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*ORIGINAL STUDY*

# Pulsed electric fields reduce bacterial attachment to stainless steel plates

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**The purpose of this study was to evaluate the capacity of pulsed bilateral electric fields to control bacterial attachment on stainless steel plates. Previously sterilized circular metal plates of stainless steel were submerged in a liquid medium with a known concentration of Staphylococcus epidermidis and incubated for 1 hour at 36ºC while a 200 Hz pulsed electric field of 18 V/cm was applied for 2.5 µseg and then sonicated for 5 minutes in 10 ml of saline. Three different models were cultured and compared: 1) negatively-charged plate, 2) positively-charged plate, and 3) control plate without electric current. A total of 39 metal plates were processed. The median adherence in the control group and the electric field group was 312 CFU/mm2 and 16,2 CFU/mm2 respectively (p < 0.001, reduction of 95% of bacterial attachment). Bilateral pulsed electric field is able to reduce bacterial attachment on stainless steel plates in in vitro conditions.**

**Keywords** : pulsed electric field ; bacterial attachment ; prosthetic joint infection ; biofilm ; adhesion.

## INTRODUCTION

Prosthetic joint infection (PJI) is probably the worst complication after joint replacement. This complication is associated with a high morbidity, economic cost and a non-negligible mortality. Currently, local or systemic antibiotics are the main strategies for reducing the infection rate while the physical methods remain disregarded.

Biofilm formation on orthopedic implant surfaces plays an important role in PJIs. Bacteria embedded in biofilms are resistant to immune system *(17)* and antibiotics *(2,25)* and implant removal are necessary in most cases to achieve success. Coagulase-negative staphylococci and

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specially Staphylococcus epidermidis are the most common isolated microorganisms in acute and chronic PJIs *(9)* and they are the main cause of infection persistence and subsequent loosening after acute PJI *(8)*.

Adhesion of planktonic bacteria to the implant surface is the first step in the biofilm formation and it is influenced by a great variety of components. There are two physical-chemical forces that play a role in bacterial adhesion on materials: the Van der Waals and the electrostatic forces. The bactericidal effect of strong electric fields and electrical enhancement of the efficacy of antimicrobials against biofilm bacteria "bioelectric effect" are well known. However, the evidence in the literature regarding the impact of an external electric field on the electrostatic interactions of bacteria with a charged substrate is scarce. To our best knowledge, only a few articles have been reported in the literature regarding this topic. The aim of this study was to evaluate the capacity of pulsed bilateral electric field to control the bacterial attachment on stainless steel (SS) plates.

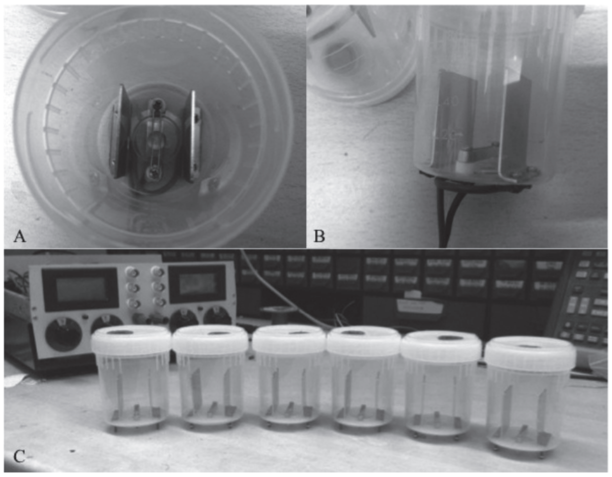


***Fig. 1.*** — Photography of the used recipients showing the different metallic and electric components of the bottles. A) Top view of the recipient, showing the circular plates introduced in the bottle and flanked by two rectangular plates. B) Lateral view of the recipient, the electric connections can be seen below. C) General view of the recipients containing the plates.

## MATERIALS AND METHODS

Metallic components of SS, circular metal plates or plate under test (PUT), metallic supports and lateral L-plates were supplied by AMES S.A. The circular plates were obtained from cutting cylindrical solid bars of 30mm of diameter and 2 m length to discs of 2mm of thickness. Each plate was mechanically polished to remove irregularities and to promote identical physical conditions at the surface.

Metallic supports of 30mm long with a central through-hole of 2.15 x 20 mm were produced. The design of the through-hole allowed the press-fit insertion and perfect subjection of the PUT in vertical and parallel configuration with lateral L-plates. A small portion of rectangular plates of 30 x 40 mm and 1 mm of thickness were bended to create L-plates.

Three SS plates were placed vertically into containers for urine sample (Aquisel SL, Abrera, Spain), placed parallel and equidistant to each other. The lateral L-plates were fixed to the container and were both electrically connected. PUT was fixed to the container using a metallic support attached to the container bottom on which PUT was inserted (Figure 1). After all the metallic and electric components and L-plates were adjusted, each bottle was sterilized using a low-temperature formaldehyde machine (130 LF, Matachana SA, Barcelona-Spain) to avoid damaging of heat sensitive components.

To apply the electric field, a power pulse wave generator was constructed. Using the suitable knobs, amplitude, width and frequency of the electric pulses could be adjusted.

Three different models (Figure 2) were tested for bacterial adherence in the PUT under three different electrical conditions: negatively charged, positively charged and uncharged. In the charged models an electric field between the PUT and the lateral L-plates with a strength of 18 V/cm for 2.5 µs at a frequency of 200 Hz was applied. In the uncharged model no electric field was applied, in order to allow comparison with the charged models.

S. epidermidis RP62A (ATCC 35984) was used to perform the study. A colony of RP62A was grown 24h at 37ºC in Trypticase Soy Broth (TSB,

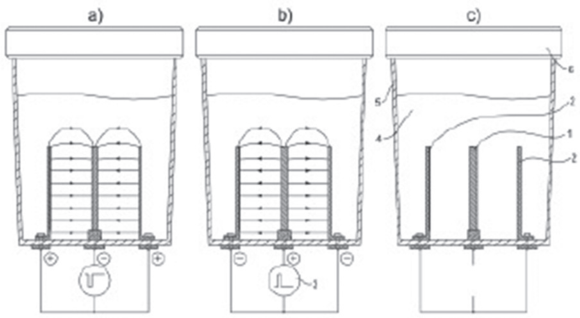


*Fig. 2.* — Drawing of the electric field distribution of the three different electric models: a) Plate negatively charged, b) Plate positively charged, c) Control plate without current). Detail of numbers: 1- Plate Under Test or circular metal plate; 2- Lateral L-plates; 3- pulse wave generator; 4- liquid medium with bacteria; 5- container for urine sample; 6- tight cover that keeps sealed the recipient.

Biomerieux, Lyon, France), then the overnight culture of S. epidermidis was diluted 1:200 in fresh TSB containing 0.25% of glucose. Metal discs previously sterilized by wet autoclaving at 135 ºC (S1000, Matachana SA, Barcelona-Spain), were inserted into the metallic supports in sterile conditions and completely submerged in 100 ml of the fresh media with S. epidermidis and incubated for 1 hour. During this period of time and depending on the concrete chosen electric model, an electric field was applied. After the incubation time, discs were washed with sterile PBS in order to remove all traces of culture media and non-adherent bacteria, and discs were sonicated (Xuba 1, Grant Instruments, Cambridge-UK) for 5 minutes at 44 kHz in 10 ml of saline serum.

Ten fold dilutions were performed to quantify adherent bacteria. Counts were done in Blood agar. Results were related to cfu/mm$^2$ of the metal disc. The experiment was made under sterile conditions.

The cfu/mm$^2$ values of each model were compared. Due to the size of the groups, a non-parametric test (Mann-Whitney U Test) was used. Statistical analysis was performed using SPSS v16 (SPSS Inc, Chicago Ill, USA) for Windows. Level of statistical significance was established at $p < 0.05$ (two-tailed).

## RESULTS

An initial medical record query yielded a list of 65 A total of 43 circular SS plates were initially used. However, due to technical mistakes during the incubation period, the application of electric fields or the microbiological process, 4 plates were excluded. Thus, 39 metal plates were finally processed and included in the study: 12 circular plates without electric field, 15 with positive polarity and 12 with negative polarity.

A statistical significant reduction of the 95% of bacterial attachment was observed when the electric field was applied (Table I). Comparing the positive PUT model and the negative one, we found statistical differences (p = 0.013) but with a difference lower than 1 log (Table II).

Table I. — Quantification of Staphylococcus epidermidis adhesion on Plate Under Test (PUT) between pulsed and uncharged electric models

| Electric Model | N | median (cfu/mm$^2$) | Min (cfu/mm$^2$) | Max (cfu/mm$^2$) | (p)* |
|---|---|---|---|---|---|
| Pulsed | 27 | 16.22 | 1.24 | 62.41 | <0.001 |
| Uncharged | 12 | 312 | 62.41 | 811.37 | |

* Mann-Whitney U Test

Table II. — Quantification of Staphylococcus epidermidis adhesion on Plate Under Test (PUT) between positively and negatively electric models

| Electric Model | N | median (cfu/mm$^2$) | Min (cfu/mm$^2$) | Max (cfu/mm$^2$) | (p)* |
|---|---|---|---|---|---|
| Negative PUT# | 12 | 11.85 | 1.87 | 44.93 | 0.013 |
| Positive PUT# | 15 | 25.58 | 1.24 | 62.41 | |

* Mann-Whitney U Test, #PUT: Plate Under Test.

## DİSCUSSİON

PJI is one of the most important problems associated with orthopedic implants. These infections are associated with a significant morbidity and with a high economic cost *(3,22,* 30). The bacterial adhesion and biofilm formation play an important role in the physiopathology of these infections, conditioning the response in patients treated with debridement and retention of the implant (5,20). Current approaches to develop new biomaterials with a low risk of becoming infected once implanted in the human body are predominantly based on developing non-adhesive surfaces (12,23). Nowadays, most methods to control, prevent and treat these infections are based in chemical methods while the physical ones have not been developed.

The bactericidal activity of the electric current has been formerly described in the literature. In 1919, Anderson et al. (1) reported the usefulness of low alternative current (AC) to sterilize milk. Since then, the advances in industrial water processing and food management have been constant and nowadays the use of electric currents (of high voltage) is common in these fields. However, it is not possible to apply these intensities of current in medicine owing to security reasons. As a matter of fact, only few incremental steps have been recently taken to develop this field of research toward the vision of a viable infection-resistant medical device system based on electricity. However, several in-vitro studies have showed the capacity of low intensity electric field ((AC and direct current (DC)) for inhibiting the bacterial growth (7) of different species: Staphylococcus aureus, Pseudomonas aeruginosa (13) and S. epidermidis (6). Such inhibition is voltage, current and frequency dependent. Moreover, the enhancement of the bactericidal action of some antibiotics by using electric fields (bioelectric effect) has been reported several times in different in-vitro studies (4). In addition, Del Pozo et al. (6) showed in an animal model using rabbits that DC is statistically significantly more effective than intravenous doxycycline for the treatment of S. epidermidis osteomyelitis. In addition, in 5 of 14 animals treated with doxycycline alone resistant bacteria were selected but not when combining doxicycline with DC. Recently, Ruiz-Ruigomez M et al. (24) published a study examining the effect of a low amount of DC exposure on different microorganisms, concluding that direct DC reduces bacterial and yeast biofilm formation. However, to apply current with the objective of killing bacteria requires longer therapeutic periods of time and this fact may pose several potential drawbacks: firstly, DC produces electrolysis of water and salts creating new hydrated ions and molecules such as H2O2 or chlorine (18) that may affect not only bacteria but also cells of the patient. Secondly, DC could modify pH levels, entailing a possible toxic consequence. Thirdly, AC could increase the temperature by inducing vibration of water molecules. In order to avoid these possible adverse effects and the toxicity associated with them, we decided to use pulse DC fields of low frequency (200Hz) and a very short duration (square waves of only 2.5 μsec). In other words, for one second of time, the electric current flows only for 0.0005 seconds (a 0.05% of the time). Moreover, as we only wanted to study the influence of electric fields in the process of adhesion of bacteria to implants, the total period of time of pulse application was of only 1 hour.

The application of electric fields to modify the bacterial adhesion or to detach the formed biofilm to implants has been less studied. Van der Borden et al reported the electric current-induced detachment of S. epidermidis biofilms from surgical stainless steel (27,28), whereas Poortinga et al (21) reported the capacity of electric fields to modify the bacterial attachment to conductive surfaces. Based on these theoretical in vitro principles, the prevention of pin tract infections in external SS fixator frames using electric current in a goat model has been reported (26). In urology, the usefulness of low electric fields to reduce bacterial attachment to medical devices has also been described: Gabi et al. (10) published the usefulness of electrical microcurrent to prevent conditioning film and bacterial adhesion to urological stents. Khan SI et al. (16) recently published a study with the purpose of investigating the effect of PEFs on biofilm-infected mesh infected with bioluminescent P. aeruginosa and treated with a PEF using a concentric electrode system to derive, in a single experiment, the critical electric field strength needed to kill bacteria. The results indicated that increased efficacy of treatment is due to increased number of pulses delivered.

The DLVO (Derjaguin, Landau, Verwey and Overbeek) theory (29) describes the adhesion of colloidal particles to describe bacterial adhesion in biomaterials and implants. Based on this theory, as the majority of bacteria cell surfaces carry a net negative charge, an electrostatic repulsion should be expected when approaching to a negative charged surface. We indeed found statistical significant differences between the model with positive PUT and the model with negative PUT, so these results were in concordance with the DLVO theory. However, we considered these differences should be interpreted with caution in the medical practice, owing to either the variability of the used bacterial quantification method and the fact that the difference was very low (lower than 1 log). Moreover, several studies have shown discrepancies between observations and theoretical expectations: Gottenbos et al. reported, both in vitro and in an animal model *(14,15)*, that positively charged biomaterial surfaces exert an antimicrobial effect on adhering Gram-negative bacteria, but not on Gram-positive ones. Gall et al. *(11)* described that, in negative potential experiments, P. aeruginosa appeared to be uniformly distributed on the electrode surface, whereas on sensors of positive electric potential experiments, the bacterial cell distribution was rather irregular with a low density of bacterial cell accumulation. In summary, the bacterial cell wall is structurally and chemically more complex than the surface of synthetic colloidal particles. Thus, this fact may probably cause an impact on bacterial adhesion to surfaces. Bacterial attachment under electric fields does not always follow the DLVO theory, especially regarding Gram-negative bacteria.

It has been recently reported that cathodic voltage-controlled electrical stimulation combined with clinically relevant lengths of vancomycin therapy may be a treatment option for implant-associated infections and allow for component retention in certain clinical scenarios *(19)*. We found that a bilateral PEF is able to avoid 95% of bacterial attachment on SS plates in in vitro conditions, while polarity of metal plate plays also a smaller role on S. epidermidis attachment. However, further studies including other electric conditions, metals, and microorganism are necessary. In our opinion, and based on a recent report of the WHO (World Health Organization) *(31)*, antibiotic resistance is no longer a prediction for the future but is happening right now, so it seems mandatory to explore new alternatives to bacterial infection management. Future efforts focused in this field may allow the design and development of electrified devices and antimicrobial material surfaces.

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