Harnessing electrical energy for anti-biofilm therapies: effects of current on cell morphology and motility

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ABSTRACT
Preferentially altering bacterial migration could be a successful approach for augmenting the natural wound-healing process. Inducing electrotactic behaviours through the application of physiologically safe currents is one possible strategy for altering bacterial movement. By controlling bacterial movement at the site of infection, healing times and the severity/extent of bacterial infection could be reduced. Here, we deployed microfluidics and atomic force microscopy to determine the effect of an applied electrical current on bacterial motility and cell morphology in wound pathogens namely Pseudomonas aeruginosa, Escherichia coli, and methicillin-resistant Staphylococcus aureus (MRSA). Nanoscale imaging combined with microfluidic platforms allows for the study of single-cell swimming dynamics. Current values of 0, 0.07, and 0.125 mA were applied to bacteria in suspension. E. coli exhibited an increase in directionality and a drop in mean cellular velocity across all voltages. P. aeruginosa showed a significant decrease in mean cellular velocity at 0.07 mA, while all currents increased the directionality of movement. Electrical current had no statistically significant effect on the width or length of individual bacterial cells. A better understanding of how electrical stimulation affects pathogenic bacteria at wound sites may lead to advancements in electrotherapy and help to identify new alternatives to traditional antimicrobial approaches.

KEYWORDS
biofilms; atomic force microscopy; microfluidics; bacteria; electrotherapy

1. Introduction
The pervasive use of antimicrobials has increased the prevalence of antimicrobial-resistant strains, making it increasingly more difficult to clear infections caused by pathogenic bacteria. This inability to effectively treat infections can lead to severe health complications for affected individuals. For these reasons, the World Health Organisation (WHO) [1] has labelled antimicrobial resistance as one of the greatest threats currently facing humanity. The WHO has outlined several principal areas of focus to help slow the rise in drug-resistant bacteria on a global scale, which include, national action plans, enhanced surveillance to identify and track resistant bacteria, better laboratory facilities to analyse population risks, maintenance of high-quality antimicrobials, strict regulations for antimicrobial usage, public awareness, and
prevention programmes [1]. In 2015, the WHO released a comprehensive report on the progress of the global community in dealing with the rising threat of antimicrobial resistance. However, public awareness remains low and antimicrobial use often does not adhere to the strict rules set forth by the WHO [1]. Therefore, antimicrobial resistance remains a chief concern and other strategies are necessary to overcome the problem of growing antimicrobial resistance.

The problem of antimicrobial resistance is costly; according to the Centres for Disease Control (CDC), complications associated with antimicrobial resistance have cost the United States in excess of $55 billion [2]. Furthermore, the CDC estimates that as many as 1 in every 100 individuals infected with resistant strains will succumb to their illness [3]. Antimicrobial resistance is a chief factor that contributes to the occurrence of recalcitrant infection, where otherwise curable infections become chronic and life-threatening [3]. New strategies that will overcome recalcitrance without further contributing to bacterial resistance will be the key to combating this new microbial threat.

Another chief contributor to recalcitrant infection is the formation of biofilms. Planktonic bacteria attach to surfaces (e.g. a wound) to form a microbial community, which, by definition, is inherently resistant to antimicrobials. Microbial appendages, such as pili and flagella help facilitate attachment. Once adhered to the surface, these bacteria produce/accumulate extracellular polymeric substances (EPS) and begin to propagate across the surface. The final result is a complex three-dimensional structure that is highly recalcitrant to removal [4]. Most naturally occurring biofilms consist of vibrant and expansive communities of various bacteria, fungi, and other microbes [4]. This complexity is yet another reason biofilm is difficult to treat and remove [5–9]. Areas where skin is damaged and vulnerable provide opportunities for pathogens to bind to the wound site. Opportunistic pathogens such methicillin-resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa thrive in these environments and are responsible for prolonged healing times in compromised individuals with both acute and chronic infections [6–8]. P. aeruginosa is a prevalent Gram-negative, rod-shaped bacterium that is highly motile in solution and on surfaces through use of a single polar flagella and Type IV pili, respectively [10]. MRSA is a non-motile, Gram-positive bacterium that is highly resistant to antimicrobials and is prevalent at wound sites in compromised individuals.

Bacterial taxis refer to the preferential migration of a cell due to a gradient of some factor in the environment. The type of factor determines the type of taxis (e.g. mechanotaxis, chemotaxis or electrotaxis) [11]. The ability to alter a bacterial movement could potentially lead to new methods of preventing bacterial infection, simply by directing bacterial cells to move away from a human tissue surface, such as wound. Over the past 20 years, the field of microfluidics has seen substantial growth. Devices with channels that are tens to hundreds of micrometres in dimension allow for small, highly controllable environments that can be functionalised to represent biological systems, such as capillaries in the human body. In addition to functionality, microfluidic platforms require extremely low quantities of volume for testing [12]. Due to these benefits, microfluidics research has been enormously influential in the study of bacterial taxis [13–18]. Combining microfluidics with optical microscopy, general migration patterns and the heterogeneous motility dynamics of bacteria can be tracked and analysed [16]. Due to the scale and flow dynamics inherent in microfluidic systems, they are an ideal testing environment for wound models.
Controlling the directionality of bacterial migration could be used to develop new applications for wound healing [19]. Manipulating bacterial electrotaxis is an emerging method that is gaining attention, because it can shunt bacterial migration towards a specific direction and is largely harmless to the environment in which it is utilised. Biological electrical fields aid in the forced directionality of cellular migration during wound healing. When a wound is created, natural barriers form over the wound surface to separate the healing tissue from the surrounding environment. The cells within this barrier become polarised as they transport ions [20]. This polarisation creates a natural electrical potential within the environment, known as bioelectricity. For this reason, artificially created devices that can produce low levels of electrical potential and current, similar to those naturally produced by the body, could enhance the function of this naturally formed barrier.

Our study focuses on the application of an electrical current as a means of controlling bacterial movement to understand how these perturbations affect the physical characteristics of the cell. A better understanding of this phenomenon could decrease our reliance on antimicrobials by helping to prevent biofilm formation at wound sites. Electrotherapy could serve as a potential alternative or could augment the chemical treatments currently utilised in the medical field. Previous studies on this topic have only focused on large bacterial populations. Therefore, our study was designed to get in-depth and critical look at how individual cell motility dynamics and kinetics are affected by the application of electricity.

2. Materials and methods

2.1. Optical imaging

Optical imaging was performed on the Nikon TI-U Eclipse Microscope Inverted Microscope, using Nikon NIS-Elements software. Cellular tracking was performed using ImageJ and the manual tracking plugin: Chemotaxis and Migration Tool 2.0 ibidi software. Ibidi μ.-icoslide III microfluidic channels were used for observation of single bacterial cells. All measurements were analysed using Origin 9.1: Data Analysis and Graphing Software for statistical analysis.

2.2. Bacterial cultures and electrical perturbation of motile bacteria

MRSA, P. aerugionsa and E. coli strains were obtained from the Centre for Public Health and Zoonoses (CPHAZ) of the Ontario Veterinary College of the University of Guelph of Canada. All the bacterial strains were streaked onto 5% blood agar plates, while samples were then taken from bacterial colonies using an inoculation loop and introduced into 5 mL of tryptic soy broth (TSB) for culturing. Samples were shaken at 37 ℃ at a rotational speed of 200 rpm, for approximately 5 h. One millilitre of bacterial solution was then pipetted into a microfuge tube and centrifuged at 1000 ×g for 5 min. Excess liquid was then decanted into a waste beaker and 1 mL of deionised water was used to resuspend the cells. This process was then repeated again. One hundred microlitre of the resuspended cells were then transferred to a fresh microfuge tube using a pipette and 900 μL of
Deionised water was added to create a 1:10 dilution for imaging. This was standardised using optical density measured using photo-spectrometer. Alteration of the amount of dilution was necessary for proper and efficient imaging. The bacterial solution was then introduced into the microfluidic channel. A copper electrode was placed in the outlet and inlet of the microfluidic device. Using a current amplifier, consistent electrical currents of 0.0, 0.075, and 0.125 mA were then applied to the microfluidic system. Optical imaging and video capture was then performed for both the *P. aeruginosa* and *E. coli* populations. *E. coli*, although often not harmful to humans, except in cases of foodborne illness, is a highly studied motile organism, which makes it an ideal tool for these studies.

### 2.3. Application of electrical potential for morphology studies

One hundred microlitre of suspended, undiluted bacterial solutions were then pipetted into a Teflon well with a copper cathode and anode. Separate *P. aeruginosa* and MRSA populations were then exposed to currents as follows: 0, 0.075, 0.125, and 0.175 mA for 60 s. The solutions were then retrieved using a pipette and deposited onto gelatin-coated sheets of mica for approximately 30 min. After incubating, the surfaces were gently rinsed using 1 mL of deionised water. The substrates were then left to dry overnight to be imaged the following day.

### 2.4. Atomic force microscopy

Samples were imaged using the Agilent 5500 series AFM, at room temperature (Figure 1). Agilent Type II MACLever tapping mode tips were used to image: force constant = 2.8 N/m, resonant frequency = 75 kHz, tip diameter ≤ 10 nm.

*Figure 1. Basic setup for an atomic force microscope. The laser is reflected off the back of the cantilever, onto the photodetector. The laser position is monitored by a feedback loop as the tip scans the surface, bending and twisting in relation to the surface morphology.*
2.5. Method for measurement acquisition of *P. aeruginosa* and methicillin-resistant *S. aureus*

A total of 20 bacterial cells were measured for each current value for *P. aeruginosa* and MRSA. Measured bacterial cells were chosen based on a lack of adjacent bacterial cells directly in contact with them (Figure 2). Bacterial cells clearly undergoing cell division were also excluded. In this, the ideal bacterial cells for measurement are single cells, separate from the bulk population.

3. Results and discussion

3.1. Effect of applied current on bacterial motility

Exposure to an electrical current changes cellular velocity in *P. aeruginosa* and *E. coli*; the velocity of a single *P. aeruginosa* cell decreases significantly when a 0.075 mA current is applied (Table 1). However, this decrease does not occur with the application of higher voltage (0.125 mA). *E. coli* cells, on the other hand, show notable decreases in mean cellular velocity at 0.075 and 0.125 mA, while the magnitude of change is highest when a 0.075 mA current is applied (Table 1).

Our findings agree with previous observations that current affects various bacterial species, while different species react uniquely to an electrical field. *E. coli* and *P. aeruginosa*, both motile organisms with single polar flagella, have been shown to migrate toward different electrodes when exposed to an electrical field [21]. *E. coli* will move towards the anode, while *P. aeruginosa* will move towards the cathode. One possible explanation is that surface roughness can impact overall bacterial charge and electrotactical behaviour. Bacterial cell membranes have a naturally negative surface potential [22], such that a

| Table 1. Values for mean cellular velocities for bacteria during exposure to electrical current. |
|---------------------------------------------------------------|
| Current (DC) | 0.0 mA | 0.07 mA | 0.125 mA |
| Mean cellular velocities (μm/s) | *P. aeruginosa* | 44 ± 3 | 34 ± 2 | 40 ± 2 |
| | *E. coli* | 9.7 ± 0.5 | 5.0 ± 0.4 | 6.0 ± 0.4 |

*Figure 2.* Methods for examining cellular velocity and directness. Atomic force microscope images of *P. aeruginosa* after application of 0.075 mA (A) and MRSA after application of 0.125 mA (B). Preferably single cells, having no adjacent cells, were chosen for dimensional measurements.
bacterial strain with a rougher surface will have a greater negative surface potential than a strain with a smoother surface. A rough strain will be more negatively charged than the flagellar bundle, causing the bacterial cell to move towards the anode at a faster rate, positioning the flagella behind the cell. This orientation causes the bacterium to be propelled towards the anode [21]. Conversely, in a smooth strain, the flagellar bundle is more negatively charged than the membrane, causing the flagella to be gradually pulled ahead of the bacteria towards the anode, and the cell is then propelled towards the cathode instead [21]. Another explanation involves eukaryotic migration; a cell exposed to an electrical current generated with two electrodes, where one side is closer to the anode than the other, will move around in solution until eventually it will orient itself such that the motility mechanism is closer to the anode. This causes a negative charge to build up on one side of the cell, hyperpolarising it. In this hyperpolarised state, positive ions from solution are drawn towards and inside the cell through diffusion, causing a contraction within the cell. This cellular contraction causes the cell to move, and in this particular case, towards the cathode [23,24]. Therefore, the impact of current on a bacterium is subject to several properties specific to the cell and strain that may dynamically influence its movement. In keeping with this observation, we found that the application of current affected two different bacterial species uniquely, while both species responded, often showing decreases in velocity with the application of current. Wound exudates are primarily in the form of ionic liquid. It is utmost important to maintain a delicate fluid balance at the wound/dressing interface to provide sufficient moisture for cellular activity during the healing process [25,26].

The choice of using current (electric field energy) as a parameter in our study is based on the existence of endogenous electric fields that has been observed to direct cell migration [27]. Not only does current affect the velocity of individual cells, but also it can change the type of movement exhibited. Not all bacterial movement is equivalent. Some cells move in the long strides (running), while others tumble in solution (tumbling), and a given cell can exhibit any combination of these behaviours. *E. coli* cells, when exposed to a 250 ms pulse of a 150 V/cm electrical field, show distinct changes in the type of motility used. For 15 s after the initial pulse, the linear speed increases, suggesting that a majority of the cells are exhibiting a running motion [28]. However, between 15 and 45 s after the pulse, cells exhibit an increased angular speed, suggesting an increase in tumbling. The type of movement displayed by bacteria in an electric field should be directly observable through the use of optical microscopy [29], which will help us to understand the heterogeneous motility dynamics exhibited by bacterial cells under the influence of an electric field. Ultimately, electrical fields could be harnessed to devise strategies to induce favourable patterns of movement in cells that will discourage colonisation and subsequently limit disease.

Cellular directness is a quantity that gives insight into the straightness of a bacterium’s path from the start to the end of tracking. This is represented mathematically by creating a ratio of the cellular displacement from the origin to the overall distance travelled. Comparatively, a low directness value, nearing zero, would imply that the bacterial cell has not moved far from the original position, and therefore its path trajectory is highly nonlinear. On the other hand, a high value, nearing 1, would imply it has travelled linearly, as far as possible. In this study, both *E. coli* and *P. aeruginosa* cells showed an increase in directness as the current was raised, which is likely associated with largely bacterial running motility.
(Table 2). Although the current applied to this system was lower than some previous studies, the increase in running behaviour with the application of current is consistent with published findings [28].

Moving forward, motile bacteria should be also tested at 0.175 mA to better explore the impact of a dynamic range of voltages on bacterial motility, examining both the velocity and type of movement. Results from the literature suggest that current affects the type of bacterial motility behaviour exhibited, such as running or tumbling, but few studies have used direct observation of bacterial movement to explore motility. Through use of optical microscopy, these heterogeneous behaviours can be observed directly. Additionally, PDMS microfluidic devices on coverslips can be used in place of the plastic ibidi micro channels to increase overall image quality when using optical microscopy. Understanding this type of behaviour is significant, but it allows us to control, to a better degree, how bacteria are moving within a solution. Precise control of bacterial movement could grant us the ability to control bacterial colonisation and infection at wound sites.

3.2. Effect of applied current cellular morphology

Understanding the effects of electricity on cell morphology is important, because not only does it give insight into how to potentially harm pathogenic bacteria, but it could also provide cautionary data as to how the eukaryotic cell membranes at the wound sites may react. Dimensional data for the height, width, and length of MRSA cells (Figure 3) and P. aeruginosa cells (Figure 4) showed no significant differences with the application of electrical current. This may be due the heterogeneity inherent in these cellular dimensions and the inability to measure the same cell before and after current application using

| Current (DC)       | 0.0 mA | 0.07 mA | 0.125 mA |
|-------------------|--------|---------|----------|
| Directness P. aeruginosa | 0.79 ± 0.03 | 0.82 ± 0.02 | 0.87 ± 0.02 |
| Directness E. coli  | 0.74 ± 0.03 | 0.90 ± 0.02 | 0.82 ± 0.02 |

Figure 3. Effect of current on MRSA cellular morphology. Height (A) and diameter (B) box graphs for MRSA, at values of 0, 0.075, 0.125, and 0.175 mA. Cellular dimensions were unaffected by applied currents.
AFM. However, these results also suggest that current does not have a strong impact on cellular morphology, which supports the idea that it may be a safe therapeutic for human tissue and will not destroy human tissue. There seems to be a consensus that electromagnetic perturbations to a system affect bacterial cell functionality, but there is still controversy as whether exposure to an electromagnetic field has any effect on the cell membrane itself [29]. Systems exposed to brief electrical impulses seem to yield results showing degradation in the cell membrane, increasing permeability and decreasing cell wall integrity. Moving forward, data on the surface roughness of bacteria could be particularly powerful. Surface roughness results could be used to assess cell membrane integrity, which may be compromised during the application of electricity. Further research in this area is warranted [30,31], while our results suggest that perhaps the application of low-voltage current does not significantly alter E. coli or MRSA cellular morphology. Flagella use contractile proteins and ion channels for movement; while electrical stimulation affects the flagella proteins and thereby inhibits the motility as the applied current causes impairment of flagella [32]. Electrical stimulation in the wound sites can cause the release of cytokines and prostaglandins [33,34], which attracts the macrophages to the site in which the stimulation is being realised. Due to the movement and attraction of blood cells

Figure 4. Effect of current on P. aeruginosa cellular morphology. Width (A), length (B), and height (C) box graphs for P. aeruginosa, at values of 0, 0.075, 0.125, and 0.175 mA. Cellular dimensions are unaffected by applied currents.
such as the macrophages, the electrical stimulation can also cause bacterial death as a secondary effect.

4. Conclusions
The ability to control the preferential migration of bacterial colonies could be a highly successful strategy to be employed by wound-healing devices. Electrotherapy is an attractive form of wound-healing treatment, as it does not contribute to overall bacterial resistance, which is one of the major problems that humanity faces today. Manipulating bacterial electrotaxis is a useful way to approach this issue, as it does not require the use of chemicals or antimicrobials. Under the influence of an electrical current in our study, bacterial cellular velocities were found to decrease, while cell directness increased. However, these same currents did not significantly affect the cellular morphology, suggesting that the impact on cellular integrity may be best revealed using different methods, and/or current does not directly impair bacterial cellular architecture, such that it may also be safe to human cells, while altering bacterial motility in such a way as to discourage colonisation and infection.

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