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

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Objectives: 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 surroundings. 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.

Methods: BAI was induced in 26 mice by subcutaneous implantation of silicone rubber discs with a biofilm of Staphylococcus aureus Xen29. During the development of BAI, half of the mice received rifampicin/vancomycin treatment. After 5 days, the infected discs were removed from all mice, and either a polymer brush-coated or pristine silicone rubber disc was re-implanted. Revision discs were explanted after 5 days, and the number of cfu cultured from the discs and the surrounding tissue was determined.

Results: None of the polymer brush-coated discs after antibiotic treatment appeared colonized by staphylococci, whereas 83% of the pristine silicone rubber discs were re-infected. Polymer brush-coated discs also showed reduced colonization rates in the absence of antibiotic treatment when compared with pristine silicone rubber discs. Tissue surrounding the discs was culture-positive in all cases.

Conclusions: 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 the colonization of pristine silicone rubber.

Keywords: implant-related infection, biomaterials, antifouling surfaces, polyethylene oxide, biofilm

Introduction

Along with the rapid expansion in the use of biomaterial implants for the restoration of function after trauma, oncological surgery or wear, the number of patients suffering from biomaterial-associated infection (BAI) increases continuously.1 BAI results from the colonization of a biomaterial 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 required 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 fold higher than for the primary implant,1 due to bacterial persistence in the 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 stresses6 and in physiological fluids.7

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In this study, the effectiveness of polymer brush-coated silicone rubber in preventing infection after BAI was compared with that of pristine silicone rubber in revision surgery, using a murine model. The 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.

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 discs, with a radius of 4 mm. Discs were rinsed with ethanol and demineralized water and subsequently sonicated for 3 min in 2% RBS 35 detergent (Omnilab International BV, Breda, The Netherlands) and rinsed thoroughly with demineralized water again, placed in 70% ethanol for 5 min and rinsed with sterile demineralized water. In order to apply a polymer brush-coating, cleaned silicone rubber discs were exposed to a sterile solution of 0.5 g/L Pluronic F-127 (Sigma-Aldrich, St Louis, MO, USA) in PBS (10 mM potassium phosphate, 150 mM NaCl, pH 6.8). The discs were kept in the solution overnight prior to the implantation.

Staphylococcus aureus Xen29 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 of tryptone soya broth (TSB) (Oxoid, Basingstoke, UK), which were incubated at 37°C for 24 h. Pristine silicone rubber discs were incubated for 72 h at 37°C on a rotary shaker at 60 rpm with 10 mL of TSB enriched with 4% NaCl and inoculated with 100 μL of the pre-culture.

In order to induce BAI in mice, colonized silicone rubber discs 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 a 3.5% isoflurane/O2 gas mixture (Zeneca, Zoetermeer, The Netherlands) that was maintained at 1.5% during the implantation procedure. Buprenorphine (0.03 mg/kg) was administered subcutaneously 30 min before surgery as an analgesic. The discs were left in situ for 5 days, during which half of the mice received daily intraperitoneal injections of 0.5 mL of an 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 of 0.9% NaCl solution. After 5 days, the infected discs were removed under similar conditions as described earlier, and either a sterile pristine or polymer brush-coated silicone rubber disc was implanted. After 5 days, animals were sacrificed and either a sterile pristine or polymer brush-coated silicone rubber discs were removed under similar conditions as described earlier, and tissue samples were collected from the implant site and tissue homogenates of a mouse sacrificed after antibiotic administration caused an inhibition zone with a diameter of 14 mm on culture plates (positive control), while a negative control yielded zero inhibition zones.

The colonization rate of pristine silicone rubber discs after revision is high, which is in line with the clinical studies reporting a high rate of infection in revision surgery after BAI of a primary implant. The infection rate of primary penile prostheses with silicone rubber tubes, for instance, was only 0.5%, compared with 6.6% in patients undergoing revision surgery. The colonization rate of pristine silicone rubber discs 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. The infection rate of primary penile prostheses with silicone rubber tubes, for instance, was only 0.5%, compared with 6.6% in patients undergoing revision surgery. The colonization rate of pristine silicone rubber discs 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. The infection rate of primary penile prostheses with silicone rubber tubes, for instance, was only 0.5%, compared with 6.6% in patients undergoing revision surgery. The colonization rate of pristine silicone rubber discs 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.

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

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Bacterial presence in tissues surrounding an implanted disc was determined after homogenization by sonication and subsequent culturing on blood agar plates and enumeration for cfu as described earlier. Tissue samples, including muscle homogenate, were further analysed for the potential presence of bactericidal antibiotic concentrations, by putting 20 μL of homogenate on Mueller–Hinton agar plates (Oxoid) inoculated with fresh S. aureus Xen29. Growth inhibition was inspected visually after incubating the plates for 24 h at 37°C.
antibiotics. However, it cannot be completely ruled out that subbactericidal concentrations of antibiotics were more effective on staphylococci loosely adhering to polymer brush-coated discs 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.

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Transparency declarations

None to declare.

References