision surgery is needed in which the primary infected implant is removed and a revision implant is inserted [2-4]. The risk of infection for the revision implant however, is several-folds higher than for the primary implant [1], due to bacterial persistence in surrounding tissue, even after antibiotic treatment [5].

Polymer brush-coatings are generally considered to attract very few bacteria and may therefore be extremely useful for application in revision surgery after BAI. Physisorbed polyethylene oxide (PEO) brush-coatings can reduce the number of adhering bacteria by one to two orders of magnitude while, moreover, bacteria adhere weakly to polymer brush-coatings [6]. Physisorbed PEO brush-coatings can be applied on different hydrophobic biomaterials, including silicone rubber, and are stable under flow induced shear stresses [6] and in physiological fluids [7].

In this study, the effectiveness of polymer brush-coated silicone rubber in preventing infection after BAI were compared with the effectiveness of pristine silicone rubber in revision surgery, using a murine model. Prevention of BAI was evaluated for polymer brush-coated and pristine silicone rubber in the absence and presence of antibiotic treatment prior to revision surgery.


Chapter 6

Materials and Methods

All materials were of analytical grade and purchased from Merck, Darmstadt, Germany unless otherwise stated. Implant-grade silicone rubber sheets (thickness 0.5 mm, Medin, Groningen, The Netherlands) were cut into circular disks with radius of 4 mm. Disks were rinsed with ethanol and demineralised water and subsequently sonicated for 3 min in 2% RBS 35 detergent (Omnilabo International BV, Breda, The Netherlands) and rinsed thoroughly with demineralised water again, placed in 70% ethanol for 5 min and rinsed with sterile demineralised water. In order to apply a polymer brush-coating, cleaned silicone rubber disks were exposed to a sterile solution of 0.5 g/L Pluronic F-127 (Sigma-Aldrich, St. Louis, MO, USA) in phosphate buffered saline (10 mM potassium phosphate, 150 mM NaCl, pH 6.8). The disks were kept in the solution overnight prior to the implantation.

*Staphylococcus aureus* Xen29 [8] was first grown aerobically overnight at 37°C on a blood agar plate from a frozen stock. Several colonies were used to make pre-cultures in 10 mL tryptone soya broth (OXOID, Basingstoke, England) which were incubated at 37°C for 24 h. Pristine silicone rubber disks were incubated, for 72 h at 37°C on a rotary shaker at 60 rpm with 10 mL TSB enriched with 4% NaCl, inoculated with 100 μL of the pre-culture.

In order to induce BAI in mice, colonized silicone rubber disks were implanted in subcutaneous pockets, prepared in the left flanks of female Balb/c OlaHsd mice (Harlan Netherlands BV, Horst, The Netherlands), as approved by the Animals Experiments Committee at the University Medical Center Groningen. Prior to implantation, the left flanks of the mice were shaved and cleaned with 70% ethanol. Anaesthesia was induced with 3.5% Isoflurane/O₂ gas mixture (Zeneca, Zoetermeer, The Netherlands) and maintained at 1.5% during the implantation procedure. Buprenorfine (0.03 mg/kg) was administered subcutaneously 30 min before surgery as an analgesic. The disks were left *in situ* for 5 days, during which half of the mice...
received daily intra-peritoneal injections of 0.5 mL antibiotic solution of 2 mg/mL vancomycin (Abbott BV, Hoofddorp, The Netherlands) and 1 mg/mL rifampicin (Rifadin, Aventis, Hoevelaken, The Netherlands) in 0.9% NaCl while the other half received injections of 0.5 mL 0.9% NaCl solution [9]. After 5 days, the infected disks were removed under similar conditions as described above and either a sterile pristine or polymer brush-coated silicone rubber disk was implanted. After 5 days, animals were sacrificed, all disks were explanted and transferred to the laboratory in Eppendorf tubes containing reduced transport fluid (NaCl 0.9 g/L, (NH₄)₂SO₄ 0.9 g/L, KH₂PO₄ 0.45 g/L, MgSO₄ 0.19g/L, K₂HPO₄ 0.45 g/L, Na₂EDTA 0.37 g/L, L-cysteine HCl 0.2 g/L, pH 6.8). In addition, tissue samples were collected from the implant site and from a muscle away of the implant site for analysis of bacterial presence and antibiotic content.

Staphylococci adhering to the disks were detached into suspension by intermittent sonication for three times 10 s at 30 W (Vibra Cell model 375; Sonics and Materials, Danbury, CT, USA). 100 µL of this suspension was spread on a blood agar plate and the number of colonies was determined after incubation for 24 h at 37°C.

Bacterial presence in tissues surrounding an implanted disk were determined after homogenization by sonication, and subsequent culturing on blood agar plates and enumeration for cfu’s as described above. Tissue samples, including muscle homogenate, were further analyzed for the potential presence of bactericidal antibiotic concentrations, by putting 20 µL of homogenate on Mueller-Hinton agar plates (OXOID, Basingstoke, England) inoculated with fresh S. aureus Xen29. Growth inhibition was inspected by eye after incubating the plates for 24 h at 37°C.
**Results and Discussion**

This study is the first to address the colonization of a non-adhesive polymer brush-coating in an infected pocket *in vivo*, mimicking revision surgery after BAI.

None (0/7) of the polymer brush-coated silicone rubber disks implanted in the antibiotic treated group showed signs of colonization by *S. aureus*, whereas 83% (5/6) of pristine silicone rubber disks were culture positive (Fig. 1a). Similarly, in the non-antibiotic treated group, bacteria were found on 43% (3/7) of the polymer brush-coated disks, while 83% (5/6) of the pristine silicone rubber disks were infected. Moreover, culture positive polymer brush-coated surfaces were generally colonized by fewer bacteria than pristine silicone rubber disks. Bacteria were found in all tissue samples surrounding disks, albeit that the average number of cfu’s in the antibiotic treated group was significantly lower than in the absence of antibiotic treatment (Fig. 1b). No significant difference was seen in bacterial persistence in tissues surrounding the pristine and the polymer brush-coated silicone rubber disks. None of the tissue homogenates caused any inhibition of bacterial growth on culture plates, indicating that all tissues were completely devoid of bactericidal levels of antibiotics. Tissue homogenate of a mouse sacrificed after antibiotic administration caused an inhibition zone with diameter of 14 mm on culture plates (positive control), while a negative control yielded zero inhibition zones.

The colonization rate of pristine silicone rubber disks after revision is high, which is in line with clinical studies reporting a high rate of infection in revision surgery after BAI of a primary implant [1]. The infection rate of primary penile prostheses with silicone rubber tubes, for instance, was only 0.5%, compared to 6.6% in patients undergoing revision surgery [10]. The colonization rate of pristine silicone rubber disks was independent of whether BAI was pre-treated with antibiotics or not. This illustrates the limitation of treating BAI with antibiotics and confirms clinical experiences that the fate of an infected implant is generally removal [2-4]. Broekhuizen *et al.* [9] also
suggested that in general the negative outcomes of revision surgery are due to bacterial persistence in tissues surrounding an infected implant. However, the current study shows that treatment of BAI with antibiotics prior to revision surgery does help to reduce infection rates in revision surgery in case of polymer brush-coatings. Likely, antibiotic treatment reduces the number of bacteria in adjacent tissue and the few bacteria remaining in the tissue are not able to colonize a newly inserted polymer brush-coated surface.

The reduced colonization rates of polymer brush-coated disks are most likely attributable to the non-adhesiveness of the coating and not due to differential effects of antibiotic remnants on pristine and polymer brush-coated silicone rubber, as none of the tissue homogenates contained bactericidal levels of antibiotics. However, it can not be completely ruled out that sub-bactericidal concentrations of antibiotics were more effective on staphylococci loosely adhering on polymer brush-coated disks than on bacteria more firmly adhering to pristine silicone rubber.

In summary, this is the first time that it has been demonstrated that polymer brush-coatings may assist in preventing infection of implant surfaces after revision surgery, by reduction of the number of bacteria adhering to a re-implanted biomaterial surface.
Fig. 1. Total number of colony-forming units (cfu) detected on pristine (SR) and polymer brush-coated (BRUSH) silicone rubber disks (a) explanted from mice as well as from surrounding tissue (b). Disks were originally implanted in pockets created by a BAI in animals that had or had not received antibiotic treatment prior to re-implantation.
References


