Available online at www.sciencedirect.com
ScienceDirect
Biomaterials 28 (2007) 2122–2126
Biomaterials
www.elsevier.com/locate/biomaterials

Prevention of pin tract infection in external stainless steel fixator frames using electric current in a goat model

Arnout J. van der Borden[a], Patrick G.M. Maathuis[b], Eefje Engels[a], Gerhard Rakhorst[a], Henny C. van der Mei[a], Henk J. Busscher[a], Prashant Kumar Sharma[a,*]

[a]*Department of BioMedical Engineering, University Medical Center Groningen and University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands*

[b]*Orthopaedic Surgery, University Medical Center Groningen and University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands*

Received 2 November 2006; accepted 1 January 2007
Available online 4 January 2007

Abstract

Pin tract infections of external fixators used in orthopaedic reconstructive bone surgery are serious complications that can eventually lead to periostitis and osteomyelitis. In vitro experiments have demonstrated that bacteria adhering to stainless steel in a biofilm mode of growth detach under the influence of small electric currents, while remaining bacteria become less viable upon current application. Therefore, we have investigated whether a 100 μA electric current can prevent signs of clinical infection around percutaneous pins, implanted in the tibia of goats. Three pins were inserted into the lateral right tibia of nine goats, of which one served for additional frame support. Two pins were infected with a *Staphylococcus epidermidis* strain of which one pin was subjected to electric current, while the other pin was used as control. Pin sites were examined daily. The wound electrical resistance decreased with worsening of the infection from a dry condition to a purulent stage. After 21 days, animals were sacrificed and the pins taken out. Infection developed in 89% of the control pin sites, whereas only 11% of the pin sites in the current group showed infection. These results show that infection of percutaneous pin sites of external fixators in reconstructive bone surgery can be prevented by the application of a small DC electric current.

Keywords: Bacterial adhesion; Infection; Electric current; Bacterial detachment; Reconstructive bone surgery; Stainless steel

1. Introduction

Pin site infections of external fixators in reconstructive bone surgery frequently occur with an incidence up to 71% [1,2] and constitute a major concern for orthopaedic surgeons. Prevention of pin site infections is also an important nursing responsibility [3], but there is no consensus on how to perform optimal pin site care [4]. When a pin site becomes infected, it is usually difficult to treat due to the formation of biofilm around the metal surface. The biofilm mode of growth shields the bacteria from the host defence mechanism and antibiotics. Literature indicates that 500–5000 times higher levels of antibiotics are needed to achieve the same antimicrobial effects on biofilm bacteria than needed for planktonic bacteria [5–7].

The development of a biomaterials-associated infection starts with the adhesion of bacteria to the biomaterials surface, as mediated by attractive Lifshitz-Van der Waals forces, acid–base interactions and electrostatic forces [8]. Because all naturally occurring surfaces, including those of bacterial cells, are generally negatively charged, the electrostatic force between bacteria and a biomaterials surface is repulsive [9]. These repulsive forces can be enhanced by application of an electric current, therewith increasing the negative charge and consequently the repulsive force [10,11].

Recently, we demonstrated that it was possible to detach more than 60–76% of staphylococci adhering to surgical stainless steel surfaces through the application of small electric currents (100 μA or less), while also staphylococci

*Corresponding author. Tel.: +31503633160; fax: +31503633159.
E-mail address: p.k.sharma@med.umcg.nl (P.K. Sharma).

0142-9612/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2007.01.001

growing in a biofilm could be detached through the application of an electric current [12], most notably in the absence of any biocide. An electric current has been known before to enhance the bactericidal effects of many biocides, an effect called the "bioelectric effect" [13,14], whereas a direct bactericidal effect of electric currents has also been described [15,16]. Recently, we have observed this direct bactericidal effect on bacteria that remained adhering after electric current induced detachment in the absence of any antibiotics [17]. Note that for human application an electric current of 100 μA is well below the limit of being dangerous.

Considering the problems that infections pose in reconstructive bone surgery using external fixators, the threat posed by the ongoing (mis-)use of antibiotics and the rise in antibiotic resistance amongst many human pathogens and the above described in vitro experiments, it is the goal of this paper to determine whether a direct electric current of 100 μA can prevent clinical infection around percutaneous pins, implanted in the tibia of goats.

2. Materials and methods

2.1. Bacterial strains

Staphylococcus epidermidis HBH276, isolated from surface sites of premature neonate in 1990 at St. Joseph's Health Centre in London, Ontario, Canada [18,19] was used for the study after approximately 10 passages and adhered firmly and formed biofilms on different surfaces [12]. Bacteria cultured in Trypton Soya Broth (TSB, OXOID, Basingstoke, UK) at 37 °C in ambient air was used for the experiments. Bacteria were inoculated from blood agar plate in a pre-culture and allowed to grow for 24 h followed by a main culture which was grown for 17 h prior to harvesting. Bacteria were centrifuged (5 min at 5000*g* at 10 °C) and washed twice in 10% TSB growth medium and re-suspended with 3.0×10^6 colony-forming units (CFU) per ml in 10% TSB growth medium. CFU per ml was determined in triplicate prior to surgery by plate counting of a 17 h old culture and subsequently adjusted by dilution.

2.2. Electric current and electrodes

Self-drilling, self-tapping surgical stainless steel pins (5038-2-080 Apex Pin, Stryker Corp, Kiel, Germany) were used as a cathode while a circular platinum electrode supported by a polycarbonate canister completed the circuit as an anode. The pins, connecting rods and electrodes were sterilized in an autoclave at 121 °C for 20 min. An aluminium housing containing a high power 9 V battery and the electronic circuit was the current source. The aluminium housing was sled over the connecting rod and connected to the negative pole of the battery. Fig. 1A shows the fixation frame with the anodes and the current source attached to it. Each applied current was controlled by its own LM334Z (National Semiconductor Corp, Silicon Valley, USA), whose output potential was adapted continuously to meet the required current. A 100 μA DC current was used for the present study.

2.3. Experimental protocol

The experiments were approved by the University of Groningen Animal Ethical Committee. Nine mature female Saanen goats were used for this study. The goats were allowed free access to food and water and were unrestrained in their cages throughout the experiments. However, prior to surgery, the animals did not have any food for 8 h. Preoperatively, the animals were sedated with Thiopental (Nesdonal, 20 mg/kg i.v., AUV, Cuijk, The Netherlands), and after intubations anaesthesia was continued with a mixture of isoflurane and oxygen. Per operative Buprenorfine-hydrochloride (Temgesic, 0.01 mg/kg i.v., AUV, Cuijk, The Netherlands)

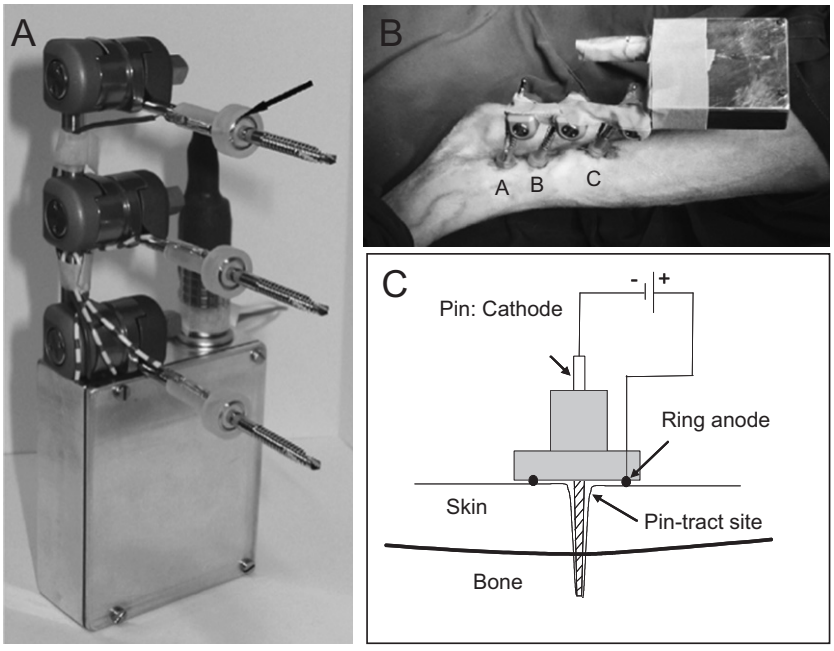

Fig. 1. Current source and its placement. (A) Photograph of the fixation frame equipped with current source. The pins act as cathodes, while the anodes are constituted by polycarbonate disks, with a platinum ring inserted to form the actual anode (arrow). (B) Fixation frame equipped with current source and secondary electrodes implanted in the right tibia of a goat. Electric current is applied to pin A, while pin B is used as control. Pin C offers additional support to the frame and power supply. (C) A schematic presentation illustrating the positioning of the pin in the bone, platinum electrode and the electrical connections.

was given for analgesia, which was continued intramuscularly once every 24 h for 2 days to prevent postoperative pain. Furthermore analgesics were given on indication.

The right hind limb of each animal was shaved and disinfected with betadine. Incisions of approximately 1 cm were made in the skin on the lateral side of the right tibia, the first (A) 3 cm above the ankle joint and the second (B) and third (C) each 3 cm above the first and second, respectively. The 3 mm external fixation pins were inserted with the aid of a hand-drill in the far cortex of the tibia. Open wounds around pins A and B were carefully dried of blood before inoculating them with 0.1 ml of a 3×10^6 CFU per ml suspension of *S. epidermidis* HBH276. The bacterial suspension was pulled into the wound and in-between the pin–skin interface by gravity. Care was taken that the bacterial suspension from one wound did not flow into the adjacent wounds. After placement of the pins and inoculation with the bacterial suspension, the sterilized anodes were installed thus shielding and covering the wound. All pins were connected to a single rod with pin-to-rod couplings (Hoffmann II Compact, Stryker Corp, Kiel, Germany) to which the current source was attached and connected to the platinum anodes (Fig. 1B). The platinum electrode around pin A received 100 μA DC current from the current source from where the current passed along the skin and wound into the pin. Pin A was connected to the negative pole of the battery via the connecting rod and aluminium housing hence completing the circuit. The current was applied from the time of implantation until the end of the experiment on a continuous basis. Pin B was used as control (receiving no current), whereas pin C served as an additional support for the frame. Before the animals returned to their cages, the electric current and voltage were measured.

After surgery, the goats were housed individually and daily clinical evaluations of each pin site began 24 h after surgery. None of the goats were observed to lick or bite the pins. Sometimes the connecters detached and the current source displaced on the connecting rod which could be due to goats bumping into the side walls or sitting on the leg. This detachment and displacement was corrected once observed and current and voltage monitored every morning.

The infection condition of a pin was annotated as one of the following: dry pin site was considered as no infection (score 1), inflammation or moist wound (score 2) or frank purulence at the pin site (score 3) [20]. The infection was observed and judged by two independent observers, although scoring was always unanimous.

On postoperative day 21, the animals were sacrificed. From the leg with pins inserted, an X-ray photo was taken. Subsequently, to allow microscopic evaluation of the biofilms on the pins, the frame was removed and the part of the pin outside the body was whipped clean with alcohol. Next the skin and the remaining tissue were carefully dissected from each pin, to allow removal of the pin without damaging a possibly existing biofilm. The explanted pin was submerged in staining fluid (LIVE/DEAD *Bac*light Bacterial Viability Kit, Molecular Probes, Leiden, The Netherlands) and incubated for 15 min in the dark. On different, randomly chosen locations on each surface, micrographs were taken with a confocal laser scanning microscope (CLSM) using a 40× ultra long working distance objective with the microscope set to FITC (excitation 488 nm and emission 500–600 nm) and TRITC (excitation 543 nm and emission 560–700 nm) to show dead and live bacteria, respectively.

3. Results and discussion

Fig. 2 summarizes the infection scores for each pin site obtained by the clinical evaluations during the 21 days of the follow-up. In the control group, 1 out of 9 goats showed no signs of infection, 2 out of 9 showed inflammation and 5 out of 9 showed frank purulence. On average, inflammation occurred after 6.9 ± 3.7 days ($\pm$standard error of the mean), while at 11.7 ± 4.6 days frank purulence was observed. In the group of pins to which an electric current was applied, 8 out of the 9 pins showed no inflammation or infection. One pin out of 9 pins showed infection from the 6th day onwards, which we considered unrelated to the experiment as this 9th goat displayed an entirely swollen and red right hind leg with clinical signs of infection, also around the third support pin and this already within 3 days after surgery. The above results clearly demonstrate a clear advantage of applying electric current to prevent pin-tract infection.

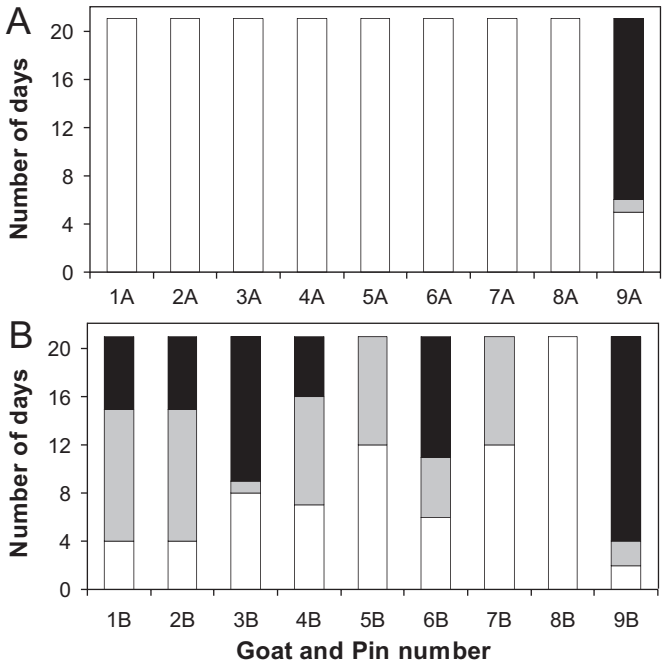

Fig. 2. The development of infection over time for each implanted stainless steel pin site and goat. Intentionally infected fixation pins are grouped according to whether they receive 100 μA DC current (A) or not (B). Pin number consists of the goat number and implantation site (A or B). White indicates an uninfected pin site (score 1), grey indicates inflammation or serious drainage without frank purulence (score 2) and black indicates frank purulence at the pin site (score 3).

Fig. 3 shows X-rays of a non-infected pin A (Fig. 3A) after having received an electric current and of an infected pin B (Fig. 3B) in the control group. Around the infected pin there is a clear osteolytic zone visible in the bone marrow. At the point of pin entry the white zone suggests reaction of the periosteum. These radiographic findings are suspect for osteomyelitis, which in this case has developed within 21 days in absence of an electric current.

Fig. 4 shows two confocal laser scanning micrographs after live/dead staining of a biofilm remaining on the pin's surface after removal from the animal. The applied electric current killed the majority of viable bacteria in the biofilms (Fig. 4A) in comparison to the absence of an electric current (Fig. 4B), while the few viable bacteria that remained evidently did not yield clinical signs of infection.

The present study tests the effectiveness of a small 100 μA DC current in preventing pin-tract infection using a goat model. The effectiveness is tested in a worst case scenario where the pin-tract wound was intentionally

infected with 3×10^5 CFU of *S. epidermidis* HBH276 and no additional wound cleaning steps were taken during the follow-up period of 21 days. We believe that if efficacy is demonstrated under the above conditions, electric current will also be effective in real situations where bacterial burdens are much lower and additional wound care is usually taken.

Interestingly, the method described can also be used as a diagnostic tool, as there is a clear correlation between the wound score and the electrical resistance of the skin (see Fig. 5 for two examples). The wound resistance was low (~10 kΩ) directly after surgery but later as the wound dried, resistance increased. Pin 7A in the electric current group for instance, did not show clinical signs of infection and the infection score remained 1, while from day 3 onwards the electrical resistance stayed high at 6 MΩ. Pin 9A, however, became infected from day 6 on, concurrent with a drop in resistance from 6 MΩ to 25 kΩ and to 10 kΩ after day 14.

The use of electric current to prevent signs of clinical infection presents major advantages in addition to or compared with current treatment modalities. The electrodes and electric circuitry are reusable and therefore the costs can be kept low. Available fixation frames and implantation techniques can be employed without any need

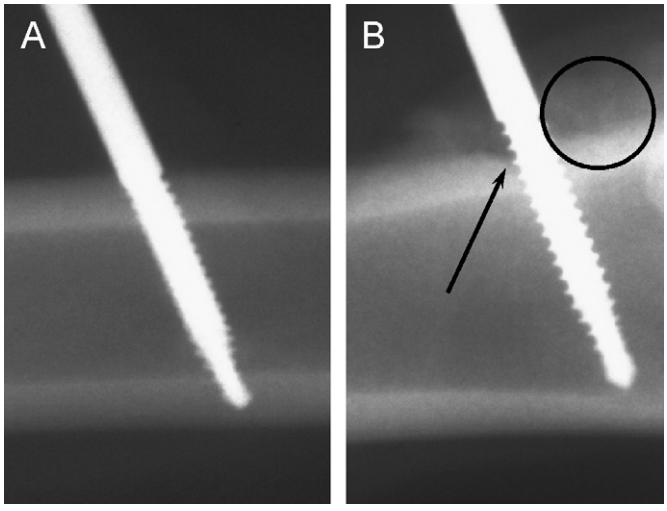

Fig. 3. X-ray examples of implanted pins. (A) A non-infected pin site, after electric current application for 21 days and (B) an infected control pin site, in the absence of electric current application. The inflammatory reaction of the periosteum is encircled and the osteolytic zone in the cortex is indicated by the arrow.

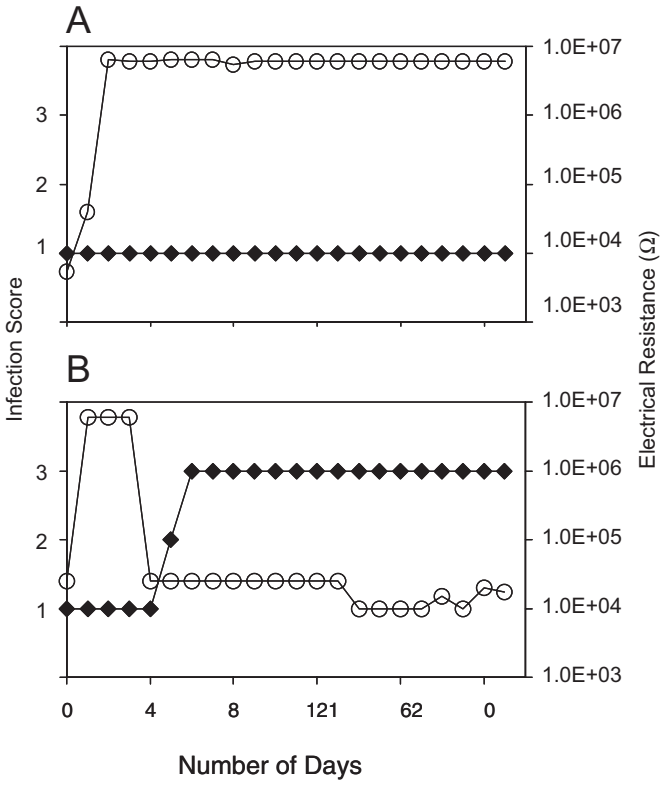

Fig. 5. Infection score (closed symbols) and electrical resistance (open symbols) for pins receiving electric current as a function of time. (A) Pin 7A, no infection developing due to current application and (B) Pin 9A, where inflammation and later infection started on the 6th day even after application of current.

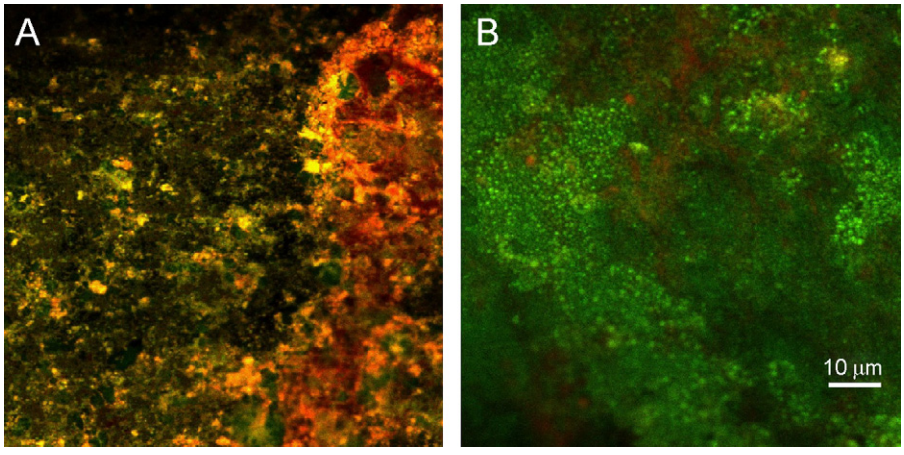

Fig. 4. CLSM micrograph of biofilms adhering to a stainless steel pin after explantation from a goat. (A) Biofilm on a pin after application of an electric current and (B) biofilm on a control pin, receiving no electric current. Green represents live bacteria and red dead ones. Bar marker indicates 10 μm.

for modification; moreover, the method does not require any antibiotics.

4. Conclusions

Small electric currents of 100 μA are able to prevent clinical signs of infection around surgical stainless steel pin sites, without the use of antibiotics in intentionally infected wounds, suggesting that electric currents will also be effective in real situations where the infection burden is much lower. The wound electrical resistance decreases with worsening of the infection from a dry condition to a purulent stage.

Acknowledgements

The help from A. Nijmeijer, A. Jansen and N. Bruinsma during the animal experiments, Dr. D. Neut with confocal laser scanning microscopy and E. Ramdin with X-rays is duly acknowledged.

References

[1] Mostafavi HR, Tornetta III P. Open fractures of the humerus treated with external fixation. Clin Orthop Relat Res 1997;337:187–97.
[2] Sims M, Saleh M. External fixation—the incidence of pin site infection: a prospective audit. J Orthop Res 2000;4:59–63.
[3] McKenzie LL. In search of a standard for pin site care. Orthop Nurs 1999;18:73–8.
[4] Gordon JE, Kelly-Hahn J, Carpenter CJ, Schoenecker PL. Pin site care during external fixation in children: results of a nihilistic approach. J Pediatr Orthop 2000;20:163–5.
[5] Anwar H, Dasgupta MK, Costerton JW. Testing the susceptibility of bacteria in biofilms to antibacterial agents. Antimicrob Agents Chemother 1990;34:2043–6.
[6] Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, et al. Bacterial biofilms in nature and disease. Annu Rev Microbiol 1987;41:435–64.
[7] Khoury AE, Lam K, Ellis B, Costerton JW. Prevention and control of bacterial infections associated with medical devices. ASAIO J 1992;38:M174–8.
[8] Hermansson M. The DLVO theory in microbial adhesion. Colloids Surf B—Biointerfaces 1999;14:105–19.
[9] Jucker BA, Harms H, Zehnder AJ. Adhesion of the positively charged bacterium *Stenotrophomonas (Xanthomonas) maltophilia* 70401 to glass and Teflon. J Bacteriol 1996;178:5472–9.
[10] Poortinga AT, Bos R, Busscher HJ. Controlled electrophoretic deposition of bacteria to surfaces for the design of biofilms. Biotechnol Bioeng 2000;67:117–20.
[11] Ueshima M, Tanaka S, Nakamura S, Yamashita K. Manipulation of bacterial adhesion and proliferation by surface charges of electrically polarized hydroxyapatite. J Biomed Mater Res 2002;60: 578–84.
[12] Van der Borden AJ, Van der Werf H, Van der Mei HC, Busscher HJ. Electric current-induced detachment of *Staphylococcus epidermidis* biofilms from surgical stainless steel. Appl Environ Microbiol 2004;70:6871–4.
[13] Blenkinsopp SA, Khoury AE, Costerton JW. Electrical enhancement of biocide efficacy against *Pseudomonas aeruginosa* biofilms. Appl Environ Microbiol 1992;58:3770–3.
[14] Costerton JW, Ellis B, Lam K, Johnson F, Khoury AE. Mechanism of electrical enhancement of efficacy of antibiotics in killing biofilm bacteria. Antimicrob Agents Chemother 1994;38:2803–9.
[15] Liu WK, Tebbs SE, Byrne PO, Elliott TS. The effects of electric current on bacteria colonising intravenous catheters. J Infect 1993; 27:261–9.
[16] Liu WK, Brown MR, Elliott TS. Mechanisms of the bactericidal activity of low amperage electric current (DC). J Antimicrob Chemother 1997;39:687–95.
[17] Van der Borden AJ, Van der Mei HC, Busscher HJ. Electric-current-induced detachment of *Staphylococcus epidermidis* strains from surgical stainless steel. J Biomed Mater Res B Appl Biomater 2004;68:160–4.
[18] Bialkowska-Hobrzanska H, Jaskot D, Hammerberg O. Evaluation of restriction endonuclease fingerprinting of chromosomal DNA and plasmid profile analysis for characterization of multiresistant coagulase-negative staphylococci in bacteremic neonates. J Clin Microbiol 1990;28:269–75.
[19] Busscher HJ, Bialkowska-Hobrzanska H, Reid G, Kuijl-Booij M, Van der Mei HC. Physicochemical characteristics of two pairs of coagulase-negative staphylococcal isolates with different plasmid profiles. Colloid Surf B—Biointerfaces 1994;2:73–82.
[20] DeJong ES, DeBerardino TM, Brooks DE, Nelson BJ, Campbell AA, et al. Antimicrobial efficacy of external fixator pins coated with a lipid stabilized hydroxyapatite/chlorhexidine complex to prevent pin tract infection in a goat model. J Trauma 2001;50: 1008–14.