

# Effect of Electric Currents on Bacterial Detachment and Inactivation

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**ABSTRACT:** Since biofilms show strong resistance to conventional disinfectants and antimicrobials, control of initial bacterial adhesion is generally accepted as one of the most effective strategies for preventing biofilm formation. Although electrical methods have been widely studied, the specific properties of cathodic, anodic, and block currents that influence the bacterial detachment and inactivation remained largely unclear. This study investigated the specific role of electric currents in the detachment and inactivation of bacteria adhered to an electrode surface. A real-time bacterial adhesion observation and control system was employed that consisted of *Pseudomonas aeruginosa* PAO1 (PAO1) with green fluorescent protein as the indicator microorganism and a flow cell reactor mounted on a fluorescent microscope. The results suggest that the bacteria that remained on the electrode surface after application of a cathodic current were alive, although the extent of detachment was significant. In contrast, when an anodic current was applied, the bacteria that remained on the surface became inactive with time, although bacterial detachment was not significant. Further, under these conditions, active bacterial motions were observed, which weakened the binding between the electrode surface and bacteria. This phenomenon of bacterial motion on the surface can be used to maximize bacterial detachment by manipulation of the shear rate. These findings specific for each application of a cathodic or anodic electric current could successfully explain the effectiveness of block current application in controlling bacterial adhesion.

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**KEYWORDS:** electric current; bacterial adhesion; inactivation; detachment

## Introduction

The formation of biofilms causes many problems such as biofouling in underwater structures (Flemming, 2002), biocorrosion in pipelines (Videla and Herrera, 2005), and infections in medical devices (Thomas et al., 2006). Once established on a surface, biofilms are often difficult to control and nearly impossible to eradicate by treatment with conventional antimicrobials and disinfectants (Costerton et al., 1995). Therefore, it is generally accepted that one of the most effective strategies for controlling biofilm formation is prevention of bacterial adhesion at an initial stage in biofilm formation (Bos et al., 1999).

Although there are various approaches for controlling bacterial adhesion, a great deal of attention has been paid to electro-assisted methods (Busalmen and de Sánchez, 2001; Nakayama et al., 1998a; van der Borden et al., 2005). Electrical methods for controlling bacterial adhesion are regarded to be environmental friendly because they use “electrons” as the nontoxic reaction mediator. These methods can be applied extensively to any conductive surface that has a low electrical energy (Rajeshwar and Ibanez, 1997). The electrical methods used for controlling bacterial adhesion can be divided into current and potential applications, and each application can be conducted in the cathodic, anodic, and block (or alternating) modes. On an electrically conductive surface over which current or potential is applied, adhesion of the bacterial cells is

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governed by three major forces, that is, electrostatic, electrophoretic, and electroosmotic (Poortinga et al., 2001). Utilization of the electro-repulsive interaction between the negative surface charge of bacteria and the cathodic surface has been applied to extensively study the prevention (Busalmen and de Sánchez, 2001; van der Borden et al., 2007) and detachment (van der Borden et al., 2004a,b, 2005) of initial bacterial adhesions. One study reported that more than 75% of initially adhering staphylococci could be stimulated to detach from surgical stainless steel by the application of less than  $6.0 \mu\text{A}/\text{cm}^2$  of cathodic or block current (van der Borden et al., 2004a). However, it was noted that the detached bacteria could again accumulate on the surface through redeposition, resulting in continuation of the bacterial adhesion problem.

On the other hand, it has been suggested that direct electron transfer between bacteria and the anode results in the inactivation of adhered bacteria present on the anodic surface. This information has been used to control marine biofouling (Matsunaga et al., 1984; Nakayama et al., 1998a). It has also been reported that bacterial clusters are formed when an anodic current is applied ( $50 \mu\text{A}/\text{cm}^2$ ; Poortinga et al., 2000). When an anodic current or potential is applied, the inactivated bacteria tend to remain on the surface. In such cases, fouling from the inactivated bacteria on the surface can provide seeds for bacterial adhesion (Wagner et al., 2004). Thus, the control of bacterial adhesion through the exclusive application of anodic current is still limited.

To overcome the limitations associated with the application of direct constant current (cathodic or anodic), the application of block current or potential, which involves the utilization of cathodic and anodic currents or potentials in turns, has been recently suggested as an effective approach for bacterial detachment and inactivation (Nakayama et al., 1998b; van der Borden et al., 2004b, 2005; Wake et al., 2006). It was also noted that one of the disadvantages of direct current over block current was the presence of excess ions on the surface of electrode, which can lead to negative osteogenesis and fixation. Further, block current is better than direct current in terms of heat dissipation. Thus, the application of block current was suggested to be as promising as the application of direct current in preventing the infection of medical implants (van der Borden et al., 2005). Despite many studies on bacterial adhesion control using electric currents, there was no report on the specific role of each electric current to detach or inactivate the adhered bacteria with the purpose of developing the optimal strategy for controlling bacterial adhesion.

The objectives of this study were to investigate the specific role of electric currents in bacterial detachment and inactivation when a constant current was applied in the cathodic, anodic, and block modes. Bacterial motion was also examined quantitatively. Indium tin oxide (ITO)-coated glass was used as the conductive surface of bacterial adhesion since it is a transparent electrode on which bacterial adhesion can be observed through a fluorescent

microscope. *Pseudomonas aeruginosa* PAO1 (PAO1) was used as the indicator microorganism.

## Materials and Methods

### Preparation of the Microorganism

PAO1 tagged with green fluorescent protein was obtained from the Center for Biofilm Engineering (Montana State University, USA) and was used in this study. Due to the presence of the green fluorescent protein, this strain can be observed under a fluorescent microscope. PAO1 was streaked on a tryptic soy agar (Difco, Franklin Lakes, NJ) plate containing 150 mg/mL of carbenicillin (Aldrich, St. Louis MO). The plate was incubated for 48 h at  $37^\circ\text{C}$  to obtain a pure strain. Subsequently, it was inoculated in 3 g/L of tryptic soy broth (Difco) containing 100 mg/mL of carbenicillin and cultured for 18 h at 110 rpm and  $37^\circ\text{C}$ . PAO1 was harvested by centrifugation for 10 min at 4,500 rpm to remove the nutrients, and the bacterial pellet was washed twice with 20 mM potassium phosphate buffer ( $\text{KH}_2\text{PO}_4$ , pH 7.1). Finally, the bacterial pellet was resuspended in 20 mM potassium phosphate buffer. The initial population of PAO1 was adjusted to  $1.0\text{--}1.5 \times 10^8$  CFU/mL by using the spread plate method. The zeta potential of PAO1 was measured as  $-20$  mV using the ELS-8000 Electrophoretic Light Scattering (Photal Otsuka Electronics, Osaka, Japan) system at 160 Hz and 80 V. This result indicates that PAO1 is a negatively charged bacterium like most other bacteria.

### Preparation of Electrode Materials and the Flow Channel

To observe bacterial behavior during the application of an electric current, ITO—a popular transparent conductive material—was coated on glass (VWR Scientific, West Chester, PA). An RF sputter (A Tech Co., Seoul, Korea) at 5 m Torr, 100% Ar gas, and 300 W power for 8 min was used to obtain ITO of thickness 200 nm. ITO-coated glass was employed as the working electrode on the top side and as the counter electrode on the bottom side of the flow channel. Both electrode surfaces had electrical resistances of 26–44 ohms, and their optical transmittances were sufficient for observing bacterial adhesion through a fluorescent microscope.

A flow cell reactor (FC 81, Bio Surface Technologies, Bozeman, MT) was used as the flow channel in this study. The top and bottom plates of the flow channel were connected to a potentiostat (PARSTAT 263, Princeton Applied Research, Oak Ridge, TN) to enable application of the electric current. To prevent leakage of the solution, silicone gaskets were placed between the flow channel and top/bottom covers. The contact area of the working electrode with the solution was  $6.5 \text{ cm}^2$ . The distance between the top and bottom was 3.0 mm. The use of a

reference electrode was precluded due to space limitations in the flow channel. With the exception of the ITO-coated glass surfaces, the flow channel and top/bottom covers consisted of nonconductive materials.

## Experimental Procedures

The real-time bacterial adhesion observation and control system employed in this study is shown in Figure 1. The FC 81 flow cell was mounted on a fluorescent microscope (Eclipse 80i, Nikon, Japan) to monitor bacterial adhesion to the ITO-coated glass surface. All fluids were flowed at a shear rate of  $1.11 \text{ s}^{-1}$  ( $1.3 \text{ mL/min}$ ) through the flow channel by means of a peristaltic pump (Gilson, Middleton, WI). Shear rate was calculated from the following equation in a rectangular flow displacement system (Busscher and van der Mei, 2006):

$$\sigma = \frac{3Q}{2(h_0/2)^2 w_0}$$

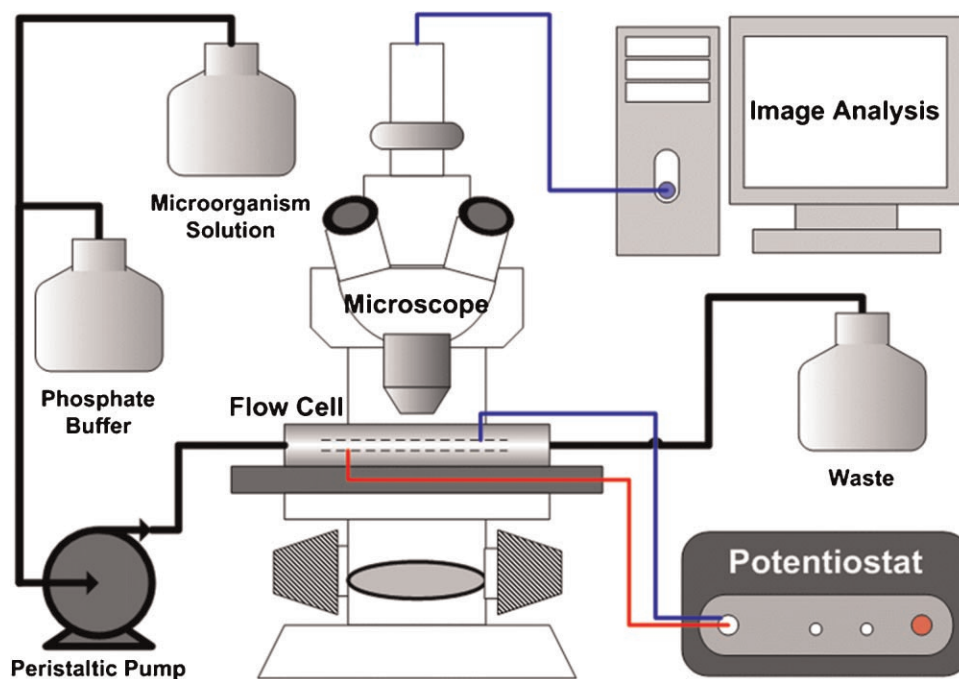
$\sigma$ , shear rate;  $Q$ , volumetric flow rate;  $h_0$ , height of channel;  $w_0$ , width of channel.

In this study, height and width of channel were 3 and 13 mm respectively. Twenty millimolars cell-free potassium phosphate buffer was flowed for 30 min to stabilize the system. The bacterial solution was then flowed for 90 min to enable PAO1 to adhere to the surface. This was followed by rinsing of the flow channel with 20 mM cell-free potassium phosphate buffer for 30 min to remove

suspended and weakly adhered bacteria from the system. The initial PAO1 population that adhered to the electrode surface was approximately  $2.4\text{--}2.7 \times 10^7$  bacteria/cm<sup>2</sup>. After initial bacterial attachment, constant electric currents (cathodic, anodic, and block) of  $15 \mu\text{A/cm}^2$  were applied for 40 min at  $1.11 \text{ s}^{-1}$  shear rate to investigate the PAO1 detachment ratio in each experiment. In this study, the block current was generated by the application of cathodic and anodic currents in turns at predetermined time intervals with no duty cycle (van der Borden et al., 2005). Simultaneously, images were captured from five equidistant locations on the electrode surface by using a digital camera (DS-2U, Nikon, Japan). The average number of bacteria that adhered per unit area (bacteria/cm<sup>2</sup>) in these locations was calculated using an image processing software (*i-solution*, IMT Technologies, Korea). Standard deviations of the number of bacteria over one substratum were noted in graphs. The results are expressed as percentages of adhered bacteria and are calculated as follows: adhered bacteria (%) = [(the number of bacteria remaining on the surface of the electrode after application of an electric current)/(the number of bacteria that initially adhered to the surface of the electrode prior to the application of an electric current)]  $\times 100$ .

## Viability Testing

In order to examine the viability of bacteria that remained on the electrode surface after application of a current, a



**Figure 1.** The real-time bacterial adhesion control and observation system consisting of a fluorescent microscope, computer-based image capturing unit, and a potentiostat for the application of an electric current. [Color figure can be seen in the online version of this article, available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

viability test was conducted using the Live/Dead BacLight bacterial viability test kit (Molecular Probes, Carlsbad, CA). The flow channel was filled with staining fluid for 15 min in the dark in order to stain the bacterial cell membrane. It was then rinsed with 20 mM potassium phosphate buffer to reduce the interference due to background fluorescence. Living bacteria appeared green when observed under a fluorescent microscope (1,000 $\times$ ) with a fluorescein isothiocyanate filter, while inactivated (or dead) bacteria appeared red with a tetramethylrhodamine isothiocyanate filter.

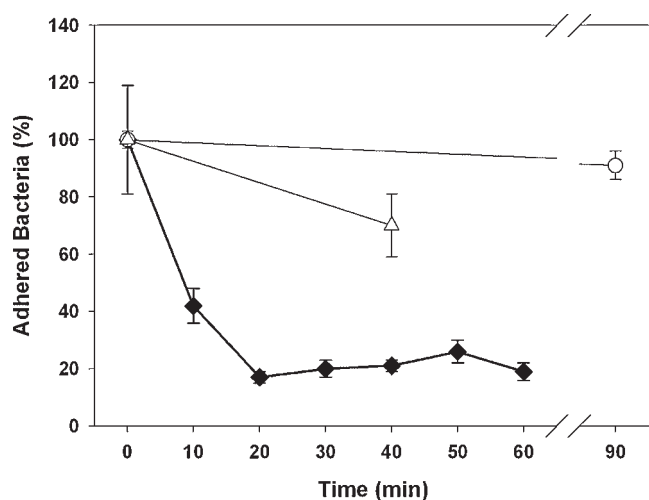
### Tracking of Bacterial Motion

To quantify the motion of the bacteria that had adhered to the electrode surface, a multiple-particle tracking (MPT) method was employed, which measures the mean square displacements by calculating the two-dimensional (2-D) coordinates,  $(x, y)$  of the bacterial cell centroids (Apgar et al., 2000). Bacterial motions were monitored using a CCD camera (C9100-02, Hamamatsu, Japan) mounted on an inverted microscope (IX71, Olympus, Japan) at 600 $\times$  for 20 s at a rate of 30 frames per second. The bacterial cell positions were matched frame-by-frame in order to identify each cell and generate its 2-D trajectory. Frame-by-frame matching assumed that the closest bacterial cell in the next frame is the same cell.

## Results and Discussion

### Effect of Electric Currents on Bacterial Detachment

Figure 2 shows the detachment levels of the PAO1 population when constant cathodic and anodic currents



**Figure 2.** Detachment of PAO1 during the application of a current (15  $\mu\text{A}/\text{cm}^2$ ;  $\blacklozenge$ , cathodic current;  $\triangle$ , anodic current;  $\circ$ , no current) at shear rate 1.11  $\text{s}^{-1}$ .

(15  $\mu\text{A}/\text{cm}^2$ ) were applied. As shown in Figure 2, when a cathodic current was applied, PAO1 detachment occurred rapidly during the first 20 min, resulting in the detachment of approximately 80% of the PAO1 population that had initially adhered to the surface. However, no further significant detachment was observed after 20 min (symbol in Fig. 2). On the other hand, when an anodic current (15  $\mu\text{A}/\text{cm}^2$ ) was applied, approximately 70% of the PAO1 cells that had initially adhered to the surface remained on it after 40 min ( $\Delta$  symbol in Fig. 2). Note that under conditions where no current was applied, the level of the PAO1 population that had adhered to the surface was stable during the 90-min observation period ( $\circ$  symbol in Fig. 2).

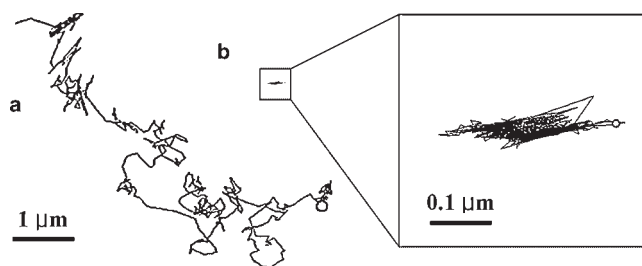
Application of a cathodic current is known to promote the detachment of bacteria from the electrode surface as a result of the electrostatic and electrophoretic repulsive forces generated (Poortinga et al., 2001). Our result in which about 80% detachment was observed is consistent with that of the previous studies of *Staphylococcus epidermidis* (van der Borden et al., 2004a,b, 2005). However, the observation that 20% of PAO1 still remained on the electrode surface indicates the existence of a population of bacteria that binds strongly to the solid surface of the electrode despite the presence of electrostatic and electrophoretic repulsive forces. This behavior can be explained by the uneven distribution of the magnitude of adhesion forces between the bacteria and the surface, although detailed quantitative information cannot be provided. This interpretation was further supported by a previous report where it was suggested that population heterogeneity in single-strain microbial cultures may influence microbial adhesion to surfaces (van der Mei and Busscher, 2001). The PAO1s still remained on the electrode surface may play an important role of additional adhesion site of suspended bacteria, and the detached PAO1s may re-adhere to the regions of less electro-repulsive forces. Thus, bacterial detachment using only constant cathodic current has limitation as an effective adhesion control method. It is necessary to control the bacteria still remained on the electrode surface.

When an anodic current ( $\Delta$  symbol in Fig. 2) was applied, the PAO1s that had adhered to the surface moved in random directions across it (data not shown). During the application of the anodic current, PAO1 motion was observed continuously. This motion is in contrast to the “bacterial clustering” that was observed with *Streptococcus salivarius* HB-C1 in a previous study (Poortinga et al., 2000). As comparing with the previous study, it was assumed that the bacterial clustering or random motion may depend on the intensity of electric current. In order to examine this assumption, the higher anodic current (30  $\mu\text{A}/\text{cm}^2$ ) was applied to the bacteria adhered electrode surface. Then, PAO1s made clusters on the surface, which was a similar result to that of Poortinga et al. (2000). In contrast, bacterial random motion was observed when applied 15  $\mu\text{A}/\text{cm}^2$ . It is considered that the extent of making bacterial clustering or movement on anodic electrode surface is related to the intensity of anodic current density which generates electro-

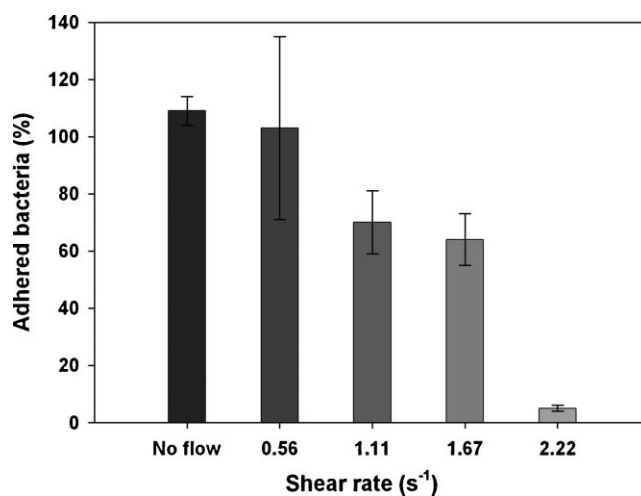
attractive forces and electro-osmotic forces. Due to the continuous movement of the PAO1 cells under the anodic current, it was difficult to distinguish the adhered bacteria from the moving bacteria on the surface. Then, the result was obtained only when application of the current was terminated in 40 min, as shown in Figure 2.

To obtain a better understanding of bacterial motion on an anodic surface, the motion of PAO1 was tracked using MPT. Figure 3 shows the 2-D trajectories of one PAO1 cell that had adhered to the anodic surface. This cell was randomly selected for 20 s, and its trajectory was compared with that observed in the absence of current. The average moving distance of the PAO1 cell on the anodic surface ( $2.87 \mu\text{m}$ ) was approximately 19 times longer than that on the surface with no current ( $0.15 \mu\text{m}$ ). Considering the size of a PAO1 cell to be  $1.5 \mu\text{m}$ , PAO1 on the anodic surface appeared to move at a rate that was approximately 10% of its length per second. To the best of our knowledge, this is the first report in which bacterial motion was quantitatively measured when a current was applied.

The bacterial motion observed under the anodic current, as shown in Figure 3, can be interpreted as the random directional motion of bacteria on the surface. There are two significant forces to be considered as anodic current is applied: One is electro-attractive forces (electrostatic and electrophoretic), and the other is electro-osmotic forces. Electro-attractive forces between negatively charged bacteria and positively polarized electrode surface work perpendicular directions to the anodic surface, attract the suspended bacteria, and make bacteria remain on the electrode surface. At the same time, electro-osmotic forces work to lateral directions to the anodic surface. Therefore, combination of both electro-attractive forces and electro-osmotic forces may drive bacterial random motion. Previous studies about polystyrene particle clustering support that lateral forces are regarded to be mainly electro-osmotic forces that are locally generated on the electrode surface (Böhmer, 1996; Trau et al., 1996). Furthermore, it was noted that although the lateral forces does not directly stimulate desorption, lateral movement of adhered bacteria over the electrode surface is associated with desorption (Poortinga et al., 2001). The lateral forces that drive bacterial motion may be associated



**Figure 3.** The 2-D trajectories of a PAO1 cell for 20 s. (a) Application of an anodic current and (b) no current (○ means final location of the tracing cell after 20 s).



**Figure 4.** Bacterial detachment during the application of an anodic current ( $15 \mu\text{A}/\text{cm}^2$ ) as a function of the shear rate for 40 min.

with the weakening of the binding force between bacteria and the surface. These forces can be used to detach a certain amount of bacteria by increasing the shear rate.

Figure 4 shows the extent of PAO1 detachment during the application of an anodic current when the shear rate was varied up to  $2.22 \text{ s}^{-1}$ . The results of Figure 2 are included in Figure 4 for comparison. As shown in Figure 4, the faster shear rate was, the easier bacteria cells detached; however, it was not in directly proportional. Bacteria that adhered weakly or were moving appear to be swept away from the surface when the shear rate was increased. When the shear rate was below  $0.56 \text{ s}^{-1}$ , almost no bacterial sweeping was observed. In contrast, a shear rate of  $2.22 \text{ s}^{-1}$  resulted in almost complete detachment of adhered PAO1s from the electrode surface. These sensitive changes in the extent of bacterial detachment were not observed when there was no current or when a cathodic current was applied.

The results shown in Figure 4 indicate that the shear rate can be an important factor that affects the level of bacteria during the application of an anodic current. These results are consistent with the interpretation from Figure 2 where it was shown that the binding force between bacteria and the solid surface varies widely. However, it would be premature to suggest that the distribution of such a force will follow a normal distribution. Although this is highly probable, it would require further investigation. Moreover, since it was reported that an increase in the shear rate during the application of a cathodic current resulted in an increase in bacterial detachment (van der Borden et al., 2004a), adjustment of the shear rate in combination with the application of an electric current may be used to effectively control bacterial adhesion.

Block current, the utilization of cathodic and anodic current in turns, has been regarded as an alternative to cathodic and anodic currents. The magnitude of detachment

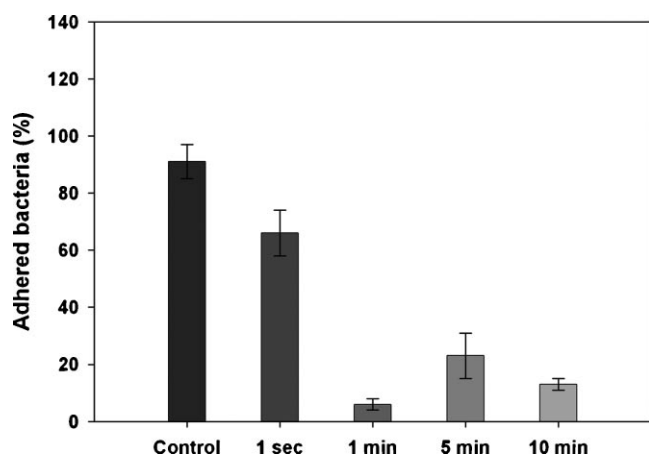
during the application of block current was larger than that observed with anodic current but similar to that obtained with cathodic current. The anodic current can reduce the binding energy, resulting in weakening the adhesion force of the adhered bacteria, then the consecutive cathodic current easily remove weakly adhered bacteria at anodic current. When using the block current method, the exchange time interval should be considered with respect to the generation of electrostatic, electrophoretic, or electroosmotic forces involved in the detachment of the adhered bacteria. The duty cycle is also a control factor (van der Borden et al., 2004b, 2005). However, in this study, the duty cycle was not considered because the primary focus was on the properties of each type of electric current in the effective control of bacterial adhesion.

Figure 5 shows the extent of PAO1 detachment as a function of the exchange time intervals of the block current. The exchange time varied from 1 s to 10 min during the application of a constant block current ( $15 \mu\text{A}/\text{cm}^2$ ). Figure 5 shows that when exchange time intervals longer than 1 min were employed, most of the PAO1s that had adhered to the electrode surface were detached. This result suggests that the detachment effect is the same or more than that observed with a cathodic current (Fig. 2). This could be due to an exchange time interval. With regard to the exchange time interval, if the time for changing the surface charge of the electrode is not sufficient, the potential required to promote bacterial detachment at the cathode and bacterial movement at the anode may not be reached. To further investigate this, the potential profiles were monitored with respect to bacterial behavior in two cases where the same block current ( $15 \mu\text{A}/\text{cm}^2$ ) was applied but in which different exchange time intervals (1 min and 1 s) were used. When the exchange time interval was 1 min, the potential difference applied between the top and bottom electrodes was measured to be 2.4 V, and bacterial motion

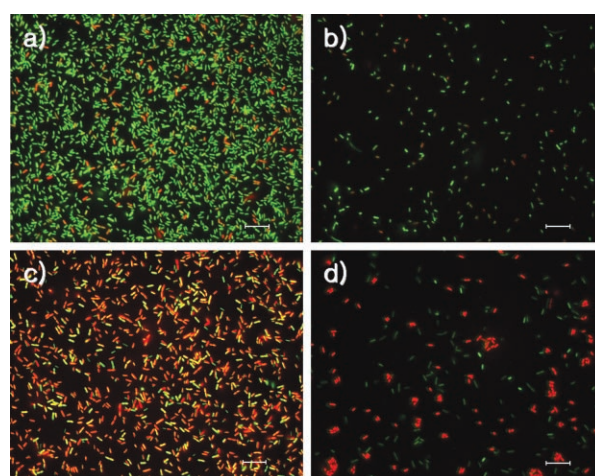
was observed in the anodic current along with vigorous bacterial detachment. In this potential, it was considered that electrostatic forces and electro-osmotic forces are sufficient to enhance bacterial movement and detachment. However, when the exchange time interval was 1 s, the potential difference was 0.5 V. It is because anodic (or cathodic) potential changes into cathodic (or anodic) potential before reaching the potential generating bacterial movement or detachment in that short exchange time interval. Even though current density and its application time were same with 1 min exchange time interval, the small potential difference at 1 s exchange time interval could not draw bacterial motion and detachment. This indicates that although the exact electrode potential could not be determined due to the absence of a reference electrode, a specific electrode potential is required to stimulate bacterial motion and detachment, and thus, proper exchange time interval is needed to obtain more effective bacterial detachment.

### Effect of Electric Currents on Bacterial Inactivation

To investigate the viability of the bacteria that remained on the solid surface after application of an electric current, the viability of PAO1 cells was measured using the Live/Dead BacLight kit. In Figure 6, the viability of PAO1 cells that remained on the electrode surface is compared with respect to each of the three current modes, which were each applied at  $15 \mu\text{A}/\text{cm}^2$  for 40 min. As expected, no significant inactivation of bacteria was observed under conditions where no current was applied (Fig. 6a). Figure 6b shows that the application of a cathodic current appears to result in the retention of live PAO1 on the electrode surface apart from achieving the significant detachment demonstrated in



**Figure 5.** Bacterial detachment resulting from the application of a block current as a function of exchange time intervals for 40 min at shear rate  $1.11 \text{ s}^{-1}$ . The x-axis represents the exchange time intervals between the cathodic and anodic currents.



**Figure 6.** Live/dead staining images of PAO1 cells that remain on the electrode surface after application of the electric current for 40 min (green color → live; red color → inactivated or dead). (a) No current (control), (b) cathodic current, (c) anodic current, and (d) block current (scale bar:  $10 \mu\text{m}$ ).

Figure 2. The result shown in Figure 6b contradicts that of previous studies in which the application of a cathodic current resulted in a bactericidal effect that may be attributed to the formation of hydrogen peroxide at the cathode by an electrochemical reaction in the presence of oxygen (Liu et al., 1997; van der Borden et al., 2004b). However, in this study, it was interpreted as that electrochemical reactions such as hydrogen peroxide production hardly occurred within 2.4 V cell potential between two electrodes, from the result of ITO cyclic voltammetric analysis which was stable between  $-0.5$  and  $2.0$  V (vs. Ag/AgCl) in 20 mM potassium phosphate buffer (pH 7.1). Even though hydrogen peroxide is generated locally on the cathodic surface, it is not likely to be involved in killing the adhered bacteria, significantly, such a short application time (40 min) of a cathodic current. This is in contrast with the previous report on the effects of bactericide produced after application of the current for 16 h (Liu et al., 1997). The viability of the remaining bacteria can be explained on the basis of no dominant electrochemical reaction for generating bactericide such as hydrogen peroxide production or due to short cathodic current application time.

If the cathodic current is unable to inactivate the bacteria that has adhered to the surface, the viable bacteria remaining on the solid surface or growth of the bacteria re-deposited on the solid surface (van der Borden et al., 2004b) will result in continuation of the bacterial adhesion problem. When an anodic current was applied (Fig. 5c), as much as 85% of the PAO1s that remained on the surface were found to be inactivated. This finding was confirmed by the difference in the color. A similar behavior was observed in the previous studies using marine Gram-negative bacterium *Vibrio alginolyticus*, which was explained on the basis of the direct electron transfer reaction of coenzyme A (CoA) that exists in the bacterial cell wall thereby leading to the inactivation of bacteria (Matsunaga et al., 1984; Nakayama et al., 1998a). The PAO1 population that adhered to the surface continued to be inactivated by application of the anodic current although the magnitude of detachment was not as much as that observed with the cathodic current as demonstrated in Figure 2.

Figure 6d shows the viability of PAO1 when a block current was applied. As shown in this figure, bacterial inactivation during the application of block current was occurred, but it was not larger than that obtained by application of an anodic current. It is because total application time of anodic current was half in comparison with only anodic current application, and direct electron transfer reaction might be obstructed by repetitive electric current change. However, in the point of view of bacterial adhesion control, bacterial detachment as well as inactivation is important. Therefore, block current can be effective adhesion control strategy, since it can achieve both bacterial detachment and inactivation.

In this study, bacterial detachment and inactivation under electric currents were investigated using *Pseudomonas*

*aeruginosa* PAO1 strain. Therefore, it needs to be cautious to generalize bacterial detachment and inactivation characteristics found in this study as tested only one strain. The detachment pattern of PAO1 was similar to that of *Staphylococcus epidermidis*, but the inactivation of PAO1 when applied cathodic current was different from the previous studies (van der Borden et al., 2004b, 2005). This difference could be due to not only the differences of electrode material and experimental conditions but also the differences of bacterial strains since metabolic pathways or cell surface charges vary depending upon strains. Thus, it is necessary to investigate the detachment and inactivation by the effect of electric current with other strains in order to further generalize the results in this study.

## Conclusions

This study clarified the properties of electric currents that contribute to the detachment or inactivation of bacteria upon application of cathodic, anodic, and block currents. Application of cathodic current promotes the detachment of adhered bacteria by electro-repulsive forces, but bacteria remained on the surface are still viable. On the other hand, the anodic current inactivates most of the remaining bacteria and weakens the binding energy of such bacteria by generating bacterial motion which may be driven by lateral forces. If bacterial motion under an anodic current is properly controlled by manipulating the shear rate, detachment effect can be obtained at that current. Through these roles of cathodic and anodic currents, a block current can achieve bacterial detachment under a cathodic current and could also result in bacterial motion and inactivation under an anodic current as long as a proper exchange time interval was employed. This is the best electrical strategy for reducing bacterial adhesion and also explains why the application of a block current can be more effective in controlling bacterial adhesion.

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## References

- Apgar J, Tseng Y, Fedorov E, Herwig MB, Almo SC, Wirtz D. 2000. Multiple-particle tracking measurements of heterogeneities in solutions of actin filaments and actin bundles. *Biophys J* 79:1095–1106.
- Böhmer M. 1996. In situ observation of 2-dimensional clustering during electrophoretic deposition. *Langmuir* 12:5747–5750.
- Bos R, van der Mei HC, Busscher HJ. 1999. Physico-chemistry of initial microbial adhesive interactions—Its mechanisms and methods for study. *FEMS Microbiol Rev* 23:179–230.
- Busalmen JP, de Sánchez SR. 2001. Adhesion of *Pseudomonas fluorescens* (ATCC 17552) to nonpolarized and polarized thin films of gold. *Appl Environ Microbiol* 67:3188–3194.
- Busscher HJ, van der Mei HC. 2006. Microbial adhesion in flow displacement systems. *Clin Microbiol Rev* 19(1):127–141.

- Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. 1995. Microbial biofilms. *Annu Rev Microbiol* 49:711–745.
- Flemming HC. 2002. Biofouling in water systems—Cases, causes and countermeasures. *Appl Microbiol Biotechnol* 59:629–640.
- Liu WK, Brown MR, Elliott TS. 1997. Mechanisms of the bactericidal activity of low amperage electric current (DC). *J Antimicrob Chemother* 39:687–695.
- Matsunaga T, Namba Y, Nakajima T. 1984. Electrochemical sterilization of microbial cells. *Bioelectrochem Bioenerg* 13:393–400.
- Nakayama T, Wake H, Ozawa K, Kodama H, Nakamura N, Matsunaga T. 1998a. Use of a titanium nitride for electrochemical inactivation of marine bacteria. *Environ Sci Technol* 32:798–801.
- Nakayama T, Wake H, Ozawa K, Nakamura N, Matsunaga T. 1998b. Electrochemical prevention of marine biofouling on a novel titanium-nitride-coated plate formed by radio-frequency arc spraying. *Appl Microbiol Biotechnol* 50:502–508.
- Poortinga AT, Bos R, Busscher HJ. 2000. Controlled electrophoretic deposition of bacteria to surfaces for the design of biofilms. *Biotechnol Bioeng* 67:117–120.
- Poortinga AT, Smit J, van der Mei HC, Busscher HJ. 2001. Electric field induced desorption of bacteria from a conditioning film covered substratum. *Biotechnol Bioeng* 76:395–399.
- Rajeshwar K, Ibanez JG. 1997. *Environmental electrochemistry*. San Diego, CA: Academic Press.
- Thomas JG, Litton I, Rinde H. 2006. Economic impact of biofilms on treatment costs. In: Pace JL, Rupp ME, Finch RG, editors. *Biofilms, infection, and antimicrobial therapy*. Boca Raton, FL: CRC Taylor & Francis. pp. 21–38.
- Trau M, Saville DA, Aksay IA. 1996. Field-induced layering of colloidal crystals. *Science* 272:706–709.
- van der Borden AJ, van der Mei HC, Busscher HJ. 2004a. Electric-current-induced detachment of *Staphylococcus epidermidis* strains from surgical stainless steel. *J Biomed Mater Res* 68B:160–164.
- van der Borden AJ, van der Werf H, van der Mei HC, Busscher HJ. 2004b. Electric current-induced detachment of *Staphylococcus epidermidis* biofilms from surgical stainless steel. *Appl Environ Microbiol* 70:6871–6874.
- van der Borden AJ, van der Mei HC, Busscher HJ. 2005. Electric block current induced detachment from surgical stainless steel and decreased viability of *Staphylococcus epidermidis*. *Biomaterials* 26:6731–6735.
- van der Borden AJ, Maathuis PGM, Engels E, Rakhorst G, van der Mei HC, Busscher HJ, Sharma PK. 2007. Prevention of pin tract infection in external stainless steel fixator frames using electric current in a goat model. *Biomaterials* 28:2122–2126.
- van der Mei HC, Busscher HC. 2001. Electrophoretic mobility distributions of single-strain microbial populations. *Appl Environ Microbiol* 67:491–494.
- Videla HA, Herrera LK. 2005. Microbiologically influenced corrosion: Looking to the future. *Int Microbiol* 8:169–180.
- Wagner VE, Kobersteinb JT, Bryer JD. 2004. Protein and bacterial fouling characteristics of peptide and antibody decorated surfaces of PEG-poly(acrylic acid) co-polymers. *Biomaterials* 25:2247–2263.
- Wake H, Takahashi H, Takimoto T, Takayanagi H, Ozawa K, Kadoi H, Okochi M, Matsunaga T. 2006. Development of an electrochemical antifouling system for seawater cooling pipelines of power plants using titanium. *Biotechnol Bioeng* 95:468–473.