Electric block current induced detachment from surgical stainless steel and decreased viability of *Staphylococcus epidermidis*

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Abstract

In vitro studies investigating the influence of electric DC current on bacterial detachment have demonstrated that continuous currents of only 25–125 μA stimulated staphylococcal strains to detach from surgical stainless steel. However, DC currents produce more power that has to be dissipated by the skin as compared to alternating currents. Also, an excess of ions on the steel can cause negative osteogenesis and fixation results. Therefore, it is the aim of this paper to examine whether detachment of *Staphylococcus epidermidis* from stainless steel surfaces in a parallel plate flow chamber can also be stimulated using electric block currents. Block currents of 15, 60 and 100 μA cause detachment of about 76% of adhering staphylococci from stainless steel, whereas in addition the remaining bacteria are less viable, as determined by culturing the remaining bacteria on agar plates. Therewith, block current-induced detachment of adhering bacteria from stainless steel appears to be an equally promising method to prevent infection of orthopaedic fixation pins and screws than application of DC currents.

Keywords: Electric block current; Detachment; Stainless steel; Bactericidal; *Staphylococcus epidermidis*; Percutaneous implant; Biofilm

1. Introduction

Biomaterials are widely used in surgical medicine for the restoration of function. However, implantation of a biomaterial creates an immuno-incompetent fibro-inflammatory zone, within which bacteria are likely to find a protective environment and ultimately cause a biomaterials-associated infection [1]. Even non-pathogenic saprophytes can become persistent pathogens in the presence of biomaterial implants. Since the biofilm mode of growth offers a protective environment, these infections are hard to treat with antibiotics. Mature biofilms require 500–5000 times higher antibiotic concentrations to achieve an effective kill than required for planktonic bacteria [2–5]. Consequently, a biomaterials-associated infection inevitably leads to the removal of an implant.

Percutaneous devices are especially prone to infection and when fixation pins or frames become infected, osteomyelitis may occur with damaging effects on the bone and surrounding tissue [6]. Review of the literature indicates that the incidence of pin-tract infections ranges from 2% to 50% [7], mostly due to staphylococcal strains [8,9]. Multiple techniques are being investigated to cure these infections, such as antibiotics, irrigation with various solutions [10], including air bubbles [11] and silver coating of pins [12,13].

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 [14]. Generally natural surfaces are negatively charged and therefore the electrostatic interactions are repulsive.
It is possible, however, to manipulate these electrostatic interactions by changing the surface polarity [16], the ionic strength conditions or by the application of an electric current [17,18]. Previously, we demonstrated that the percentage detachment of staphylococci from surfaces achieved by application of a 100 μA DC electric current after 150 min was highest (95%) in 1 mM potassium phosphate and decreased to 15% when the ionic strength exceeded 40 mM. Furthermore, different isolates of Staphylococcus epidermidis and Staphylococcus aureus could be stimulated to detach, although strains showed different patterns of current-induced detachment. The magnitude of the current only had an influence on the detachment rate, and not on the final detachment percentages achieved, which amounted to 64% on average [19]. In addition, others have shown bactericidal effects after application of a 10 μA DC current for 16 h to bacteria on human skin or on agar plates [20,21].

So far, however, only DC currents have been used for stimulating bacterial detachment or killing. A disadvantage of DC current over alternating current is the excess of ions on the steel surface, which can lead to negative osteogenesis and fixation results [22]. Furthermore, DC current consumes more power than block currents of the same magnitude, because of their continuous character. The produced power has to be dissipated by the environment, and is more likely to cause tissue damage in case of DC currents than in case of block currents. Little is known about the influence of alternating and block currents on detachment and killing of bacteria. The application of a block current will result in alternating ion movement and electro-osmotic fluid flow, which may lead to extra forces on the adhered bacteria.

The aim of this paper is to determine whether it is possible to stimulate bacterial detachment from surgical stainless steel using block currents with 5–50% duty cycle and frequencies between 0.1 and 2 Hz. Furthermore, it will be determined whether these currents yield enhanced killing of bacteria that remain adhering after current application.

2. Materials and methods

2.1. Bacterial strains

Experiments were conducted with S. epidermidis HBH276. This strain was previously shown to detach from stainless steel using DC currents. The strain was cultured in Trypton Soya Broth (OXOID, Basingstoke, UK) at 37 °C in ambient air. For each experiment, the strain was inoculated from blood agar in a batch culture and grown for 24 h. This culture was used to inoculate a second culture that was grown for 16 h prior to harvesting. Bacteria were harvested by centrifugation (5 min at 6000g at 10 °C), washed twice with demineralised water and suspended to a concentration of 3 × 10⁸/ml in phosphate-buffered saline (PBS, 10 mM potassium phosphate and 0.15 M NaCl at pH 7). Before suspending, bacteria were sonicated at 30 W for 10 s while cooling on an ice/water bath, to obtain single cells.

2.2. 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, USA) for 3 and 1.5 min, respectively. Grinding and polishing were done using a polishing machine with a 30 N load and with oppositely rotating axes (Phoenix Beta and Vector grinder/poisher, Buehler, Lake Bluff, USA). The polished steel was cleaned by 5 min sonication in 2% alkaline cleaning agent (RBS 35 in water, Omniclean) followed by thorough rinsing with tap water, sonication in ethanol and rinsing in Millipore filtered demineralised water. After cleaning, the steel was passivated according to ASTM F86-91 and thoroughly rinsed with Millipore filtered demineralised water and dried in an oven at 80 °C, prior to use as an electrode surface.

2.3. 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 plate [23]. The bottom plate consisted of the surgical stainless steel electrode (area 21 cm²), while the top plate, employed as a counter electrode, was made of an indium tin oxide (ITO), DC sputtered (Philips Natlab, Eindhoven, The Netherlands) glass. 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, USA).

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), while fluids were recirculated using a peristaltic pump. First, PBS was flowed through the chamber for 20 min followed by the bacterial suspension until 1.0 × 10⁷/cm² bacteria were found adhering to the stainless steel bottom plate, which took approximately 90 min under the experimental conditions used. Then, the chamber was perfused for 40 min with a cell-free 10 mM potassium phosphate buffer (pH 7.0) to remove planktonic bacteria from the system. Subsequently, an electric block voltage (1.5–1.7 V), varying both in frequency and duty cycle, was applied between the two electrodes, using the stainless steel bottom plate as a cathode and yielding electric currents of 15, 60 and 100 μA. The electric current was kept constant for 150 min with the aid of an LM344Z (National Semiconductor Corp, Silicon Valley, USA) and was monitored continuously during the experiments with conventional electric multimeters. The LM344Z output potential is adapted continuously to meet the required and adjusted current irrespective of the applied voltage to the
The limited space in 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 vs. the time during application of an electric current. All experiments were done in triplicate with separately cultured bacteria and freshly prepared bottom and top plates at room temperature.

2.4. Viability testing

In order to determine the number of viable staphylococci that remained adhering after current application, two parallel plate flow chambers were simultaneously operated with a bacterial suspension taken from the same culture. One flow chamber was used as a control in the absence of an electric current, while the second flow chamber was connected to the current source. Subsequently, experiments were carried out as described above. During the time of current application, the control flow chamber was perfused with 10 mM potassium phosphate buffer, although no current was applied.

After the experiment, both chambers were disconnected and the stainless steel surfaces were taken out. Bacteria were swabbed from each surface and suspended in demineralised water. The density of the suspensions obtained from both plates were determined using a counting chamber after which a dilution series was made on blood agar and grown for 24 h at 37°C in ambient air. After incubation the amount of CFUs were compared per plate, taking into account the total number of bacteria present on each surface.

3. Results

Fig. 1 illustrates the number of adhering S. epidermidis HBH276 during the course of three experiments in which either the duty cycle or frequency was varied. As can be seen, bacterial detachment is absent during flow with a cell free suspension (5000–8000 s), while detachment commences at the onset of the electric current at 8000 s. Note that detachment is negligible at a duty cycle of 5% and that a similar end-point is reached for both frequencies with a duty cycle of 50%, albeit with different kinetics.

The time dependence of the detachment process allows one to calculate so-called initial detachment rates \( \dot{j}_{\text{det},0} = \frac{dn}{dt} (\text{cm}^{-2} \text{s}^{-1}) \) and total detachment percentages \((1-N_{\text{after}}/N_{\text{prior}}) \times 100\%\), in which \(N_{\text{prior}}\) and \(N_{\text{after}}\) are the 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 HBH276 are given in Figs. 2 and 3, respectively, as a function of frequency, duty cycle and current. Initial detachment rates for both 60 μA (Fig. 2A) and 100 μA (Fig. 2B) currents do not vary with frequency for 5% and 25% duty cycle, but for...
the 50% duty cycle a linear increase with increasing frequency is observed. Increases in duty cycle also yield an increase in detachment rate for both 60 and 100 mA, but detachment remains well below the level achieved by DC currents. Note, that a 5% duty cycle does not stimulate any detachment.

Total detachment percentages for both 60 (Fig. 3A) and 100 mA (Fig. 3B) currents are almost independent of frequency or duty cycle. Only a 0.1 Hz block current does not meet these findings and has a slightly lower detachment. The detachment achieved after 150 min for the 100 μA block currents (excluding 0.1 Hz and 5% duty cycles) amounts to 76% on average, whereas DC currents [19] between 100 and 125 μA only caused 64% detachment on average (p<0.05). Block currents of 15 μA however showed no detachment at all for all combinations of duty cycles and frequencies.

During the course of an experiment, the number of viable, adhering bacteria decreased to 25 000 CFUs/cm² in the absence of any current to less than 2500 CFUs/cm² in the presence of a 100 μA DC current, while a 100 μA 2 Hz block current with 50% duty cycle yielded around 400 CFUs/cm², representing a 60-fold decrease in the number of viable bacteria with respect to the control.

4. Discussion

In this paper, it was demonstrated that an electric block current can stimulate detachment of *S. epidermidis* HBH276 from surgical stainless steel, while in addition it yields enhanced killing of remaining bacteria. Previous results had already demonstrated that a variety of staphylococcal strains could be stimulated to detach from surgical stainless steel by the application of a direct electric current (DC). The detachment mechanism was based on an ionic strength-dependent transfer of electrons [18,24,25], which gave an understanding of the increased detachment rate with increasing current. This mechanism of charge transfer clearly explains the influence of current on detachment rates, and causes that block currents, with its imputed advantages, yield lower detachment rates (see Fig. 2A and B) due to the lower amount of electrons that are pumped through the system.

The potential for both DC and high block currents are more or less equal and therefore the detachment force needed to overcome the attractive interaction forces too. In the case of very low duty cycles and currents however, the potential is too low and therefore detachment is absent, for the time given to the electrode configuration is too short, compared to the time constant (>1 min) of the set-up, to reach sufficient potential values. Therefore, from this and previous results [19] we infer that there is a step-wise increase from zero to the average detachment percentage, but there is no possibility to give an estimate for each quantity, for both current and duty cycle influence the potential. Nevertheless, 100 μA block currents stimulate more detachment than their DC counterparts, which cannot be explained by the applied potential. The gain in detachment percentages by using block currents compared with DC currents can be explained by electroosmotic fluid flow directed to and from the surface [18]. The alternating field causes hydrated ions to move along the applied field dragging water with them. The creation of the so-called osmotic fluid flow gives an additional force that stimulates detachment.

The experiments done with the two parallel flow chambers show a bactericidal effect of the electric currents. Literature indicates that this bactericidal effect can be caused by the formation of small quantities of H₂O₂ at the cathode [21], in the presence of oxygen. The half-cell reaction \[ \text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2\text{O}_2 (+0.68 \text{ V}) \]
describes the electrolysis of hydrogen in combination with oxygen. However, the production of H₂O₂ cannot explain the extra efficiency of block currents because these currents produce per definition less H₂O₂. Consequently, there must be an additional mechanism operative at the cathode. Possibly, the rapid changes occurring during block electric currents result in high electric field gradients, disrupting the integrity of the bacterial membrane, therewith inducing the extra viability decrease [21].

5. Conclusions

A method has been described by which bacteria can be stimulated to detach from surgical stainless steel and the viability of the remaining bacteria can be decreased with a low amperage block current. This method seems to be promising in preventing or curing infection of orthopaedic percutaneous fixation pins and screws, which may lead to the widening of the inclusion criteria for patients to be eligible for percutaneous treatment.

References