Surface & Coatings Technology 394 (2020) 125912

Contents lists available at ScienceDirect

Surface & Coatings Technology

journal homepage: www.elsevier.com/locate/surfcoat

Antibacterial properties of metal and PDMS surfaces under weak electric fields

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ARTICLE INFO

Keywords:
Electrical field
Bacterial adhesion
PDMS
Metal surface
Bactericidal effect

ABSTRACT

The adhesion of several species of bacteria on two parallel rectangular electrodes under weak electric fields was studied. The electrodes were based on native metal or PDMS coated Cu. After 2 h of contact at a voltage of 0.2 to 1 V without any current, the Zn cathode showed a bacterial repellent effect with a difference in bacterial adhesion of about 1.5 to 2 log CFU/cm2 on the anode. Al electrodes were inactive due to their passivation by the alumina layer. At 1 V, both Zn and Al exhibited more than 80% mortality of suspended bacteria. The Cu electrodes showed a very high bactericidal effect even at 0 V, and the bacterial adhesion on its surface was too weak to see a difference between the two electrodes. A similar study carried out on PDMS surfaces, covering Cu electrodes, revealed that a difference of 1 log CFU/cm2 of bacterial adhesion between the cathode and anode surfaces can be obtained by applying a voltage ranging from 10 to 30 V. This cathodic repellent effect was specific to staphylococcus species, suggesting that in the presence of a PDMS coating, the electrostatic forces on the surface are too low to be the main factor governing bacterial adhesion.

1. Introduction

Microbial contamination of surfaces often leads to the formation of a biofilm, resulting in serious problems for human health (e.g. device-related infections, healthcare-related infections) as well as for industrial sectors (e.g. corrosion of metal surfaces, food contamination, deterioration of pipes…), leading to a negative socio-economic impact [1–3].

However, it should be noted that bacteria in their biofilms require antibiotic concentrations 100 to 1000 times higher than those of planktonic bacteria to achieve effective eradication [4]. Therefore, the development of materials capable of preventing the adhesion of bacteria and/or the formation of biofilms on their surface is a key element to avoid contamination. Gottenbos et al. have reported that the initial attachment of bacteria depends on the result of non-specific interactions such as Van der Waals, electrostatic, acid-base interactions and Brownian motion forces [5]. Dunne et al also reported that once the bacteria approached surfaces at a critical distance (usually less than 1 nm), adhesion is determined by the net sum of attractive or repulsive forces, including electrostatic and hydrophobic interactions, steric hindrance, van der Waals forces etc. [6].

Three types of antibacterial surfaces are described in the literature: anti-adhesive surfaces, contact-killing surfaces and release-based surfaces [7].

Anti-adhesive surfaces are designed to repel bacteria by optimizing their physico-chemical properties such as the surface charge (negative charges), the wettability or the topography (micro/nano-patterned surfaces) [8–11]. However, they cannot affect the viability of pathogen that can be released to contaminate others. Furthermore, the use of physical surface modifications (particularly surface topography) as non-specific methods to prevent the bacterial adhesion is much more complex than we can imagine [12].

Contact-killing surfaces which are generally based on cationic biocidal agents covalently bonded to substrate surfaces that attract negatively charged bacteria and kill them by disrupting their cell membrane [13–15]. However, these surfaces quickly become inactive after being buried under a layer of dead bacteria.

Released-based surfaces are designed to leach antibacterial agents that kill not only adhered bacteria but also planktonic bacteria [16–18]. The disadvantage of this kind of surfaces is its limited activity owing to the limited amount of the loaded antibacterial compounds. However, excessive use of antibiotics may lead to the phenomenon of bacterial resistance [19].

It is noteworthy that the use of an electrical method to prevent infection of medical devices, without traumatising the patient by removal of the device, is of great interest In addition, over time, bacteria should

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https://doi.org/10.1016/j.surfcoat.2020.125912
Received 3 April 2020; Received in revised form 3 May 2020; Accepted 9 May 2020
Available online 11 May 2020
0257-8972/

not adapt as well with electrical treatment as with antibiotics [20]. Direct and alternating currents [21,22] as well as acoustic (by piezoelectric actuators) [23] and radio frequency wave [24] treatments have already proven to be effective techniques to prevent initial bacterial adhesion and growth, through in vitro and in vivo experiments. On the other hand, the electrical current has been found to increase the efficacy of antibiofilm agents in a synergistic action called 'the bioelectric effect' [25–29], which can lead to a dramatic decrease in the concentration of the antibiofilm compounds used. Thus, the development of electrically stimulated surfaces that prevent bacterial adhesion and biofilm formation is an emergent and attractive pathway.

Busalmen et al. [30] have studied, using an optical microscope, the influence of an electrical field on the adhesion of *Pseudomonas fluorescens* onto the surface of gold electrodes, under flow conditions at two different ionic strengths (0.01 and 0.1 M NaCl; pH 7). By applying negative electric potentials (−0.5 and −0.2 V relative to a reference electrode [Ag/AgCl-KCl saturated solution]), they have evidenced a decrease in bacterial adhesion of one Log (CFU/mm^2) after 15 min of contact, compared to adhesion at a potential of 0.2 V. In another work [31], using the same system, they demonstrated the impact of this electrical field on the growth of planktonic cells and biofilms of *Pseudomonas fluorescens* (cell morphology, size at cell division, time to division, and biofilm structure). Gall et al. [32] have studied, using Quartz Crystal Microbalance with Dissipation analysis (QCM-D), the effect of applying an electrical field, perpendicular to the flow of a *Pseudomonas fluorescens* suspension, on the adhesion of bacterial cells to the gold electrode. Surprisingly, they demonstrated that the tested bacteria were rigidly attached to the negatively charged surface, unlike to the positively charged one, suggesting that the applied electric potential could influence the conformation of the bacterial cell surface, allowing the cells to overcome the electrostatic energy barrier.

In this study, we investigated the adhesion of different bacterial species, in a static mode (without flow), on metallic and PDMS surfaces under an electrical field (without any current), generated by two parallel metal electrodes (as in a capacitor) immersed in an aqueous suspension of bacteria. The impact of the applied electrical field on bacterial survival has also been studied.

2. Materials and methods

2.1. Materials

Sylgard® 184 was purchased from DOW chemical company (USA) and was used as the coating material. Copper, zinc and aluminum blades (10 × 100 × 1.5 mm^3, Jeulin, France) were used as electrodes. *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 35984), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (PA14) and *Escherichia coli* (K12 MG1655) strains were stored as frozen aliquots in brain heart infusion broth (BHI, Bacto, France) and 30% of glycerol at −20 °C. Ultrapure water, obtained from a Milli-Q system (Siemens, France), was used in all cases.

2.2. Preparation of PDMS-coated copper or zinc electrodes

15 g of the two parts of Sylgard® 184, i.e. silicone elastomer base and curing agent, in a ratio of 10:1 (w/w) were mixed and casted into a low-density polyethylene petri dish square (120 × 120 × 17 mm^3). Then, the mixture was degassed under vacuum, until all air bubbles were removed, and cured at 70 °C for 3 h to form a first PDMS layer. Six blades were then put on top of this layer and covered with a second layer of PDMS (20 g) using the same process. The resulting PDMS-coated electrodes (12 × 3.5 × 40 mm^3) were cut out by using a scalpel. The thickness of the coating was around 1 mm.

2.3. Preparation of bacterial suspensions

For each experiment, all bacterial species were pre-cultured in BHI at 37 °C under shaking at 140 rpm for approximately16 h. Then, bacteria were harvested by centrifugation (Sigma® 3-16KL, rotor 19776, Germany) under 1600 g for 15 min at 20 °C and resuspended at a concentration of 10^7– 10^8 CFU/ml in Milli-Q water to avoid the influence of charged particles in BHI or Phosphate Buffered Saline (PBS, Gibco, UK).

2.4. Zeta potential measurements

Zeta potentials of bacteria were measured with a Zetasizer Nano-ZS system (Malvern Panalytical, Ltd., UK) at 25 °C in Milli-Q water solution at 10^7– 10^8 CFU/ml. Three measurements were carried out for each bacterial suspension.

2.5. Assessment of bacteria attachment onto PDMS and metallic blade surfaces under an electric field

The process used to evaluate the attachment of bacteria to the surface of metallic or PDMS-coated electrodes is shown in Fig. 1. The PDMS-coated electrodes were sterilized in 70% ethanol overnight and washed twice with Milli-Q water before use. Uncoated electrodes were

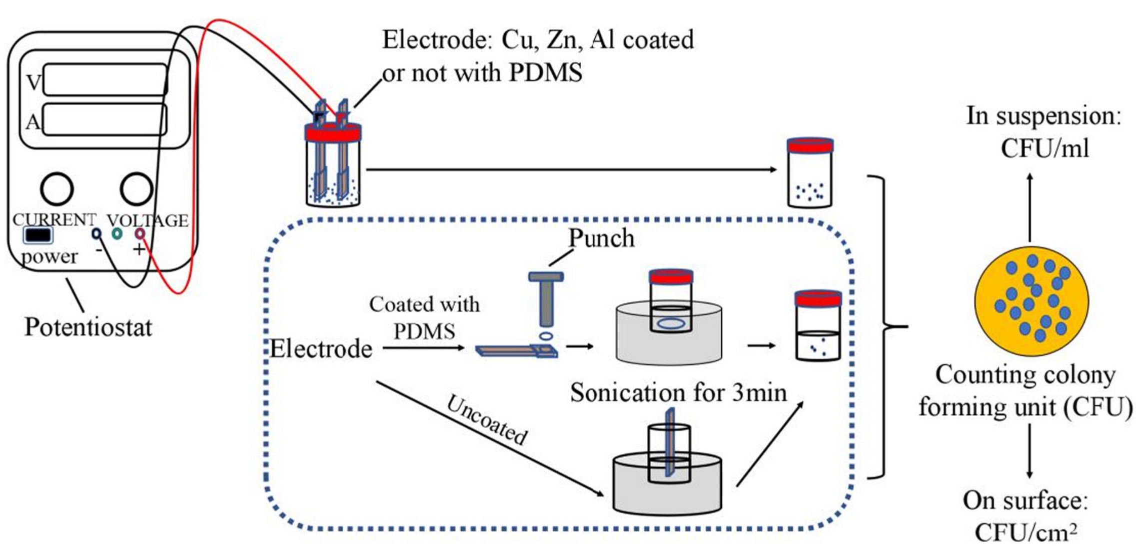

Fig. 1. Process used to evaluate the adhesion of bacteria to the surface of metallic or PDMS-coated electrodes.

sterilized in 70% ethanol for 20 min, followed by sonication for 30 min, and washed twice with Milli-Q water before use.

The bacterial adhesion was studied in a system of parallel plates with a distance of 1 cm between the two electrodes connected to the potensiostat (0–30 V, Velleman®). The electrodes were immersed (1 cm) in 7 ml of the previous prepared bacterial suspension during 2 h. Then, to remove unattached bacteria, these electrodes were washed in 15 ml of sterile Milli-Q water under slight shaking while the potentiostat was still on. They were then detached from the potentiostat. To recover the bacteria fixed to the surface, metallic electrodes were directly immersed in 7 ml of PBS solution, under sonication for 3 min. For PDMS-coated electrodes, a sample of PDMS was taken with a circular punch (Ø = 1 cm) and was immersed in 3 ml of PBS solution under sonication for 3 min. Decimal dilutions of the resulting bacterial suspensions, containing bacteria detached from the surface, as well as those where the experiments were conducted, containing unattached surviving bacteria, were spread (20 µl or 100 µl) on BHI agar plates and incubated at 37 °C for 24 h before colony counting. All experiments were performed in at least triplicate.

3. Results and discussion

Bacterial adhesion onto a surface is often explained by the Derjaguin, Landau, Verwey and Overbeek (DLVO) theory of colloidal stability. It describes initially repulsive electrical double layer interactions (electrostatic repulsion) between bacteria and similarly charged surfaces. At the same time, bacteria are attracted by van der Waals forces and approach surface contact as soon as they cross the electrostatic energy barrier by decreasing the interfacial distance. [5,6]

In this work, we aimed to use an electrical field to increase electrostatic forces between bacteria and cathodic (negatively charged) surfaces, in order to decrease bacterial adhesion and colonization. Thus, we have used two parallel electrodes, as in a capacitor, immersed in an aqueous suspension of bacteria (Fig. 2). Cu, Al and Zn were used as models for metallic electrodes; PDMS was used as representative electrodes for plastic-based biomaterials.

3.1. Bacterial adhesion to metal electrodes

The results of the enumeration of live *S. aureus* adhering to electrodes of Cu, Zn and Al, after immersion in bacterial suspensions for 2 h at an electrical voltage of 0 to 1 V, are shown in Fig. 3 (A1). Above 1 V, the presence of current and electrolysis reactions can be observed, making it unnecessary to study electrostatic interactions. The survival of suspended bacteria was also monitored before and after immersion of the electrodes (Fig. 3(A2)).

For copper electrodes at 0 V, the concentration of adhered *S. aureus* was only about 2 log of CFU/cm^2 (Fig. 3 (A1)). This low adhesion can be explained by the well-known bactericidal properties of copper [33–35], which is confirmed by the percentage of bacterial surviving in solution (Fig. 3 (A2)). Indeed, less than 20% of bacteria in solution are still alive (or cultivable) after 2 h. However, a slight difference in bacterial adhesion between anode (positively charged) and cathode (negatively charged) at 0.2 and 0.4 V was observed. Furthermore, the increase of voltage to 1 V, suppressed the adhesion *of S. aureus* onto the surface and increased the suspended bacterial mortality to more than 95%. These results suggest that the bactericidal effect of copper could be enhanced by the application of an electric field and are consistent with the “bioelectric effect” [25–29].

For the two other electrodes, a bacterial adhesion of approximately 4.5 log of CFU/cm^2 was achieved at 0 V. In the case of Al, no difference in bacterial adhesion between cathode and anode was observed regardless of the applied voltage. This could be explained by the well-known passivation of the aluminum surface by oxidation leading to the formation of a thin layer of alumina, which is an ionic salt (Al_2O_3). The electrical properties of this layer are very different from those of its pure metal, and its electrochemical static polarization is more difficult to achieve. For Zn electrodes, at all applied voltages, there was a decrease in the adhesion of *S. aureus* on the cathode compared to bacterial adhesion at 0 V. At the same time, a slight increase on the anode was observed inducing a difference of more or less 2 log of CFU/cm^2 between these two electrodes. This result suggests that electrostatic interactions are, in this case, quite predominant. Therefore, this result also indicates that bacterial adhesion could be modulated on the Zn electrodes by the use of an electric field. Moreover, for a voltage ranging from 0 to 0.4 V, an insignificant decrease, of about 10 to 40%, of live bacteria in suspension was observed for both Al and Zn electrodes. However, at 1 V, a decrease in the live bacteria population in solution of about 80% was observed for both electrodes. Soumya et al. [36] reported that potentials at about 0.9 V would affect the redox potential across the cell membrane and disrupt redox homeostasis, thereby accelerating the production of endogenous reactive oxygen species (ROS) and inhibiting bacterial growth. In summary, these results show that the nature of the electrode and the voltage values have an impact on the adhesion of *S. aureus* to the surface as well as on suspended bacterial mortality.

On the other hand, in order to determine whether the structure of the external membrane of bacteria cells has any influence on these results, we studied the adhesion of *E. coli,* as a model of Gram-negative bacteria, to zinc electrodes under voltage values ranging from 0 to 1 V.

Fig. 4 shows that *E. coli* behaves almost similarly to *S. aureus* in terms of adhesion to the Zn electrodes after 2 h of contact. Indeed, a difference in *E. coli* adhesion of about 1.5 to 2 log of CFU/cm^2 between the anode and cathode was observed. In addition, the percentage of killing of *E. coli* in suspension was also quite similar to that of *S. aureus*, i.e. low for a voltage of 0, 0.2 or 0.4 V, and higher than 80% at 1 V.

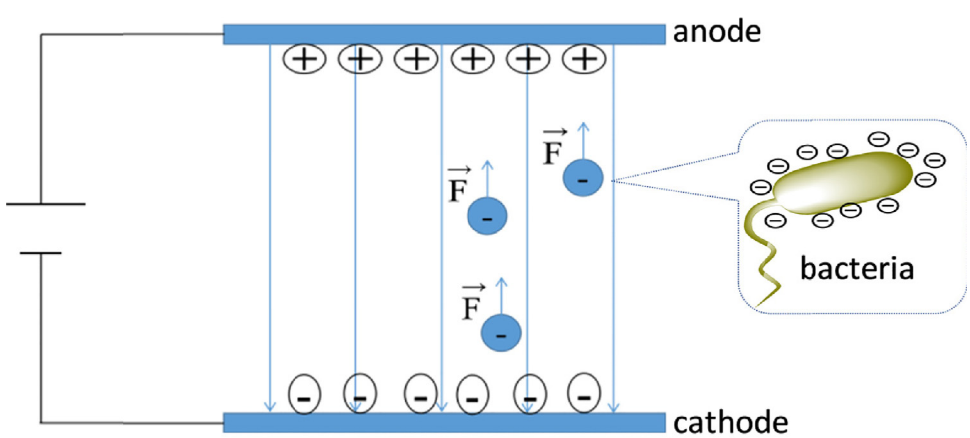

Fig. 2. Schematic principle of antibacterial action of cathode.

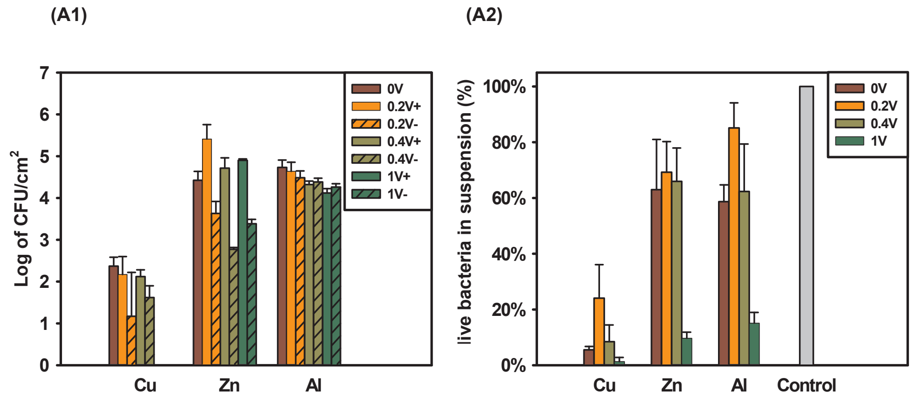

Fig. 3. Adhesion of *S. aureus* onto the surface of Cu, Zn and Al after 2 h of contact under 0, 0.2, 0.4 and 1 V: (A1) live bacteria adhered to the surface; (A2) live bacteria in suspension; control: live bacteria in suspension without any electrode.

Experiments were then carried out with different types of bacteria to verify whether these results are universal or not on zinc electrodes. The bacteria chosen are the most representative species involved in the phenomenon of contamination in the hospital environment. *S. epidermidis* and *E. faecalis* are Gram-positive bacteria and mainly cause skin and endocardial infections, respectively [37,38]. Gram-negative bacteria such as *P. aeruginosa* and *E. coli* account for more than 30% of nosocomial infections [39]. *P. aeruginosa* causes several infections in human organs such as the urinary, blood, respiratory and gastrointestinal systems [40]. *E. coli* can also cause urinary or bloodstream infections for example [41]. These experiments were performed only at 0 and 0.2 V.

As shown in Fig. 5, at 0.2 V, a difference of bacterial adhesion was obtained between the cathode and the anode for each strain, with a lower adhesion on the cathode. These results suggest that bacterial adhesion could be controlled under an electric field whatever the bacterial species. Furthermore, no significant decrease of bacterial concentration in solution was observed for all tested species.

3.2. Bacterial adhesion to the surface of PDMS under an electrical field

In a second part, we studied the bacterial adhesion, under the effect of an electric field, on the surface of PDMS, which is a material widely used as biomaterial. Previous copper electrodes were coated with a PDMS layer of a thickness around 1 mm and used as electrodes. The applied voltage ranged from 0 to 30 V, which is the maximum voltage provided by the potentiostat. The copper electrodes were chosen because they can easily reveal whether the metal surface was completely covered by the PDMS or not due to the disappearance of the bactericidal activity of the Cu in suspension.

The results of the adhesion of *S. aureus* to the surface of the PDMS under an electric field are shown in Fig. 6. It can be seen that without an electrical voltage, the bacterial adhesion was about 3 log CFU/cm^2, which is higher than its value on the surface of the uncoated copper (Fig. 3). The difference in bacterial adhesion between cathode and anode at 1 and 5 V was not significant. On the other hand, at higher applied voltages (10 to 30 V), a lower bacterial adhesion on the cathode, around 2 log CFU/cm^2, was observed, probably due to the electrostatic repulsion between the negative charges on the surface of the PDMS and the negatively charged bacterial cells. In addition, the percentage of live bacteria in suspension was approximately 90% regardless of the applied voltage, which is quite consistent with the percentage observed in milli-Q water. These results show that the PDMS coating on the copper electrodes leads to (i) the disappearance of the bactericidal effect of copper in the bacterial suspension, meaning that the electrodes were fully coated, and (ii) no significant bacterial stress at applied high voltage, probably due to the chemical and electrical insulating properties of PDMS. Consequently, the applied voltage must be increased above 30 V to achieve a greater anti-adhesion effect.

The study of adhesion of different bacterial species onto PDMS surfaces was also carried out at 30 V (Fig. 7). No copper bactericidal activity was detected for all species. However, *S. epidermidis* appears to be quite sensitive to the applied electric field and showed a reduction of about 50% in the concentration of live bacteria in suspension. For surface adhesion, only *S. epidermidis* showed the same behaviour as *S. aureus* with a difference of about 1 log CFU/cm^2 between cathode and anode. This result is in contradiction with the previous result on the zinc electrodes at 0.2 V. To understand this specificity of the

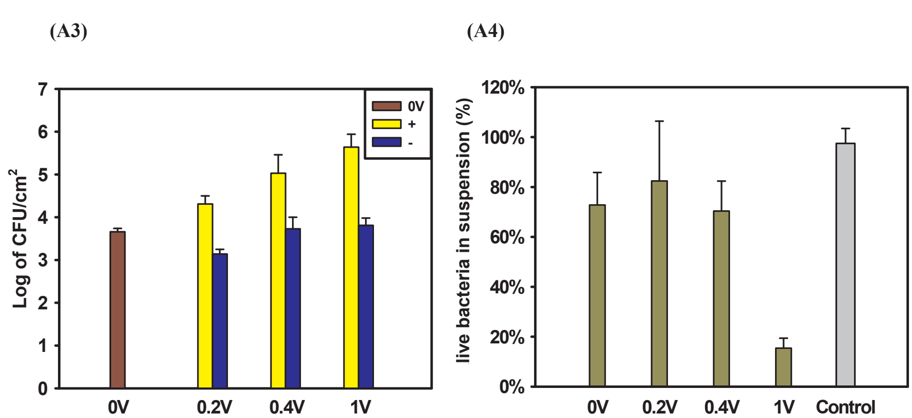

Fig. 4. Adhesion *of E. coli* to the surface of zinc electrodes after 2 h of contact at voltage values between 0 and 1 V: (A3) live bacteria adhered to the surface; (A4) live bacteria in suspension; control: live bacteria in suspension without any electrode.

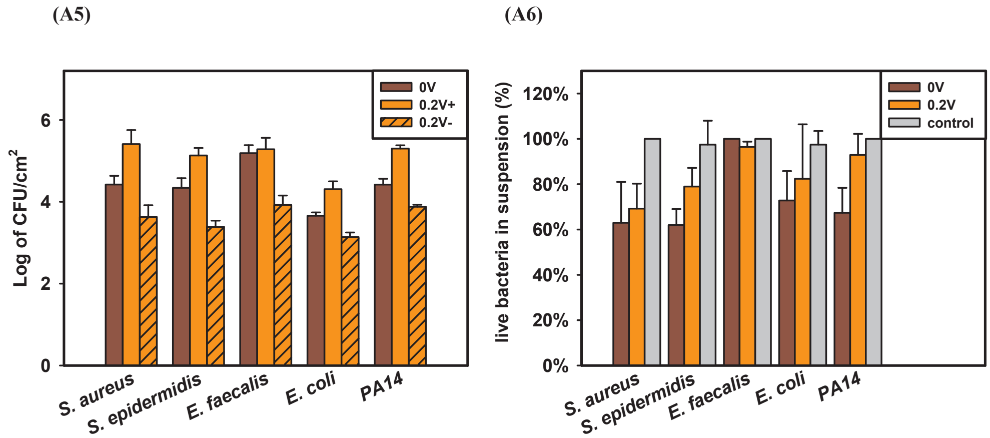

Fig. 5. Adhesion of different bacterial species to the surface of zinc after 2 h of contact at voltage values of 0 and 0.2 V: (A5) live bacteria adhered to the surface; (A6) live bacteria in suspension, control: live bacteria in suspension without any electrode.

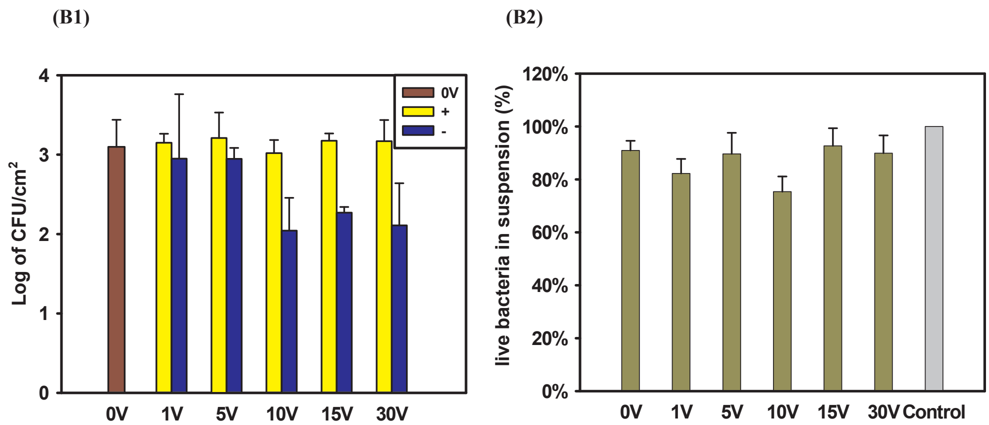

Fig. 6. Adhesion of *S. aureus* to the PDMS-coated copper surface (1 mm) after 2 h of contact at voltage values between 0 and 30 V: (B1) live bacteria adhering to the surface; (B2) live bacteria in suspension; control: live bacteria in suspension without electrode.

Staphylococcus genus, measurements of the zeta potential of the bacterial membrane were performed (Table 1).

As expected, all bacterial membranes are negatively charged, but *E. coli* and *P. aeruginosa* bacteria have the lowest zeta potential and therefore the highest overall negative charge. Theoretically, with a higher negative charge, *E. coli* and *P. aeruginosa* should undertake stronger repulsive electrostatic interactions with the PDMS surface, resulting in lower adhesion to the anode surface, which is in contradiction with our experimental results (Fig. 7). Therefore, these observations suggest that, in the presence of a PDMS coating, the electrostatic interactions are too weak to be the predominant factor governing bacterial adhesion. There are two possible reasons why Staphylococcus species are more sensitive to the low surface polarization of PDMS: (1) they are Gram+ bacteria, which have only one external phospholipidic membrane, unlike Gram- bacteria, which have two; (2) they are spherical in shape with a lower contact surface, while

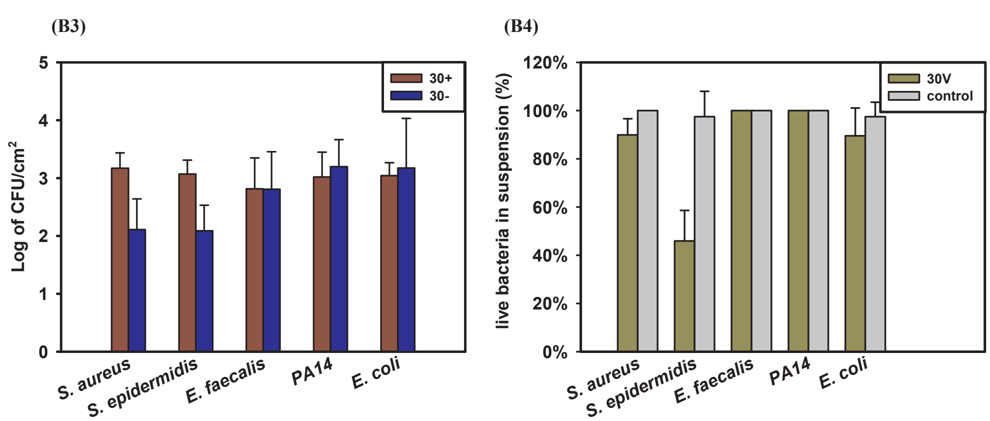

Fig. 7. Adhesion of different bacterial species to the surface of PDMS coated copper (1 mm) after 2 h of contact at 30 V: (B3) live bacteria adhering to the surface; (B4) live bacteria in suspension; control: live bacteria in suspension without any electrode.

Table 1
Zeta potential of bacteria in sterile milli-Q water at a concentration of 10^7–10^8 CFU/ml.

Bacteria	Zeta potential (mV)	Percentage
S. aureus (ATCC 29213)	−39.3 ± 0.9	100%
S. epidermidis (ATCC 35984)	−38.5 ± 1.7	51.80% ± 0.02
	−26.8 ± 1.2	48.20% ± 0.02
E. faecalis (ATCC 29212)	−35.8 ± 1.0	100%
E. coli (K12 MG1655)	−50.6 ± 1.0	100%
P. aeruginosa (PA 14)	−41.6 ± 0.9	100%

the other bacteria are bacillus-shaped.

4. Conclusion

In this work, we have studied the bacterial adhesion of Gram positive (*S. aureus, S. epidermidis and E. faecalis*) and Gram negative (*E. coli* and *P. aeruginosa*) bacteria to metal and PDMS electrode surfaces under an electric field. After 2 h of contact with each of these bacteria, the Zn electrodes showed a difference of 1.5–2 log of UFC/cm^2 between cathode and anode at voltages of 0.2 and 0.4 V, without significant killing of bacteria in suspension. Under the same conditions, the Al electrodes were found to be inactive in limiting and directing bacterial adhesion, probably because of surface oxidation causing its passivation. However, at 1 V, the Zn and Al electrodes became bactericidal by killing bacteria in suspension. On the other hand, the bactericidal effect of Cu electrodes was very high at 0 V and seems to be reinforced by the electrical filed. Nevertheless, the bacterial adhesion on its surface was too weak to see a difference between the cathode and the anode. Once coated with PDMS, the bactericidal effect of Cu disappeared and a difference of 1 log of UFC/cm^2, specific to staphylococcus species, was observed between the two electrodes above 10 V. Finally, this study demonstrates that the electrostatic force is the predominant factor governing the bacterial adhesion to Zn surfaces but not to those of Al and PDMS. For future works, high voltage should be used in order to increase the electrostatic bacterial repellent effect onto PDMS surfaces. Furthermore, metal electrodes could be used as physical disinfecting agents at 1 V without current.

CRediT authorship contribution statement

Yuzhen Lou:Investigation, Formal analysis, Data curation, Writing - original draft.**Pascal Thebault**:Conceptualization, Methodology, Validation, Supervision, Writing - review & editing.**Fabrice Burel**:Project administration, Writing - review & editing.**Nasreddine Kebir**:Funding acquisition, Project administration, Conceptualization, Methodology, Validation, Supervision, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was funded by China Scholarship Council (CSC).

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