Electric-Current–Induced Detachment of *Staphylococcus Epidermidis* Strains from Surgical Stainless Steel

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Abstract: Infection of percutaneous biomaterial implants, such as fixation frames used for the repair of complicated fractures in orthopedics, is a major complication that almost inevitably leads to replacement of the implant. As antibiotic therapy usually has little impact on biomaterial-associated infections, it is the aim of this article to examine whether implant-associated *Staphylococcus epidermidis* and *Staphylococcus aureus* strains could be stimulated to detach from a surgical stainless steel anode during application of an electric current. First, bacteria were allowed to adhere from a flowing suspension of physiological ionic strength in a parallel plate flow chamber to a stainless-steel surface, after which the suspension was replaced by a bacterium-free solution with a specified ionic strength (0.5–150-mM potassium phosphate). DC currents ranging from 15 to 125 μA were applied to induce bacterial detachment. Initial detachment decreased with increasing ionic strength at 100 μA. The percentage detachment achieved by application of an electric current after 2.5 h was highest (95%) in 1-mM potassium phosphate and decreased to 15% when the ionic strength exceeded 40 mM. The electric current did not significantly affect the percentage detachment, but initial detachment rates increased with increasing current from 1000 cm$^{-2}$ s$^{-1}$ at 15 μA to 7000 cm$^{-2}$ s$^{-1}$ at 125 μA. Although different isolates of *S. epidermidis* and *S. aureus* showed different patterns of current-induced detachment, all strains could be stimulated to detach. The results of this study define ionic-strength conditions and electric currents yielding staphylococcal detachment from surgical stainless steel and therewith point to a pathway for the treatment and prevention of percutaneous metal-implant infection. © 2003 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 68B: 160–164, 2004

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INTRODUCTION

Biomaterial implants in the human body constitute passive surfaces that are prone to bacterial adhesion as the onset of a biomaterials-associated infection. Antibiotic treatment of infected biomaterial implants is rarely successful clinically, because the biofilm mode of growth of the infecting bacteria protects the adhering organisms. Minimal inhibitory concentrations of antibiotic 50–500 times higher than required to extinguish planktonic bacteria have been reported for bacteria in a biofilm. Consequently, these infections usually persist until the biomaterials implant is removed. Percutaneous implants, such as bone screws used in orthopedics or dental implants, constitute a special class of implants with infection rates exceeding the infection rate of deep-body implants by far. Especially, fixation frames, used in orthopaedic surgery for the treatment of complicated fractures, inevitably become infected during the course of a treatment, which can take up to 15 months. Review of the literature indicates that the incidence of pin-tract infections ranges from 12 to 50%, mostly due to *Staphylococcus epidermidis*, a nonpathogenic skin saprophyte that becomes a persistent pathogen in the presence of percutaneous biomaterial implants.

Bacteria interact with surfaces through attractive Lifshitz–Van der Waals forces, acid–base interactions, and electrostatic forces, as outlined in the DLVO (Derjaguin, Landau, Verwey, and Overbeek) theory. The electrostatic interactions are generally repulsive, because most natural surfaces are negatively charged. However, the electrostatic interactions can be manipulated relatively easily by changing the surface polarity, the ionic-strength conditions, or by the application of an electric voltage and current. Recently, Poortinga et al. have demonstrated that it is possible to stimulate bacterial detachment from conducting indium tin...
oxide (ITO) -coated glass in low-ionic-strength solutions by applying electric currents of 10 μA cm⁻². Both anodic and cathodic surfaces, with bacteria adhering in a density of around 10⁷ cm⁻² could be almost totally cleaned, even in the presence of an adsorbed conditioning film. In addition, bacterialicidal effects have been shown after application of a 10μA DC current for 16 h to bacteria on human skin or on agar plates.¹⁴,¹⁵

The aim of this article is to determine the influence of a DC current, voltage, and ionic strength on current-induced detachment of different Staphylococcus epidermidis and Staphylococcus aureus strains from surgical stainless steel, as a potential means to prevent or cure biofilm infection of percutaneous fixation frames as used in orthopaedic surgery.

**MATERIALS AND METHODS**

**Bacterial Strains**

Experiments were conducted with 10 staphylococcal isolates, comprising Staphylococcus epidermidis HBH₂ 277, ATCC 35984, ATCC12228, 242, 298, 3399, 169, HBH 276, Staphylococcus aureus ATCC12800, and a freshly isolated clinical Staphylococcus aureus IL01. Strains were cultured in Trypton Soya Broth at 37 °C in ambient air. For each experiment, a strain was inoculated from blood agar in a batch culture and grown for 24 h. This culture was used to inoculate a second strain was inoculated from blood agar in a batch culture and grown for 24 h. This culture was used to inoculate another culture that was grown for 16 h prior to harvesting. Bacteria were harvested by centrifugation (5 min at 6480 g at 10 °C), washed twice with demineralized water, and suspended to a concentration of 3 × 10⁸ ml⁻¹ in 150-mM potassium phosphate-buffered saline. Before suspension, bacteria were sonicated at 30 W for 10 s while cooling on an ice/water bath, to obtain single cells.

**Stainless Steel**

AISI 316 LVM stainless steel (Stryker Corp, Kiel, Germany) was ground down to grit number 1200, and subsequently polished with a 6- and 3-μm diamond water-based suspension (Metadi 3- and 6-μm diamond suspension and Trident polishing cloth, Buehler, Lake Bluff) for 3 and 1.5 min, respectively. Grinding and polishing were done with the use of a polishing machine with a 30-N load and with oppositely rotating axes (Phoenix Beta and Vector grinder/polisher, Buehler, Lake Bluff). The polished steel was cleaned by 5-min sonication in 2% alkaline cleaning agent followed by thorough rinsing with tap water, sonication in ethanol, and rinsing in Millipore filtered deionized water. After cleaning, the steel was passivated according to ASTM F86-91 and thoroughly rinsed with Millipore filtered deionized water and dried in an oven at 80 °C, prior to use as an electrode surface.

**Parallel Plate Flow Chamber and Detachment Experiments**

Bacterial adhesion and subsequent detachment was studied in a parallel plate flow chamber with a distance of 0.6 mm between the top and the bottom plates.¹⁶ The bottom plate consisted of the surgical stainless-steel electrode (area 21 cm²), whereas the top plate, employed as a counter electrode, was made of an indium–tin oxide (ITO), DC-sputtered (Philips Natlab, Eindhoven, The Netherlands) glass plate. The ITO-coated glass plates were cleaned in the same way as the stainless steel, and an electrical wire was glued to the surface with silver dag (Electrodag 1415, Acheson, Port Huron).

Adhering bacteria were observed with a CCD-MXR camera mounted on a metallurgical microscope equipped with a 40 × ultra-long working distance objective. All fluids used were circulated through the chamber under the influence of hydrostatic pressure at a flow rate of 0.021 ml/s (shear rate 10 s⁻¹), whereas fluids were recirculated with a peristaltic pump. First, buffer was flowed through the chamber for 20 min followed by the bacterial suspension until 1.0 × 10⁷ cm⁻² bacteria were found adhering to the stainless-steel bottom plate, which took approximately 1.5 h under the experimental conditions used. Then, the chamber was perfused for 40 min with a cell-free potassium phosphate buffer (pH 7.0) with an ionic strength ranging between 0.5 and 150 mM (depending on the experiment) to remove planktonic bacteria from the system. Subsequently, an electric voltage (1.5–1.7 V) was applied between the two electrodes, with the stainless-steel bottom plate used as a cathode, yielding electric currents between 15 and 125 μA. The electric current was kept constant for 2.5 h with the aid of a LM344Z (National Semiconductor Corp, Silicon Valley) and was monitored continuously during the experiments with conventional electric multimeters. The limited space within the parallel plate flow chamber did not allow the use of a reference electrode.

During an experiment, images were grabbed from bacteria adhering to the stainless steel bottom plate and stored in the computer to obtain the number of bacteria adhering per unit area versus the time during application of an electric current. All experiments were done in triplicate at room temperature.

**RESULTS**

Figure 1 illustrates the number of adhering S. epidermidis HBH 276 during the course of an experiment for two different ionic strengths (2.5 and 100-mM potassium phosphate). As can be seen, bacterial detachment is absent during flow with a cell-free suspension, whereas detachment commences at the onset of the electric current. Note that detachment is negligible at an ionic strength of 100 mM. The time dependence of the detachment process allows the so-called initial detachment rates (jₜₐₐt₀ = dn/dt [cm⁻² s⁻¹]) and total detachment percentages (1 – N_end/N_begin) × 100% to be calculated, in which N_begin and N_end are number of bacteria adhering prior to and after the application of an electric current, respectively. The initial detachment rates and the total detachment percentages for S. epidermidis HBH 276 are given in Figure 2 as a function of ionic strength. Initial detachment rates [Figure 2(a)] decrease strongly with increasing ionic strength until an ionic strength of about 20 mM, beyond
which the initial detachment rates remain more or less constant. Similarly, a decrease in total detachment percentage with ionic strength is observed [Figure 2(b)], although the total detachment percentage levels off at a higher ionic strength of about 40 mM. Note, however, that the total detachment percentage is kinetically controlled in the current experiments and determined by the point in time at which the electric current was switched off.

Figure 3 illustrates the number of adhering *S. epidermidis* HBH 276 in a 10-mM potassium-phosphate buffer for different electric currents. The initial detachment rates increase with increasing current [Figure 4(a)], whereas the total detachment percentages after 2.5 h [Figure 4(b)] are virtually independent of the electric current applied. Table I compiles the initial detachment rates and total detachment percentages for the eight different *S. epidermidis* and the two *S. aureus* strains involved in this study, as established by the application of a 100-μA electric current in a 10-mM potassium-phosphate buffer. There is no correlation between the total detachment percentage and the initial detachment rates, and this is true for both the eight different *S. epidermidis* and the two *S. aureus* strains. The total detachment percentages vary less between the different strains (factor three) and are 64% on average. There is no correlation between the total detachment percentage and the initial detachment rates (correlation coefficient 0.38, p > 0.1).

**DISCUSSION**

In this article, it was demonstrated that a variety of different staphyloccocal strains, isolated from biomaterials-associated infections, could be stimulated to detach from surgical stainless steel by the application of an electric current. Previously,
Poortinga et al. demonstrated that it was possible to stimulate detachment of *Streptococcus oralis* from ITO surfaces with the use of electric currents and forwarded a detachment mechanism based on an ionic-strength-dependent transfer of electrons during initial bacterial adhesion that has to be reversed in order for detachment to occur. The idea of charge transfer during bacterial adhesion to conducting surfaces was new, and the necessity to reverse the charge transfer in order for detachment from metallic surfaces to occur shows why the detachment rate increases with increasing electric current (see Figure 4(a)). At the same time, the applied potential remains influential on the detachment process, because the detachment force is proportional with voltage and needs to overcome attractive Lifshitz–Van der Waals and acid–base forces between the adhering bacteria and the surface. In the present experiments, the applied potential varies little compared with the resulting variation in electric currents, which results in a constant detachment percentage throughout the electric current series. Note that, in the absence of any current, detachment does not occur, from which it can be inferred that there is a stepwise increase from zero to the average detachment value measured at a certain threshold current between 0 and 15 μA (unpublished control experiment).

The ionic-strength dependence of the bacterial detachment observed is caused by the fact that the electric double layer is very thin for high ionic strengths, yielding close contact between adhering bacteria and the stainless-steel surface, and, as a consequence, greater attractive forces. In addition, higher ionic strengths yield a higher conductivity of the fluid and hence a lower voltage is required to establish a certain electric current, that is, a lower attracting force. Alternatively, this lower voltage causes a decrease in the detachment-force operative and a lower detachment. Current-induced detachment of adhering bacteria from stainless-steel surfaces as established here, not only involves detachment, but also the prevention of re-deposition of detached bacteria. Under high flow conditions, detached bacteria are more readily transported away from the surface than under low flow conditions, so re-deposition is unlikely. This was demonstrated in a separate experiment, during which two different flow rates were applied during the detachment phase. Figure 5 shows that there is no re-deposition of *S. epidermidis* HBH 276 after detachment from a stainless-steel surface by a current of

**Figure 4.** Electric-current-induced detachment in a 10-mM potassium-phosphate buffer of *Staphylococcus epidermidis* HBH 276 as a function of the electric current applied. (a) Initial detachment rate ($j_{det,0}$ cm$^{-2}$ s$^{-1}$), (b) total detachment percentage $(1 - N_{end}/N_{begin}) \times 100\%$. Error bars denote the standard error over three experiments with separately cultured bacteria.

**Figure 5.** Example of the time series involved in the adhesion and electric-current-induced detachment of *Staphylococcus epidermidis* HBH 276 for two different flow rates (0.021 ml s$^{-1}$, indicated by triangles and 0.108 ml s$^{-1}$, indicated by circles) for a 100-μA DC current in a 10-mM potassium-phosphate buffer.

**TABLE I.** Initial Detachment rates $j_{det,0}$ and Total Detachment Percentages $(1 - N_{end}/N_{begin}) \times 100\%$ of Staphylococci from a Stainless-Steel Cathode during Application a 100-μA Electric Current in a 10-mM Potassium-Phosphate Buffer. Results with Standard Error Indicated by ± Sign Have Been Conducted in Triplicate with Separately Cultured Organisms

<table>
<thead>
<tr>
<th>Strain</th>
<th>$j_{det,0}$ (cm$^{-2}$ s$^{-1}$)</th>
<th>Total Detachment Percentage (%)</th>
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<tr>
<td><em>S. epidermidis</em> HBH 277</td>
<td>13776 ± 4266</td>
<td>78 ± 10</td>
</tr>
<tr>
<td><em>S. epidermidis</em> 3399</td>
<td>9317</td>
<td>59</td>
</tr>
<tr>
<td><em>S. epidermidis</em> ATCC 35984</td>
<td>9176 ± 850</td>
<td>54 ± 31</td>
</tr>
<tr>
<td><em>S. epidermidis</em> 298</td>
<td>8490 ± 2576</td>
<td>72 ± 20</td>
</tr>
<tr>
<td><em>S. epidermidis</em> HB 276</td>
<td>3746 ± 2960</td>
<td>54 ± 21</td>
</tr>
<tr>
<td><em>S. epidermidis</em> 242</td>
<td>2075</td>
<td>73</td>
</tr>
<tr>
<td><em>S. epidermidis</em> ATCC 12228</td>
<td>1234</td>
<td>83</td>
</tr>
<tr>
<td><em>S. epidermidis</em> 169</td>
<td>1051</td>
<td>53</td>
</tr>
<tr>
<td><em>S. aureus</em> IL01</td>
<td>9876</td>
<td>54</td>
</tr>
<tr>
<td><em>S. aureus</em> ATCC12800</td>
<td>955</td>
<td>30</td>
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100 μA in 10-mM potassium-phosphate buffer when the flow rate is 0.108 ml s⁻¹. However, the flow rates employed throughout this article are about fivefold lower, that is, 0.021 ml s⁻¹, and although initial detachment is as high as for the higher flow rate, re-deposition is clearly present within 800 s and yields a new equilibrium situation. Note that this equilibrium is established at a lower level than attained in the absence of an electric current. Evidently, under the mass transport conditions in the parallel plate flow chamber, re-deposition during current application occurs as a result of high bacterial concentrations near the surface, which must be transported away for effective and lasting cleansing of the surface. Summarizing, electric-current-induced detachment of staphylococcal strains from surgical stainless steel is effective at low ionic strengths, requiring currents of only 25–125 μA. Therewith the results of this study suggest that regular application of an electric current to percutaneous metal implants, such as orthopaedic fixation frames, may be a feasible alternative to antibiotic treatment for the prevention of infection.

**REFERENCES**