International Journal of Oral Science (2014) 6, 7–14

www.nature.com/ijos

ORIGINAL ARTICLE

# The growth of *Staphylococcus aureus* and *Escherichia coli* in low-direct current electric fields

**Dunya Zituni[1], Heidi Schütt-Gerowitt[2], Marion Kopp[1], Martin Krönke[2], Klaus Addicks[3], Christian Hoffmann[3], Martin Hellmich[4], Franz Faber[5] and Wilhelm Niedermeier[1]**

**Electrical potentials up to 800 mV can be observed between different metallic dental restorations. These potentials produce fields in the mouth that may interfere with microbial communities. The present study focuses on the impact of different electric field strengths (EFS) on the growth of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) *in vitro*. Cultures of *S. aureus* and *E. coli* in fluid and gel medium were exposed to different EFS. Effects were determined by calculation of viable counts and measurement of inhibition zones. In gel medium, anodic inhibition zones for *S. aureus* were larger than those for *E. coli* at all field strength levels. In fluid medium, the maximum decrease in the viable count of *S. aureus* cells was at 10 $V \cdot m^{-1}$. Field-treated *S. aureus* cells presented ruptured cell walls and disintegrated cytoplasm. Conclusively, *S. aureus* is more sensitive to increasing electric field strength than *E. coli*.**

*International Journal of Oral Science* (2014) **6,** 7–14; doi:10.1038/ijos.2013.64; published 6 September 2013

**Keywords:** direct current; *Escherichia coli*; electrogalvanism; gold electrode; *Staphylococcus aureus*

## INTRODUCTION

Different metallic restorations are used in dentistry to restore decayed, fractured and missing teeth. When different alloys are placed in the oral cavity, a galvanic current is induced at the time of their contact with saliva which acts as an electrolyte.[1–3] This phenomenon is called oral galvanism.[4–7] Dental alloys develop an anodic and cathodic pole depending on the position of metals in electrochemical series and individual variations of saliva.[8] A potential as high as 950 mV has been measured in the oral cavity between an aluminium splint and a gold crown.[9] Such potential can decrease the proliferation rate of oral mucosa cancer cells lines[10] and also cause local or systemic adverse effects on biological structures like pain and discomfort, metallic or salty taste, burning mouth syndrome, erythema, xerostomia, glossitis and oral mucosal lesions[9,11–14]. It may also cause general medical symptoms and diseases due to the absorption of ionized toxic metals.[9] In addition, such potentials may induce changes in oral homeostasis through their direct or indirect interference with oral ecosystems. Since changes in environmental factors can stimulate the development of adaptive responses in individual microorganisms and introduce more pathogenic microorganisms into the microbial community,[15] the effect of electric fields on microbial communities was the area of interest.

*Staphylococcus aureus* are roughly spherical Gram-positive cocci that can be frequently isolated from the oral cavity of particular patient groups such as children and elderly.[16–17] Smith *et al.*[18] isolated methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* from the oral cavity by an oral rinse and a tongue swab respectively. They reported that *S. aureus* might be a more frequent isolate from the oral cavity than hitherto suspected. In addition, Ohara-Nemoto *et al.*[19] demonstrated that the most frequently isolated species in saliva was *S. aureus* followed by *S. epidermidis* and confirmed the highly significant occurrence of oral staphylococci in systemically and periodontally healthy adult. On the other hand, *Escherichia coli* are Gram-negative rods and are an extensively studied model organism; probably the best-understood bacterium at all.[20]

The present study was conducted to investigate the effects of low-direct current (LDC) electric fields and their electrochemical products on *S. aureus* and *E. coli in vitro* within fluid and gel media to explain the similar or different effects of galvanic currents on normal growth of oral bacteria in different salivary constituents that influence the equilibrium state of oral cavity and thus producing diseases.

## MATERIALS AND METHODS

### Voltage generated by electrodes in tryptic soy broth, NaCl and distilled water

To test in absence of direct current (DC) supply, if tryptic soy broth (TSB) with immersed gold electrodes play a role in producing potentials, two holes were drilled into the cover of 9.4-cm Petri dish, each is

[1]Department of Prosthetic Dentistry, School of Dental and Oral Medicine, Cologne University, Cologne, Germany; [2]Institute for Medical Microbiology, Immunology and Hygiene, Cologne University, Cologne, Germany; [3]Institute for Anatomy I, Cologne University, Cologne, Germany; [4]Institute for Medical Statistics, Informatics and Epidemiology, Cologne University, Cologne, Germany and [5]Department of Pre-Clinical Dentistry, School of Dental and Oral Medicine, Cologne University, Cologne, Germany
Correspondence: Dr W Niedermeier, Department of Prosthetic Dentistry, School of Dental and Oral Medicine, Cologne University, Kerpener Street 32, Cologne 50931, Germany
E-mail: wilhelm.niedermeier@uk-koeln.de
Dr D Zituni, Department of Prosthetic Dentistry, School of Dental and Oral Medicine, Cologne University, Glas Street 79, Cologne 50823, Germany
E-mail: dunya.zituni@yahoo.de
Accepted 14 June 2013

0.5 cm apart from the centre (area of field homogeneity) and two measuring gold electrodes were passed through the drilled holes (Figure 1). Three Petri dishes, each with four gold electrodes (Solaris Goldbad, 99.9% pure gold; DeguDent, Hanau-Wolfgang, Germany) were used. A–24 mL of each; TSB (positive control 1), 0.9% NaCl (positive control 2) and distilled water (Aqua bidest DAB; Servoprax, Am Marienbusch, Wesel, Germany) (24 mL of each) as a negative control were poured in the dishes that were incubated at 37 ℃ for 24 h. The two measuring electrodes were connected to a sensitive voltmeter (2 000 Multimeter, ID-NR: 20009867; Keithley, Cleveland, OH, USA) and the potential difference in every dish was measured every hour for 8 h and at 24 h.

**Voltage generated by electrodes in TSB containing bacteria**

To measure the voltage generated by gold electrodes that were not connected to a DC supply, 24 mL of TSB and 1 mL of bacterial suspension ($10^{-1}$ dilution in 0.9% sterile saline of McFarland 0.5) were poured in Petri dish. The dish was then incubated with four gold electrodes at 37 ℃ for 24 h and the potential difference between two measuring electrodes was measured every hour for 8 h and at 24 h.

**Survival rates of *S. aureus* and *E. coli* at different electric field strengths**

Strains of *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were provided as cultures grown overnight on blood agar plates. Three Petri dishes were used, 24 mL of TSB and 1 mL of the bacterial suspension ($10^{-1}$ dilution in 0.9% sterile saline of McFarland 0.5) were poured into every dish and a figure eight movement was done to evenly distribute bacteria. The three dishes were for: field-stimulated bacteria (FSB), electrode-stimulated bacteria (electrodes not connected to an external DC power supply (ESB)) and negative control bacteria (neither current nor electrodes (NCB)). Gold electrodes were immersed perpendicularly in the periphery of the first two dishes, four electrodes in each (Figure 1). The distance between each two opposing electrodes was 9.3 cm. The first dish electrodes were connected to a DC supply (two anodes and two cathodes) that generates a DC voltage between 0 and 2.5 V.

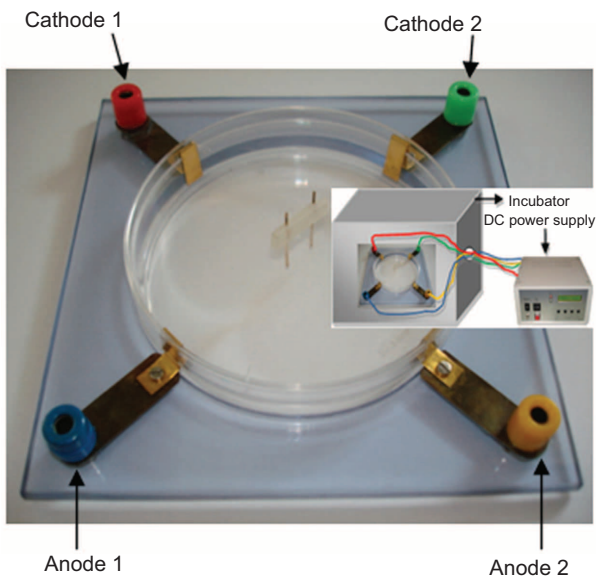


**Figure 1 Four gold electrodes placed at equal distances of 90° each.** The distance between each two opposing electrodes =9.3 cm with two measuring gold electrodes in the center of Petri dish.

During the experiments, *S. aureus* and *E. coli* were exposed separately to electric field strengths (EFS) of 2–10 $V \cdot m^{-1}$. The three dishes were incubated at 37 ℃ for 24 h. pH and temperature were measured using pH test strips and a thermometer (THM 912; Oregon Scientific, Rue du Bosquet, Louvain-La-Neuve, Belgium). Aliquot 0.5 mL samples were taken at the first anode from the first dish, at the first electrode from the second dish and from the negative control dish every hour during the initial 8 h and at 24 h. For each sample, serial dilutions up to $10^{-6}$ dilutions were performed using sterile saline (0.9%). Each diluted sample was pipetted onto the surface of blood agar and incubated at 37 ℃ for 24 h. The survival rate in terms of bacterial count was expressed as lg ($CFU \cdot mL^{-1}$).

**Electric currents produced in TSB containing bacteria after DC voltage induction**

In EFS experiments of 2–10 $V \cdot m^{-1}$, the intensity of arising electric currents in TSB containing bacteria was measured using DasyLab software programme (DASY*Lab*; DATALOG, Mönchengladbach, Germany).

**Inhibition zones for *S. aureus* and *E. coli* at different electric field strengths**

One milliliter of bacterial suspension ($10^{-5}$ dilutions in 0.9% sterile saline of McFarland 0.5) was cultivated on two Mueller-Hinton agar (MHA) plates, 0.5 mL in every plate. The two MHA plates were used for FSB and ESB. After the absorption of the bacterial suspension, the two plates were incubated at 37 ℃ for 24 h. EFS of 5–27 $V \cdot m^{-1}$ were applied on FSB plate, each for 24 h. The radius of each inhibition zone was measured.

**Transmission electron microscopy**

Microbial growth and cell survival dynamics under electric field treatment of (4, 6 and 10 $V \cdot m^{-1}$), electrode treatment and the relative negative controls were observed using transmission electron microscopy (TEM). The collected cells were harvested by centrifugation for 10 min at 6 440*g* in a Hettich Rotanta/S centrifuge and fixed using glutaraldehyde for 24 h. Then, the cells were rinsed, postfixed in osmium tetroxide for 3 h, dehydrated in ethanol in ascending grades and embedded in Epon Araldite. Following this, the ultrathin sections were examined using TEM (Zeiss TEM 902; Carl Zeiss Lithos, Oberkochen, Germany).

**Statistical analysis**

Data was recorded and analyzed in the data module of the Statistical Package for Social Science (IBM SPSS Statistics 19) for Windows (IBM, Somers, NY, USA). The results are presented as means±s.d. Significant differences in survival rate were assessed by repeated measures analysis of variance (ANOVA) with Bonferroni (step-down) Holm correction. Inhibition zone experiments were evaluated statistically using univariant ANOVA with Bonferroni (step-down) Holm correction. A value of $P \leqslant 0.05$ was used to determine statistical significance between different means of different cells (FSB, ESB and NCB) and between the inhibition zones for both strains; $P \leqslant 0.05$ was considered significant.

## RESULTS

**Voltage generated by electrodes in TSB, NaCl and distilled water**

The voltage generated by the electrodes in TSB without any connection to a DC supply was 260.55 mV (2.8 $V \cdot m^{-1}$)±47.59 mV, in 0.9%

**Table 1 Voltage generated by gold electrodes in different media without DC voltage induction**

| | Voltage generated by electrodes in different media/mV | | |
|---|---|---|---|
| Time/h | TSB (positive control 1) | NaCl (positive control 2) | Distilled water (negative control) |
| 0 | 191 | 22.5 | 15 |
| 1 | 209 | 33 | 11.5 |
| 2 | 268 | 50 | 6.5 |
| 3 | 299 | 67.5 | 3 |
| 4 | 200 | 79 | 5 |
| 5 | 346 | 87.5 | 2.5 |
| 6 | 336 | 71 | 2.5 |
| 7 | 248 | 63 | 2.5 |
| 8 | 296 | 58.5 | 3 |
| 24 | 269 | 50 | 3 |
| Mean±s.d. | 266±51.75 | 58.2±19 | 5.45±4.16 |
| Corr. value | 260.55±47.59 | 52.75±14.84 | |

TSB, tryptic soy broth; Corr. value, corrected value=mean of positive control (1 or 2)–mean of negative control; DC, direct current; s.d., standard deviation.

NaCl was 52.75 mV (0.6 V·m$^{-1}$)±14.84 mV and in distilled water was 5.45 mV (0.06 V·m$^{-1}$)±4.16 mV (Table 1, $n$=2).

### Voltage generated by electrodes in TSB containing bacteria

The voltages generated from electrodes-stimulated *S. aureus* and *E. coli* were 274.69 mV (2.9 V·m$^{-1}$)±68.33 mV and 226.53 mV (2.4 V·m$^{-1}$)±33.38 mV, respectively. Thus, the electrodes in TSB containing bacteria in absence of current produce EFS of ⩽3 V·m$^{-1}$ ($n$=5).

### Survival rates of *S. aureus* and *E. coli* at different electric field strengths

Electrical field treatment (4–10 V·m$^{-1}$) led to a decrease of the survival rate of *S. aureus* from (7.11±0.22) lg (CFU·mL$^{-1}$) to (6.01±0.2) lg (CFU·mL$^{-1}$). For ESB, the survival rate decreases from (6.88±0.25) lg (CFU·mL$^{-1}$) to (6.10±0.4) lg (CFU·mL$^{-1}$) when compared to the initial bacterial load from (7.56±0.26) lg (CFU·mL$^{-1}$) to (7.00±0.39) lg (CFU·mL$^{-1}$). In contrast for *E. coli*, EFS of 2–10 V·m$^{-1}$ and electrodes alone showed no change in the survival rate (Figure 2, $n$=2). In all experiments, there was no significant change in pH and temperature; the range of pH (from 7 to 6) and temperature (from 36.9 ℃ to 37.2 ℃) measured in all treated and control dishes were similar.

### Electric currents produced in TSB containing bacteria after DC voltage induction

In TSB containing *S. aureus*, the intensity of current increased gradually from (106.17±0.69) μA to (567.56±86.71) μA with the increase in field strength from 2 to 10 V·m$^{-1}$ (Table 2, $n$=5). On the other hand, the intensity of current in TSB containing *E. coli* was (289.13±215.02) μA at 10 V·m$^{-1}$.

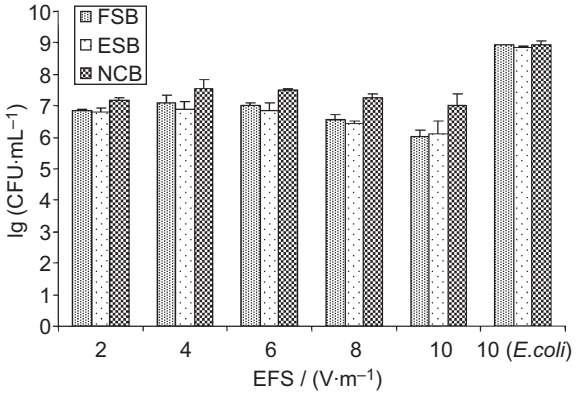


**Figure 2 Survival rates of *S. aureus* and *E. coli* after the exposure to different EFS in 24 h.** The first 2, 4, 6, 8 and 10 V·m$^{-1}$ for *S. aureus* and 10 (*E. coli*) is a representative experiment to *E. coli* tests. CFU, colony-forming unit; EFS, electric field strength (V·m$^{-1}$); ESB, electrode-stimulated bacteria; FSB, field-stimulated bacteria; NCB, negative control bacteria.

**Table 2 The intensity of current and the resistance of bacteria in TSB at different electric field strengths**

| Bacteria | EFS/(V·m$^{-1}$) | EP/mV | Intensity of current/μA | Resistance/kΩ |
|---|---|---|---|---|
| *S. aureus* | 2 | 200 | 106.17±0.69 | 1.88 |
| | 4 | 400 | 204.05±3.67 | 1.96 |
| | 6 | 600 | 289.52±37.09 | 2.07 |
| | 8 | 800 | 383.29±45.71 | 2.09 |
| | 10 | 1 000 | 567.56±86.71 | 1.76 |
| *E. coli* | 10 | 1 000 | 289.13±215.02 | 3.46 |

EFS, electric field strength; EP, electric potential; TSB, tryptic soy broth.

### Inhibition zones for *S. aureus* and *E. coli* at different electric field strengths

Zones of inhibition of varying sizes were observed at the positive electrode (anode) at all field strengths for both bacterial strains (Table 3). Electrical field treatment (5–27 V·m$^{-1}$) of *S. aureus* and *E. coli* increases the inhibition zone from (4.00±3.83) to (13.75±1.50) mm and from (1.63±0.74) to (12.25±1.83) mm, respectively. No zones of inhibition around the cathode or around sham electrodes (no current) were observed. The radius of zones for *S. aureus* was more than for *E. coli* at all field strength levels ($n$=5).

**Table 3 Inhibition zones produced around the anode at different EFS and *P*-value using univariate ANOVA with Bonferroni (step-down) Holm correction**

| | | Inhibition zones/mm | | IZ*s* *vs.* IZ*e* | |
|---|---|---|---|---|---|
| EFS/(V·m$^{-1}$) | EP/mV | IZ*s* | IZ*e* | ANOVA | ANOVA+Bonferroni-H |
| 5 | 500 | 4.00±3.83 | 1.63±0.74 | 0.019 9* | 0.039 8* |
| 10 | 1 000 | 7.00±2.16 | 2.88±0.84 | 0.000 12* | 0.000 48* |
| 16 | 1 500 | 7.75±1.71 | 4.25±1.17 | 0.000 86* | 0.002 58* |
| 22 | 2 000 | 11.25±1.89 | 6.50±0.76 | 0.000 01* | 0.000 05* |
| 27 | 2 500 | 13.75±1.50 | 12.25±1.83 | 0.135 03 | 0.135 03 |

ANOVA, analysis of variance; EFS, electric field strength; EP, electric potential; IZ*e*, inhibition zones for *E. coli*; IZ*s*, inhibition zones for *S .aureus*.
*Significant ($P$⩽0.05).

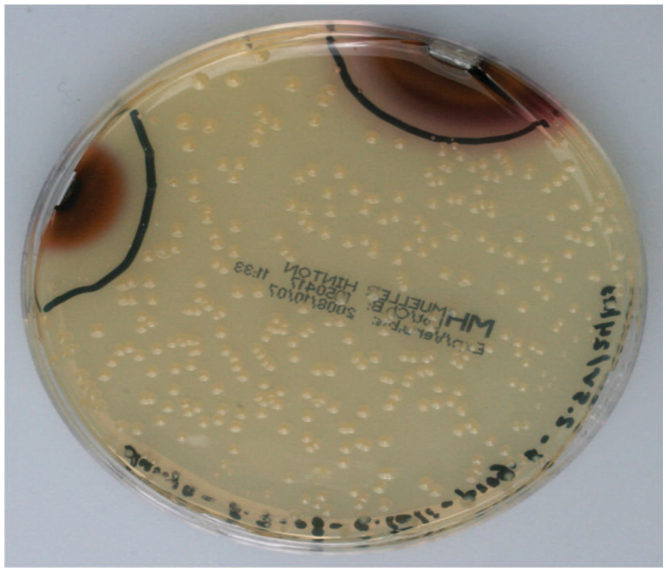

**Figure 3 Discoloration of MHA medium observed at anodes when bacteria (*E. coli*) were exposed to 27 V·m$^{-1}$.** MHA, Mueller-Hinton agar.

Discoloration of the medium was found at anodes in the experiments with 16, 22 and 27 V·m$^{-1}$ (Figure 3).

### TEM

The LDC electric field treatment applied to *S. aureus* cultures caused alterations in the morphology of cells, rupturing of membrane and loss of cell organization when compared with the control (Figures 4 and 5). Ultrastructurally, the electric field treated cells presented thinner discontinuous or ruptured cell walls with leakage of cytoplasmic material. The cytoplasmic material leaking out of *S. aureus* cells is seen as debris between the cells as shown in Figure 4a and 4b. Blebbing with disintegration of cell material, and shrinkage of the cell were also evident (Figure 5a). Furthermore, the rupturing of the membrane system with loss of cell organization was either evident in electrode treated *S. aureus* cells (Figures 4 and 5c and 5d). The insoluble gold compounds were seen directly in the cell membrane (Figure 6a), inside the cells as small black granules (Figure 6b and 6c), and indirectly as electron dense vacuoles or bodies (Figure 6b). The yellow-orange precipitate (gold crystalline deposits) in Petri dish after evaporation of TSB supports the present findings (Figure 7a and 7b). On the other hand, untreated control cells presented as compact dense cells with thick cell walls, distinct organells and normal cell division (Figures 4 and 5e and 5f).

The electric field treated *E. coli* showed little changes in cell morphology when compared to control like discontinuous cell membrane with some degrees of disintegration in cytoplasmic materials, which exhibited as vacuoles formation and non homogenous cytoplasm (Figure 8a and 8b). Moreover, the electrode treated cells showed more morphological changes characterized by granulated cytoplasm with some signs of cytoplasmic retraction and extensive vacuoles formation (Figure 8c and 8d) in contrast to the smooth and continuous double membrane structure of the untreated *E. coli* cells (Figure 8e and 8f).

### Statistical analysis

EFS of 4–10 V·m$^{-1}$ led to significant reduction in the growth of *S. aureus* ($P \leqslant 0.05$) with high reduction at 8–10 V·m$^{-1}$, and non-significant reduction in the growth of *E. coli* ($P=0.905$). Moreover, electrodes alone in absence of currents provided (in four out of five cases) significant reduction in *S. aureus* growth ($P \leqslant 0.001$) and non-significant reduction in *E. coli* growth ($P=0.348$). The difference between FSB and ESB was non-significant ($P \geqslant 0.292$) for both strains

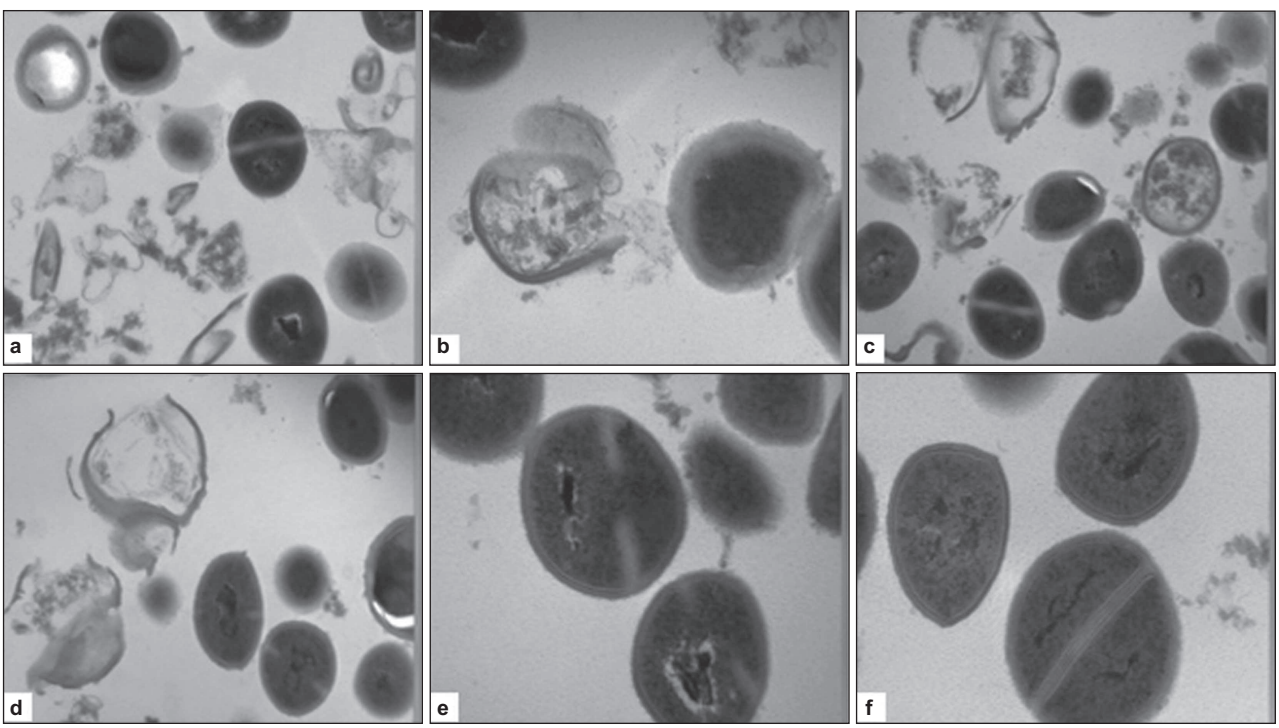


**Figure 4 *S. aureus* cells treated with 4 V·m$^{-1}$.** (**a** and **b**) Electric field-treated cells; (**c** and **d**) electrode-treated cells; (**e** and **f**) untreated cells. (**a**, **c** and **d**) ×30 000; (**b**, **e** and **f**) ×50 000.

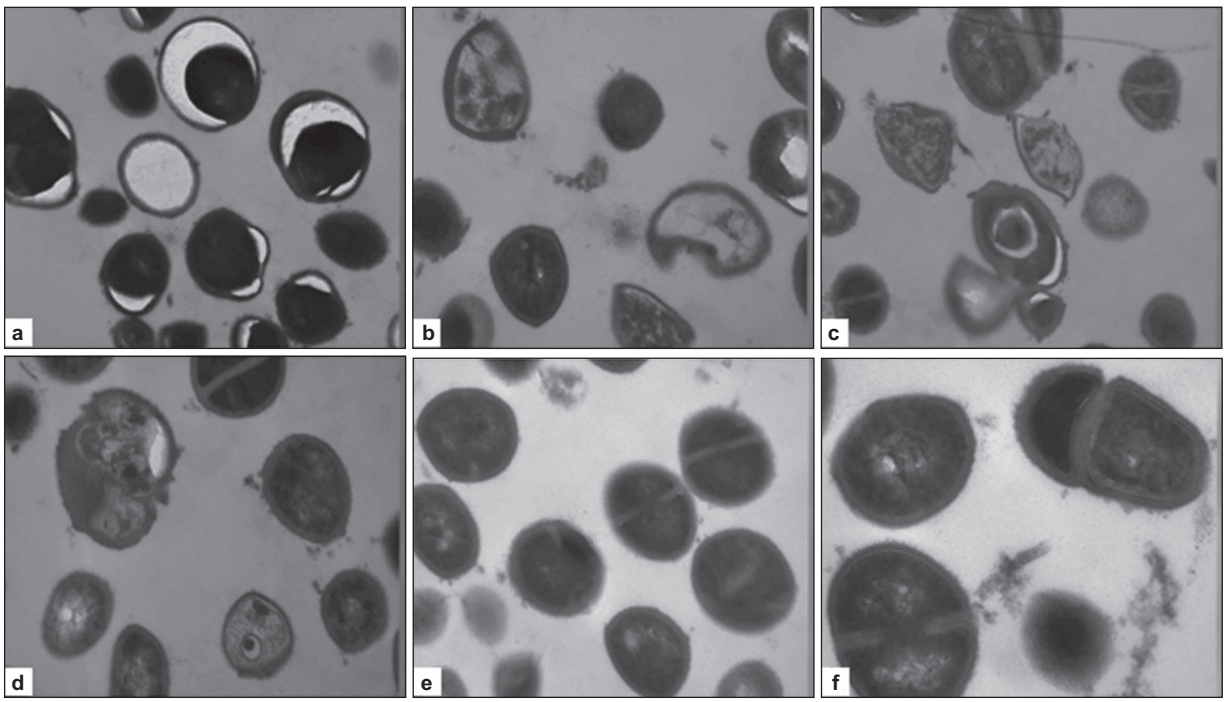


**Figure 5** ***S. aureus*** **cells treated with 6 V·m$^{-1}$.** (**a** and **b**) Electric field-treated cells; (**c** and **d**) electrode-treated cells; (**e** and **f**) untreated cells. (**a** and **c**) ×20 000; (**b**, **d** and **e**) ×30 000; (**f**) ×50 000.

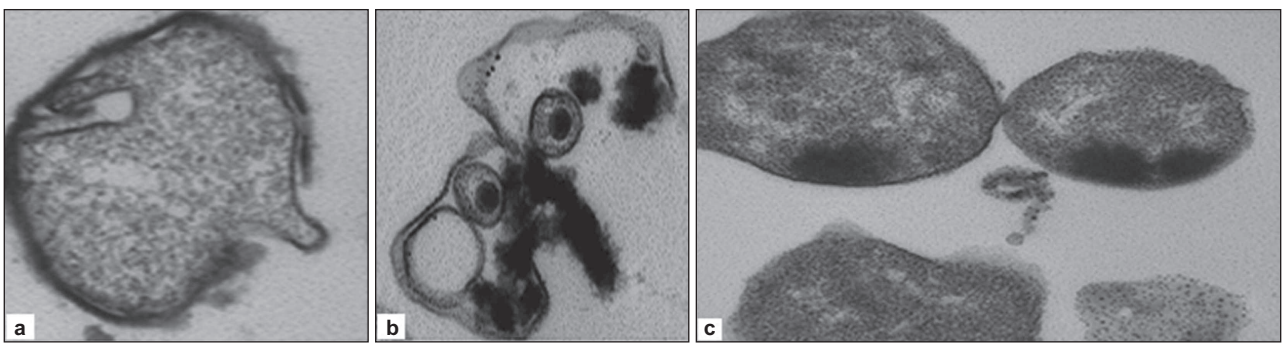


**Figure 6 Electrode treated** ***S. aureus*** **cells.** (**a**) Gold compounds precipitate in the cell membrane; (**b**) two electron dense bodies as well-defined opaque vacuoles; (**c**) gold granules as small black particles inside the cells. (**a**–**c**) ×85 000.

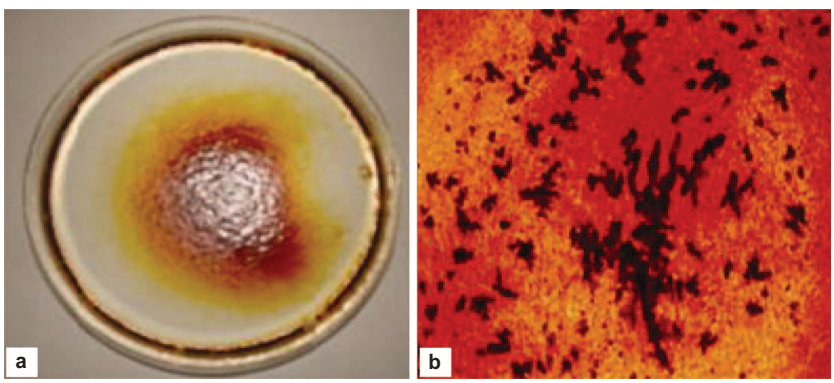


**Figure 7 The precipitation of gold after evaporation of bacterial media (TSB) of electrode-treated** ***S. aureus*** **cells.** (**a**) Macroscopic gold precipitate; (**b**) microscopic gold precipitate (×100). TSB, tryptic soy broth.

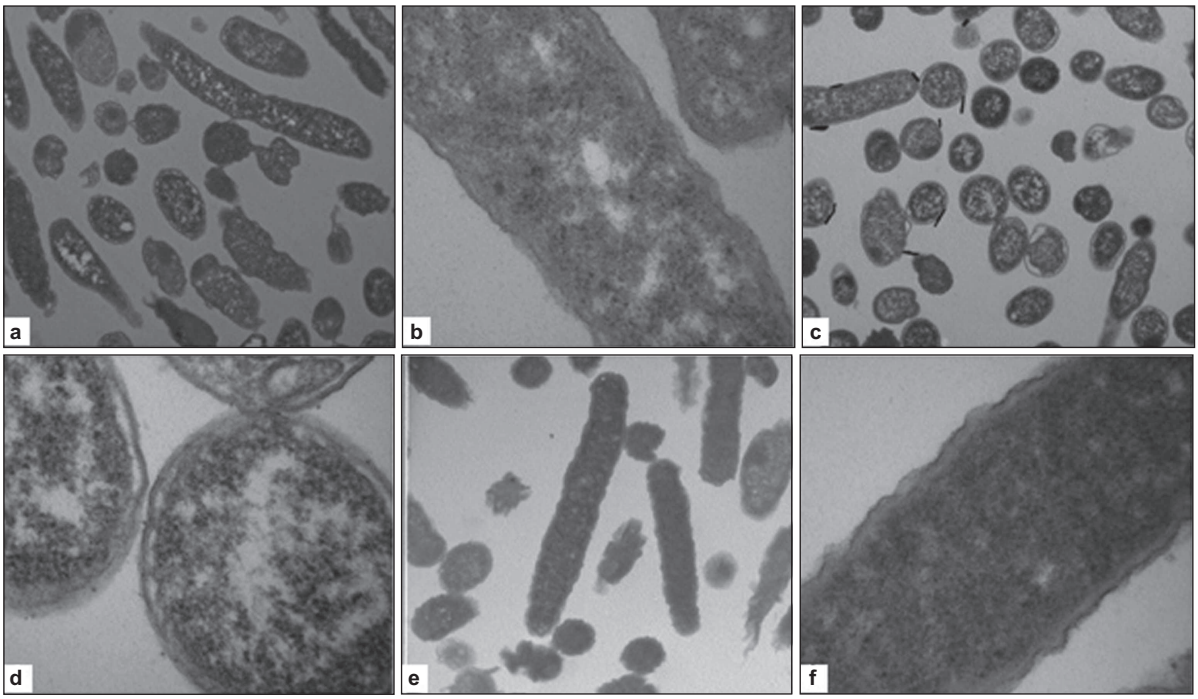


**Figure 8** ***E. coli*** **cells treated with 10 V·m$^{-1}$.** (**a** and **b**) Electric field-treated cells; (**c** and **d**) electrode treated cells; (**e** and **f**) untreated cells. (**a**, **c** and **e**) ×12 000 (**b**, **d** and **f**) ×85 000.

**Table 4** ***P*-value for viable counts in different electric field experiments using repeated measures ANOVA with Bonferroni (step-down) Holm correction**

| | | | FSB *vs.* NCB | | ESB *vs.* NCB | | |
|---|---|---|---|---|---|---|---|
| Bacteria | EFS/(V·m$^{-1}$) | EP/mV | ANOVA | ANOVA +Bonferroni-H | ANOVA | ANOVA +Bonferroni-H | FSB *vs.* ESB |
| *S. aureus* | 2 | 200 | 0.174 | 0.348 | 0.129 | 0.258 | 0.861 |
| | 4 | 400 | 0.050* | 0.15 | 0.006* | 0.018* | 0.292 |
| | 6 | 600 | 0.028* | 0.112 | 0.006* | 0.024* | 0.455 |
| | 8 | 800 | 0.006* | 0.03* | 0.002* | 0.01* | 0.578 |
| | 10 | 1 000 | 0.000 3* | 0.001 8* | 0.001* | 0.006* | 0.675 |
| *E. coli* | 10 | 1 000 | 0.905 | 0.905 | 0.348 | 0.348 | 0.399 |

ANOVA, analysis of variance; EFS, electric field strength; EP, electric potential; ESB: electrode-stimulated bacteria; FSB, field stimulated bacteria; NCB, negative control bacteria.
*Significant ($P \leqslant 0.05$).

(Table 4). Univariate ANOVA revealed significant difference between the inhibition zones for *S. aureus* and *E. coli* ($P \leqslant 0.01$) at EFS of 5, 10, 16 and 22 V·m$^{-1}$ (Table 3).

## DISCUSSION

This study investigated effects of LDC (μA) electric fields and their electrochemical products on the growth of *S. aureus* and *E. coli*. The growth behaviour of some bacterial species can be influenced by low electric fields. The antibacterial effect of both alternating and direct electric current (AC and DC) and metal electrodes have been previously demonstrated in salt solutions[21] and in synthetic urine.[22] Rosenberg *et al.*[23] noted the inhibition of *E. coli* by electrolysis products when AC has been conducted through platinum electrode. Pareilleux *et al.*[21] studied the effects of 10–200 mA on *E. coli* viability and found the minimum current required to obtain bacterial death to be 25 mA. Liu *et al.*[24] showed the antibacterial effect of LDC (10–100 μA) on *S. aureus* in agar.

In the oral cavity, bacteria adhere to surfaces to form complex communities called biofilms.[25–26] The growth of oral bacteria as a biofilm almost increases their resistance to antibacterial treatment compared with planktonic cultures that is grown in liquid media.[27] However, It is possible to manipulate bacterial–surface electrostatic interactions by changing the surface polarity, the ionic strength conditions or by the application of an electric current.[28–29] Electric manipulation of bacteria is possible since bacterial cells are generally negatively charged, which dictates their electrophoretic movement in DC fields[30] and the enhancement of reversible adhesion.[31] Poortinga *et al.*[28] reported electrical detachment of biofilm formations from surgical implants. van der Borden *et al.*[32] demonstrated that DCs of only 25–125 μA stimulate detachment of staphylococcal strains from stainless steel. Moreover, del Pozo *et al.*[33] published the marked decrease in the viability of *S. aureus*, *S. epidermidis* and *P. aeruginosa* biofilms after prolonged exposure to low-intensity electrical current of 20–2 000 μA.[33]

Regarding metal ions released from galvanic reactions, it was confirmed that the electrochemical products from low current (4.0 μA) stimulated gold electrodes in agar medium are bacteriostatic.[34] Moreover, gold iontophoresis at 400 μA was the most effective in eliminating or reducing bacterial growth.[35] Generally, direct electric currents could inhibit biofilms on metal surfaces by elimination of planktonic cells before they adhere to the surface and initiate biofilm formation (attachment process) or by their interference with colonization and growth formation processes.

The intensity of evolving DC depends on different factors such as the electrode potential, polarization and distance between the electrodes, structure of the electrode surface, aeration, temperature, pH and composition of the electrolyte. In the present study, the difference in field produced by gold electrodes in TSB ($\leqslant 3$ V·m$^{-1}$) and 0.9% NaCl normal saline (0.6 V·m$^{-1}$) was attributed to the difference in the chemical composition of media. Some electrodes may corrode and their corrosion products may interfere with the bactericidal effect of electrical current.[22] In the present study, the gold alloy was chosen as electrode material to overcome this interference with current treatment.

In fluid media, the data of FSB revealed a significant decrease in the viability of *S. aureus* with a maximum reduction in their growth at 10 V·m$^{-1}$ ($P$=0.000 3), whereas no significant effect was observed on *E. coli* ($P$=0.905) in the same EFS, although over 24 h, they did not reach its initial inocula levels. The effect of EFS is strongly dependent on the following parameters: bacterial structure, inoculum size, bacterial growth rate, growth medium composition and bacterial resistance.[35–36] Thus, the difference in resistance between *E. coli* (3.46 kΩ) and *S. aureus* (1.76 kΩ) in the same EFS of 10 V·m$^{-1}$ may be one of reasons for the difference effect of EFS on tested bacteria. The second cause might be the induction of a heat shock response (stress protein synthesis) that protects cells against environmental stimuli including electric field.[37–38]

Earlier studies were in agreement with our findings; Davis *et al.*[35–36] suggested that lower current of 325–375 μA delivered *via* gold wire could kill *E. coli* at low concentration of $1\times10^3$ CFU·mL$^{-1}$ within 2 days, but was variable in killing *E. coli* at high concentrated levels of $1\times10^7$ CFU·mL$^{-1}$. Thus, bacteria have a high survival rate in the denser inoculum than in the less-dense inoculum.[36] As the reduction in bacterial growth was related directly to the intensity of current and inversely to bacterial concentration,[35] high bacterial concentration at the beginning of the experiment ($1\times10^6$ CFU·mL$^{-1}$) should be considered as a third cause. Furthermore, Obermeier *et al.*[39] showed the reduction of *S. aureus* growth in fluid medium under the influence of DC electric field within 24 h, and they noted that the DC electric field has strongest effects. No noteworthy corrosion, pH shifts and change in temperature were seen for gold electrodes in TSB at any level of DCV induction (data not shown).

In the present study, we proposed that the applied electric field increases the electric component of membrane potential ($\Delta\Psi$, negative inside) that hindered proton translocation to the outer side of the membrane, thus lowering both pH and adenosine triphosphate (ATP) synthesis. Additionally, due to the increased $\Delta\Psi$, the transport of electrons down the transport chain would also be prevented, and reduced compounds such as nicotinamide adenine dinucleotide (NADH) or flavin adenine dinucleotide (FADH) would be accumulated.[40] These compounds would lower both rates of main oxidative metabolic pathways and the nutrient consumption.[41] This effect could be responsible for the shift-down-like response of tested bacteria to positive polarization.[40]

The data of ESB in this study revealed a significant inhibition of *S. aureus* growth ($P\leqslant0.001$), despite no current flow when compared to *E. coli* ($P$=0.348). This inhibition is probably due to the formation of electrochemical reaction products particular to gold when used as sham electrodes in fluid media (oligodynamic reaction).

Once electrodes had immersed in TSB, the negatively charged bacteria interact with gold surfaces through attractive forces (van der Waals forces, acid–base interactions and electrostatic forces),[42] and an open circuit potential of ($\leqslant0.3\pm0.07$) V were produced. The adhesion of bacteria at this potential was irreversible and increased with the time of exposition.[43] During the adhesion, bacteria are able to uptake gold ions for extracellular precipitation and transport.[44] After transport process, gold ions combine readily with intracellular fluids to form a relatively insoluble compound that indicated as black particles in TEM.[45–47] It has been demonstrated that Gram-positive bacterial cell wall is more efficient metal chelators because it contains a thick peptidoglycan layer responsible for bacterial adhesion to surfaces.[44] One of the microbial responses to metals is the synthesis of intracellular metal-binding proteins.[44]

On agar medium (MHA), the influence of electric fields on the growth of *S. aureus* and *E. coli* was represented by the formation of inhibition zones only around the anode, which confirms the antibacterial effect of EFS at the anode.[24] The zones of inhibition for *S. aureus* were larger than that for *E. coli* in all applied electric fields that clarify the ability of *E. coli* to withstand electric fields in agar medium too because of the reasons mentioned before. The gradual increase in zone's size may be due to the increase of electrochemical products that are directly proportional to the subjected DC electric field.[24] Discoloration of agar at EFS of 16, 22 and 27 V·m$^{-1}$ suggested that physical changes related to electric field (discoloration at the anode and gas formation at the cathode) occur immediately in the area surrounding electrodes.[48–49]

Electric field-treated *S. aureus* revealed more ultrastructural changes than *E. coli* that exhibited little changes in cell morphology with no significant reduction in cell count. Changes like loss of membrane integrity with leakage of intracellular contents as a result of membrane damage were observed.[24,50] Ultrastructural changes of tested bacteria are a result of their response to the environmental stresses generated by DC.[51] The large surface area, double-membrane structure (inner- and outer-membrane) and motility provide *E. coli* the ability to protect themselves against LDC electric field in comparison to non-motile *S. aureus* cocci that has a single-membrane (inner-membrane) with small spheroid surface area.

## CONCLUSION

According to the present research, it was concluded that *S. aureus* is more sensitive to increasing electric field strength than *E. coli* and the influence of EFS treatment on *S. aureus* within fluid medium was significantly higher than in gel medium. The present data suggest that changes in oral ecosystem could be generated from small chronic influence of arising galvanic currents on bacterial growth. Therefore, it is recommended to prevent the occurrence of oral galvanism by avoidance of different metallic restorations in the oral cavity. Further studies are needed to determine if these galvanic currents can produce more resistant bacteria through studying the change in their genetic material.

1 Kucerová H, Dostálová T, Procházková J *et al*. Influence of galvanic phenomena on the occurrence of algic symptoms in the mouth. *Gen Dent* 2002; **50**(1): 62–65.
2 Nogi N. Electric current around dental metals as a factor producing allergic metal ions in the oral cavity. *Nippon Hifuka Gakkai Zasshi* 1989; **99**(12): 1243–1254.

3 Toumelin-Chemla F, Lasfargues JJ. Unusual *in vivo* extensive corrosion of a low-silver amalgam restoration involving galvanic coupling: a case report. *Quintessence Int* 2003; **34**(4): 287–294.
4 Certosimo AJ, O'Connor RP. Oral electricity. *Gen Dent* 1996; **44**(4): 324–326.
5 Momoi Y, Asanuma A, Kohno A *et al*. A measurement of galvanic current and electrical potential in extracted human teeth. *J Dent Res* 1986; **65**(12): 1441–1444.
6 Nilner K, Glantz PO, Ryge G *et al*. Oral galvanic action after treatment with extensive metallic restorations. *Acta Odontol Scand* 1982; **40**(6): 381–388.
7 Ravnholt G, Holland RI. Corrosion current between fresh and old amalgam. *Dent Mater* 1988; **4**(5): 251–254.
8 Nordenström BE. *Biologically closed electric circuits (BCEC): clinical, experimental and theoretical evidence for an additional circulatory system*. Stockholm: Nordic Medical Publications, 1983: 112–172.
9 Schriever W, Diamond LE. Electromotive forces and electric currents caused by metallic dental fillings. *J Dent Res* 1952; **31**(2): 205–229.
10 Wartenberg M, Wirtz N, Grob A *et al*. Direct current electrical fields induce apoptosis in oral mucosa cancer cells by NADPH oxidase-derived reactive oxygen species. *Bioelectromagnetics* 2008; **29**(1): 47–54.
11 Söremark R, Ingels O, Plett H *et al*. Influence of some dental restorations on the concentrations of inorganic constituents of the saliva. *Acta Odontol Scand* 1962; **20**: 215–224.
12 Snyder DE. Incidence of painful oral electrogalvanism. *Gen Dent* 1987; **35**(3): 198–199.
13 Sun ZL, Wataha JC, Hanks CT. Effects of metal ions on osteoblastlike cell metabolism and differentiation. *J Biomed Mater Res* 1997; **34**(2): 29–37.
14 Wataha JC. Alloys for prosthodontic restorations. *J Prosthet Dent* 2002; **87**(4): 351–363.
15 Takahashi N. Microbial ecosystem in the oral cavity: metabolic diversity in an ecological niche and its relationship with oral diseases. *Int Congr Ser* 2005; **1284**: 103–112.
16 Bagg J, Sweeney MP, Harvey-Wood K *et al*. Possible role of *Staphylococcus aureus* in severe oral mucositis among elderly dehydrated patients. *Microbiol Ecol Health Dis* 1995; **8**: 51–56.
17 Miyake Y, Iwai M, Sugai M *et al*. Incidence and characterisation of *Staphylococcus aureus* from the tongues of children. *J Dent Res* 1991; **70**(7): 1045–1047.
18 Smith AJ, Robertson D, Tang MK *et al*. *Staphylococcal aureus* infection in the oral cavity: a 3-year retrospective analysis of clinical laboratory data. *Br Dent J* 2003; **195**(12): 701–703.
19 Ohara-Nemoto Y, Haraga H, Kimura S *et al*. Occurrence of staphylococci in the oral cavities of healthy adults and nasal–oral trafficking of the bacteria. *J Med Microbiol* 2008; **57**(Pt 1): 95–99.
20 Lee PS, Lee KH. *Escherichia coli*: a model system that benefits from and contributes to the evolution of proteomics. *Biotechnol Bioeng* 2003; **84**(7): 801–814.
21 Pareilleux A, Sicard N. Lethal effects of electric current on *Escherichia coli*. *Appl Microbiol* 1970; **19**(3): 421–424.
22 Davis CP, Wagle N, Anderson MD *et al*. Bacterial and fungal killing by iontophoresis with long-lived electrodes. *Antimicrob Agents Chemother* 1991; **35**(10): 2131–2134.
23 Rosenberg B, van Camp L, Krigas T. Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. *Nature* 1965; **205**: 698–699.
24 Liu WK, Brown MR, Elliott TS. Mechanisms of the bactericidal activity of low amperage electric current (DC). *J Antimicrob Chemother* 1997; **39**(6): 687–695.
25 Chen C. Periodontitis as a biofilm infection. *J Calif Dent Assoc* 2001; **92**(5): 362–369.
26 Costerton JW, Lewandowski Z, Caldwell DE *et al*. Microbial biofilms. *Annu Rev Microbiol* 1995; **49**: 711–715.
27 Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999; **284**(5418): 1318–1322.
28 Poortinga AT, Smit J, van der Mei HC *et al*. Electric field induced desorption of bacteria from a conditioning film covered substratum. *Biotechnol Bioeng* 2001; **76**: 395–399.
29 Ueshima M, Tanaka S, Nakamura S *et al*. Manipulation of bacterial adhesion and proliferation by surface charges of electrically polarized hydroxyapatite. *J Biomed Mater Res* 2002; **60**(4): 578–584.
30 DeFlaun MF, Condee CW. Electrokinetic transport of bacteria. *J Hazard Mater* 1997; **55**: 263–277.
31 Marshall KC, Stout R, Mitchell R. Mechanism of the initial events in the sorption of marine bacteria to surfaces. *J Gen Microbiol* 1971; **68**: 337–348.
32 van der Borden AJ, van der Mei HC, Busscher HJ. Electric block current induced detachment from surgical stainless steel and decreased viability of *Staphylococcus epidermidis*. *Biomaterials* 2005; **26**(33): 6731–6735.
33 del Pozo JL, Rouse MS, Mandrekar JN *et al*. Reduction of *Staphylococcus* and *Pseudomonas* biofilms by prolonged exposure to low-intensity electrical current. *Antimicrob Agents Chemother* 2009; **53**(1): 41–45.
34 Spadaro JA, Berger TJ, Barranco SD *et al*. Antibacterial effects of silver electrodes with weak direct current. *Antimicrob Agents Chemother* 1974; **6**(5): 637–642.
35 Davis CP, Arnett D, Warren MM. Lontophoretic killing of *Escherichia coli* in static fluid and in a model catheter system. *J Clin Microbiol* 1982; **15**(5): 891–894.
36 Davis CP, Weinberg S, Anderson MD *et al*. Effects of microamperage, medium, and bacterial concentration on iontophoretic killing of bacteria in fluid. *Antimicrob Agents Chemother* 1989; **33**(4): 442–447.
37 Mager WH, de Kruijff AJ. Stress-induced transcriptional activation. *Microbiol Rev* 1995; **59**(3): 506–531.
38 Wójcik-Sikora A, Laubitz D, Pierzynowski S *et al*. Exposure of *Escherichia coli* to intestinal myoelectrical activity-related electric field induces resistance against subsequent $UV_{254nm}$ (UVC) irradiation. *Mutat Res* 2001; **496**(1/2): 97–104.
39 Obermeier A, Matl FD, Friess W *et al*. Growth Inhibition of *Staphylococcus aureus* induced by low-frequency electric and electromagnetic fields. *Bioelectromagnetics* 2009; **30**(4): 270–279.
40 Busalmen JP, de Sánchez SR. Electrochemical polarization-induced changes in the growth of individual cells and biofilms of *Pseudomonas fluorescens* (ATCC 17552). *Appl Environ Microbiol* 2005; **71**(10): 6235–6240.
41 White D. *The Physiology and Biochemistry of Prokaryotes*. 2nd ed. New York: Oxford University Press, 1995: 534–544.
42 Hermansson M. The DLVO theory in microbial adhesion. *Colloids Surf B Biointerfaces* 1999; **14**: 105–119.
43 Busalmen JP, de Sánchez SR. Adhesion of *Pseudomonas fluorescens* (ATCC 17552) to nonpolarized and polarized thin films of gold. *Appl Environ Microbiol* 2001; **67**(7): 3188–3194.
44 Gadd GM. Heavy metal accumulation by bacteria and other microorganisms. *Cell Mol Life Sci* 1990; **46**: 834–840.
45 Seltzer S, Green DB, Weiner N *et al*. A scanning electron microscope examination of silver cones removed from endodontically treated teeth. *Oral Surg Oral Med Oral Pathol* 1972; **33**(4): 589–605.
46 Reith F, Lengke MF, Falconer D *et al*. The geomicrobiology of gold. *ISME J* 2007; **1**(7): 567–584.
47 Lengke M, Southam G. Bioaccumulation of gold by sulphate-reducing bacteria cultured in the presence of gold (I)–thiosulfate complex. *Geochim Cosmochim Acta* 2006; **70**: 3646–3661.
48 Barranco SD, Spadaro JA, Berger TJ *et al*. *In vitro* effect of weak direct current on *Staphylococcus aureus*. *Clin Orthop Relat Res* 1974; **(100)**: 250–255.
49 Merriman HL, Hegyi CA, Albright-Overton CR *et al*. A comparison of four electrical stimulation types on *Staphylococcus aureus* growth *in vitro*. *J Rehabil Res Dev* 2004; **41**(2): 139–146.
50 Shimada K, Shimahara K. Leakage of cellular contents and morphological changes in resting *Escherichia coli* B cells exposed to an alternating changes. *Agric Biol Chem* 1985; **49**: 3605–3607.
51 Sanin SL, Sanin FD, Bryers JD. Effect of starvation on the adhesive properties of xenobiotic degrading bacteria. *Process Biochem* 2003; **38**: 909–914.