

# 1 **Electroceutical disinfection strategies impair the motility of pathogenic**

## 2 ***Pseudomonas aeruginosa* and *Escherichia coli***

3 Kristina Doxsee<sup>1,a)</sup>, Ryan Berthelot<sup>1,b)</sup> and Suresh Neethirajan<sup>1,c)</sup>

4 <sup>1</sup>BioNano Laboratory, School of Engineering, University of Guelph, 50 Stone Road East,

5 Guelph, Ontario N1G 2W1, Canada

6 <sup>a)</sup>kdoxsee@gmail.com, <sup>b)</sup>rberthel@uoguelph.ca, <sup>c)</sup>Author to whom correspondence should be  
7 addressed. Electronic mail: sneethir@uoguelph.ca

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9 Electrotaxis or galvanotaxis refers to the migration pattern of cells induced in response to  
10 electrical potential. Although it has been extensively studied in mammalian cells, electrotaxis has  
11 not been explored in detail in bacterial cells; information regarding the impact of current on  
12 pathogenic bacteria is severely lacking. Therefore, we designed a series of single and multi-cue  
13 experiments to assess the impact of varying currents on bacterial motility dynamics in  
14 pathogenic multi-drug resistant (MDR) strains of *Pseudomonas aeruginosa* and *Escherichia coli*  
15 using a microfluidic platform. Motility plays key roles in bacterial migration and the colonization  
16 of surfaces during the formation of biofilms, which are inherently recalcitrant to removal and  
17 resistant to traditional disinfection strategies (e.g. antibiotics). Use of the microfluidic platform  
18 allows for exposure to current, which can be supplied at a range that is biocidal to bacteria, yet  
19 physiologically safe in humans (single cue). This system also allows for multi-cue experiments  
20 where acetic acid, a relatively safe compound with anti-fouling/antimicrobial properties, can be  
21 combined with current to enhance disinfection. These strategies may offer substantial therapeutic  
22 benefits, specifically for the treatment of biofilm infections, such as those found in the wound  
23 environment. Furthermore, microfluidic systems have been successfully used to model the

24 unique microfluidic dynamics present in the wound environment, suggesting that these  
25 investigations could be extended to more complex biological systems. Our results showed that  
26 the application of current in combination with acetic acid has profound inhibitory effects on  
27 MDR strains of *P. aeruginosa* and *E. coli*, even with brief applications. Specifically, *E. coli*  
28 motility dynamics and cell survival were significantly impaired starting at a concentration of 125  
29  $\mu\text{A}$  DC and 0.31% acetic acid, while *P. aeruginosa* was impaired at 70  $\mu\text{A}$  and 0.31% acetic  
30 acid. As these strains are relevant wound pathogens, it is likely that this strategy would be  
31 effective against similar strains *in vivo* and could represent a new approach to hasten wound  
32 healing.

33

## 34 **Introduction**

35 Antibiotic resistance, although a long-standing issue, continues to be a growing problem.  
36 The number and prevalence of multi-drug (MDR) resistant bacterial strains is steadily increasing,  
37 and more than 2 million people are infected each year in the United States by resistant strains,  
38 causing some 23,000 deaths<sup>1</sup>. An important contributor to antibiotic resistant infection is biofilm  
39 infection; by definition biofilms are inherently resistant to antimicrobials, warranting up to 1000  
40 times the dose required to clear non-biofilm infections<sup>2</sup>. Therefore, alternative strategies to  
41 disinfection (e.g. electrocidal, electroceutical, and mechanical approaches) have gained interest  
42 and represent potentially powerful weapons against recalcitrant biofilm infection. Although the  
43 impact of current on mammalian cells is fairly well understood, information regarding the effect  
44 on pathogenic MDR bacterial strains is lacking.

45 A number of studies have investigated bacterial responses to electrical fields and applied  
46 current. For example, the motility of *E. coli* cells has been evaluated in capillaries as a facet of

47 soil remediation initiatives<sup>3</sup>, and *Tetrahymena pyriformis* electrostatic behaviors have been  
48 investigated with the goal of developing a microrobot<sup>4</sup>. The efficacy of electrical stimulation as a  
49 means of bacteriostatic control in the areas of wound healing<sup>5-9</sup> and biofilm remediation<sup>10-13</sup> has  
50 also been investigated in recent years. However, data on the cellular motility and electrostatic  
51 and galvanotactic behaviors MDR bacterial species (in response to the external application of  
52 current) is rather limited. Electrical stimulation, particularly in the 50-500  $\mu$ A direct current (DC)  
53 range, has been shown to reduce the number of viable cells, as determined by colony forming  
54 units (CFUs). However, the underlying mechanisms by which electrical currents exert these  
55 effects in bacterial cells are poorly understood. Not only can current affect viability, but it can  
56 also impact the motility of bacterial organisms. However, the effect of current on the motility of  
57 pathogenic bacteria has not been extensively explored.

58 Cell migration/motility plays important roles in wound healing. Diverse cellular factors,  
59 such as chemical and electrical cues, guide the directional migration of several organisms<sup>14</sup>. For  
60 example, leukocytes (e.g. lymphocytes and neutrophils) can detect and follow gradients of  
61 tissue-derived chemoattractants<sup>15</sup> and electric fields generated endogenously at wound sites to  
62 foster healing and antimicrobial defense<sup>16</sup>. Therefore, the strategic application of current could  
63 augment natural wound healing responses in mammalian cells, while targeting pathogenic  
64 bacteria. MDR strains of *Pseudomonas aeruginosa* and *Escherichia coli* are of particular interest  
65 in the wound environment, as they are common wound pathogens that possess motility systems  
66 critical to biofilm formation; directed motility towards a surface and eventual loss of the flagella  
67 are essential steps in biofilm formation in these organisms<sup>17</sup>. *Pseudomonas aeruginosa*, a major  
68 cause of nosocomial infections, frequently infects open wounds and can cause sepsis and  
69 necrosis<sup>18</sup>. This organism infects approximately one-third of all burn wounds<sup>19</sup>. Additionally,

70 *Pseudomonas aeruginosa* has negative industrial and environmental influences, causing dairy  
71 spoilage<sup>20</sup> and issues in water treatment systems<sup>21</sup>. Therefore, applying electric potential could be  
72 a complementary approach in addition to using chemical-based drugs to overcome the issue of  
73 multi-drug resistance. Novel electroceutical technologies incorporating low voltage/current  
74 within a wound dressing have shown some promise and could be implemented as a wearable  
75 electrical-based treatment system<sup>22</sup>. Therefore, understanding electrotaxis behaviors in MDR  
76 strains of bacteria will provide researchers with relevant information concerning the ranges of  
77 electric potential that could be applied to chronic or acute wound infections, depending on the  
78 infecting strains and their relative sensitivities.

79 Such electroceutical strategies combine chemical disinfection with the application of  
80 current. Acetic Acid (AA) is one promising chemical candidate that may be effective in such  
81 strategies, as it is an effective, safe, and economical antimicrobial agent capable of inhibiting  
82 pathogenic and MDR strains of bacteria, even when these strains grow as a biofilm<sup>19,23</sup>. Although  
83 studies strongly support the use of AA in combination with electrical stimulation (ES) as a  
84 disinfection strategy, additional measurement of the chemotactic and electrotactic behaviors of  
85 MDR strains is needed to engineer and fabricate an effective wound healing device. Therefore,  
86 we chose to compare the effects of AA or ES alone (single cue) or a combination of AA and ES  
87 (multi-cue) on the chemotaxis of pathogenic species relevant to wound infection.

88 Our results show that combinations of AA and ES are highly effective in reducing the  
89 motility of MDR strains of *P. aeruginosa* and *E. coli*, which is likely to impede infection and  
90 biofilm formation. Our results help to deepen our understanding of the effects of electroceutical  
91 approaches on pathogenic bacteria, suggesting that motility is one heavily impacted factor. This  
92 information may aid in the design of highly effective wound healing devices/strategies.

## 93 **Materials and Methods**

94

### 95 **Experimental Parameter Design**

96 Care was taken in the selection of various experimental parameters, which will be  
97 discussed herein. Studies support that both *E. coli* and *P. aeruginosa* show little to no response to  
98 alternating current (AC) stimulation, but a bacteriostatic effect is observed when exposed to  
99 anodal and cathodal direct current (DC)<sup>7,24</sup>. Delivery of 200  $\mu$ A for 4 h/day over 4 days reduced  
100 *P. aeruginosa* biofilms on Teflon and titanium discs<sup>12</sup>, and exposure to 100  $\mu$ A resulted in  
101 observable biofilm reductions after 4 days<sup>18</sup>. Further, *in vivo* studies have shown that skin ulcers  
102 colonized with *P. aeruginosa* and treated with  $\mu$ A cathodal DC resulted pathogen-free ulcers  
103 within days of treatment<sup>16</sup>. Additional studies revealed that delivery of current through carbon-  
104 filled electrodes to microorganisms in intact human skin at 75 and 100  $\mu$ A resulted in  
105 bactericidal effects at 4 and 24 hours, beneath the positive electrode<sup>24</sup>. We selected *P.*  
106 *aeruginosa* strain BK-76, isolated from canine ear skin infections, and *E. coli* strain ATCC 8099,  
107 because these are relevant wound pathogens that exhibit antimicrobial resistance.

108 To minimize the risk of pH or temperature fluctuations, while selecting an effective  
109 current range, we elected to administer 75, 125, or 175  $\mu$ A DC to cells, as that doses of 75-200  
110  $\mu$ A have bactericidal effects. Based on a study performed at the Mayo Clinic, the deployment of  
111 low dose electric current in the urinary tract was determined to be safe; a study of electrified  
112 catheters in sheep resulted in no chemical or physical changes/trauma to tissues or urine within  
113 the urinary tract when administering 400  $\mu$ A of current. A similar effect was observed in a  
114 human population using an electrified urinary tract catheter trial<sup>9</sup>. However, it is unknown as to  
115 how inflamed and possibly necrotized skin wounds would respond to currents as great as 400  $\mu$ A

116 DC, which is why we selected a lower 175  $\mu$ A dose as the upper limit. The bacterial suspension  
117 consisted of either *P. aeruginosa* or *E. coli* suspended in filtered deionized water. This was done  
118 to reduce the complexity of the electrochemical products produced at the anode/cathode. This  
119 allowed for a more clear observation of the bacterial response to current in terms of their  
120 chemotactic behavior. When the bacteria were observed microscopically in the prepared  
121 solution, they exhibited a high level of free motility.

122 Previous studies have shown that a minimum inhibitory concentration (MIC) of 0.31%  
123 Acetic Acid to be an effective treatment against a multitude of pathogens tested, including MDR  
124 strains of *E. coli* and *P. aeruginosa*<sup>19</sup>. This same concentration of AA was also found to be  
125 effective in inhibiting biofilm formation<sup>19</sup>. Therefore, we chose to use 0.31% as the chemical  
126 component of our electrochemical approach (multi-cue experiments).

127 Electrotaxis and chemotaxis have been largely been studied separately due to the  
128 complications in designing simultaneous chemical mixing and electrical field gradients in a  
129 single device<sup>14</sup>. The development of specialized microfluidic devices have allowed researchers to  
130 test impact of electric fields as well as controlled chemical gradients, although still challenging  
131 for practical application in a wound dressing<sup>14</sup>. Therefore, we elected to use a single uniform  
132 concentration of AA in combination with the application of various currents.

133

## 134 **Cell Culture**

135 Bacterial suspensions were prepared by centrifuging cultures grown in 5 mL of Tryptic  
136 Soy Broth medium in the shaker at 200 rpm for 5 h at 37°C. The media was extracted and  
137 centrifuged at 3750 rpm for 5 min to concentrate the cells. Upon pouring off the supernatant and  
138 redistributing the cells in filtered deionized water, the process was repeated 2 times. The cells

139 were subsequently diluted with filtered deionized water and injected into the microfluidic device  
140 for viewing. The above procedure was also followed for the culturing of the cells for the  
141 electroceutical experiments, except 6.9  $\mu\text{L}$  of 45% Acetic Acid was added to 993.1  $\mu\text{L}$  of the  
142 bacterial solution to achieve the 0.31% AA concentration prior to injection into the microfluidic  
143 channel.

144

### 145 **Metrics Acquisition**

146 Copper electrodes (diameter, 4 mm) were inserted into ports A & B and into the bacterial  
147 suspension of the glass bottom fused silica microfluidic system (Figure 1). The desired ampere  
148 ranges were achieved by use of a current amplifier (Figure 1) and at the following progression  
149 and exposure times:  $I = 0$  mA (10 min), 3 min of rest, 0.07 mA (10 min), 3 min of rest, 0.125  
150 mA (10 min), 3 min of rest and 0.175 mA (10 min). A Nikon Ti-U Eclipse Microscope (Nikon  
151 Instruments Inc., Melville, NY) was used to image the cells at the following settings: Phase 2  
152 contrast with the use of D & GIF filters, 40x Objective with collar ring set to 1.3 mm, 1.5X  
153 magnification, 1.0 Gain, recorded at 90 fps by use of the Nikon NIS-Elements software for real  
154 time imaging. Regions along the entire microfluidic channel were imaged and recorded for 40  
155 seconds. The use of a flow-free microfluidic device composed of a fused silica chip, minimizes  
156 flow-induced shear stress on cell migration and movement in a static gradient environment<sup>26-29</sup>  
157 and allows quantitative evaluations of cell migration in spatiotemporally complex  
158 chemoattractant fields that mimic *in vivo* situations<sup>14,30</sup>. In addition, the miniaturization  
159 drastically reduces the Joule heating effect<sup>31</sup>, thereby reducing the chance of any thermotaxis by  
160 the cells. Data analysis conducted in this study was similar to Wright et al<sup>38</sup>. The cellular  
161 characteristic analyzed in this study includes Forward Migration Index (FMI), where FMI X and

162 FMI Y indicate the efficiency of the forward migration of cells and how they relate to the  
163 direction of both axes.

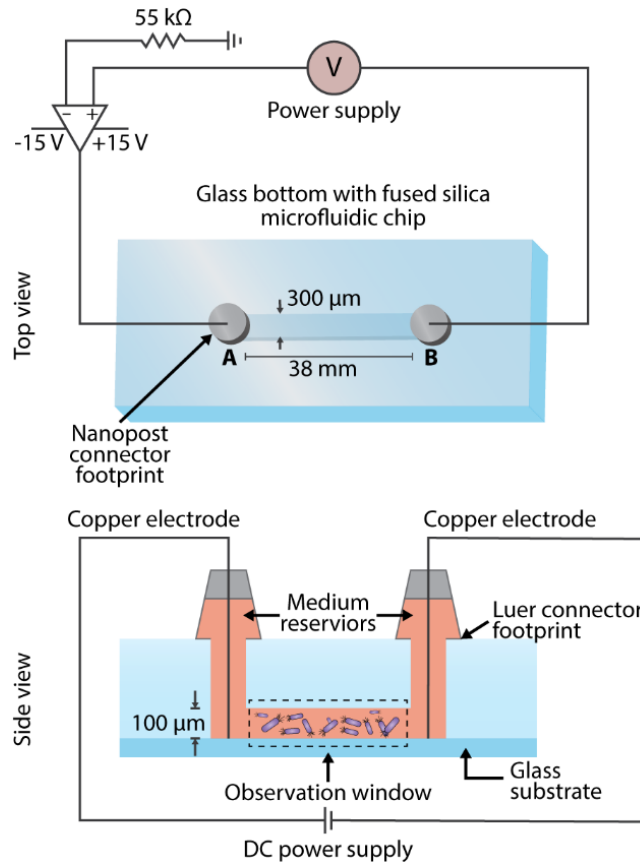
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### 165 **Statistical Tracking and Data Analysis**

166 ImageJ (<http://rsb.info.nih.gov/ij/>) was used to track the cellular frame by frame coordinates,  
167 and Ibidi Chemotaxis and Migration Tool software (Ibidi Software, Munich, Germany) was used  
168 to calculate the cellular motility metrics of the combined cell tracks. The total number of cell  
169 tracks for each setting was 70. At least five independent experiments were carried out. All  
170 quantitative data were presented as the mean value±standard deviation. Student's t-test was  
171 applied to compare two distinct groups. P value <0.05 was considered to be statistically  
172 significant.

173





174

175 **Fig. 1.** Illustration of the microfluidic experimental setup for bacterial electrotaxis

176 assays. Schematic of the circuit used for generating the desired electric current in the

177 investigation of swimming dynamics of individual bacterial cells.

178

## 179 Results and Discussion

### 180 Single-cue Electrotactic Experimental Metrics

181 Both *E. coli* and *P. aeruginosa* experience a reduction in cellular speed (Figure 2A, B)

182 and an increase in the forward migration index (FMI) in response to the application of DC, along

183 the chemotactic gradient plotted on the y-axis (Figure 2C). There is also a significant increase in

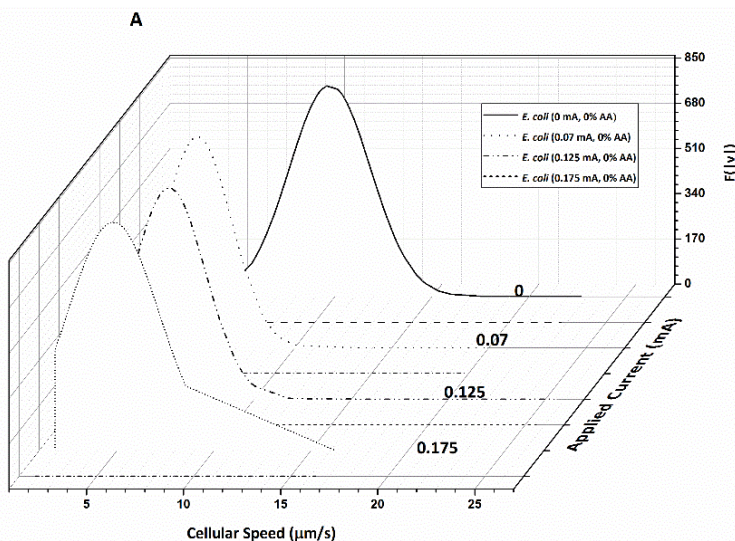
184 directness with current application (Figure 4A, B), relative to the baseline (0 mA). Resulting

185 average cellular speeds for *E. coli* were  $9.7 \pm 0.5 \mu\text{m/s}$ ,  $5.0 \pm 0.4 \mu\text{m/s}$ ,  $6.0 \pm 0.4 \mu\text{m/s}$ , and  $4.6 \pm$

186 0.3  $\mu\text{m/s}$  for 0 mA, 0.07 mA, 0.125 mA, and and 0.175 mA DC, respectively (Figure 3C).  
187 Resulting average cellular speeds for *P. aeruginosa* were  $44 \pm 3 \mu\text{m/s}$ ,  $34 \pm 2 \mu\text{m/s}$ ,  $40 \pm 2 \mu\text{m/s}$ ,  
188 and  $75 \pm 3 \mu\text{m/s}$  for 0 mA, 0.07 mA, 0.125 mA, and 0.175 mA DC, respectively (Figure 3D).  
189 Differences in the response to current between *E. coli* and *P. aeruginosa*, as measured by in the  
190 change cellular speed, likely reflects differences in their motility and chemo/electrotactic sensing  
191 systems<sup>32</sup>. This may help to explain why there is an increase in cellular speed in *P. aeruginosa* at  
192 0.175 mA (relative to baseline), while the application of any current reduces *E. coli* cellular  
193 speed. However, 0.07 and 0.125 mA currents reduced the motility of both organisms, indicating  
194 that there may be an optimal range of current that will predictably and consistently impact  
195 several pathogenic bacterial species.

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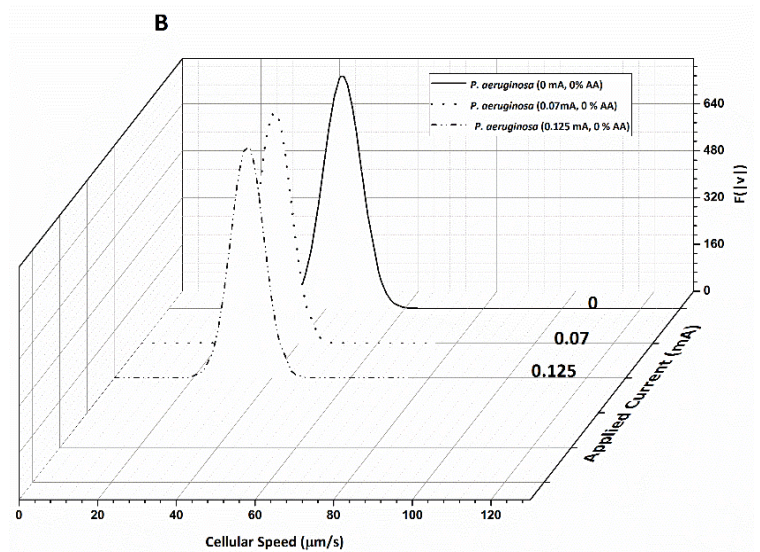


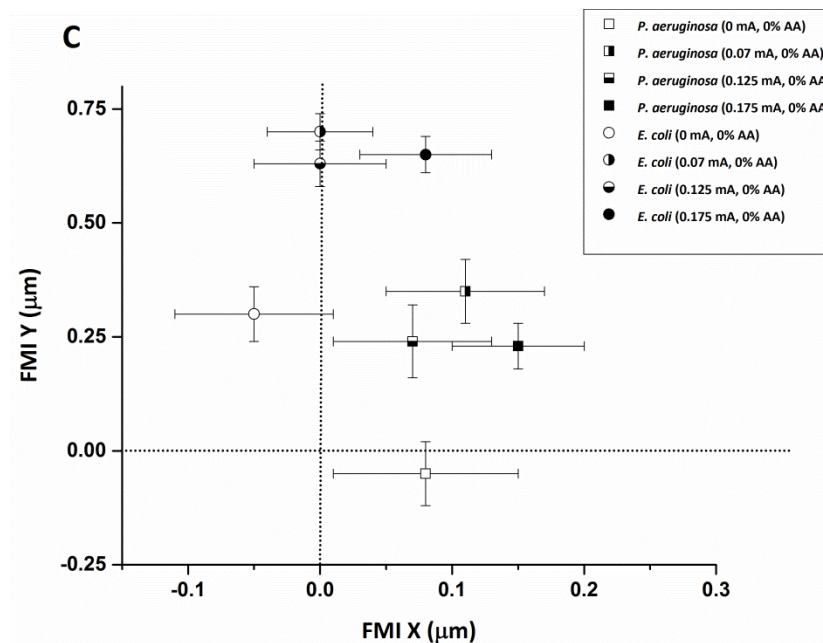
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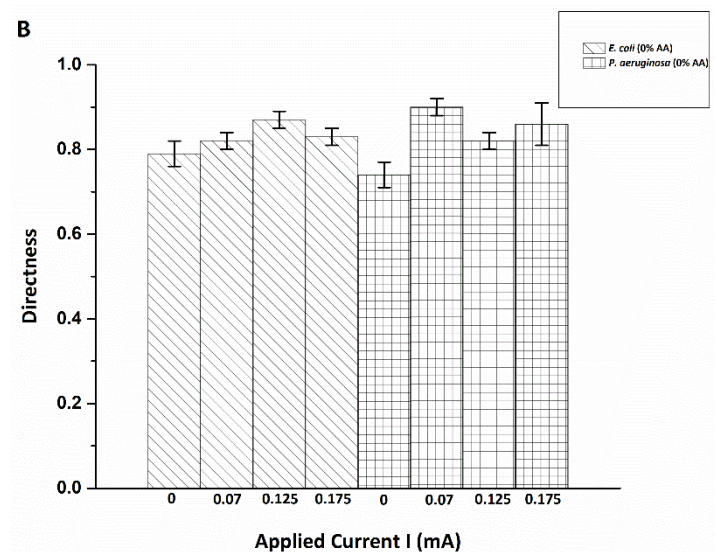
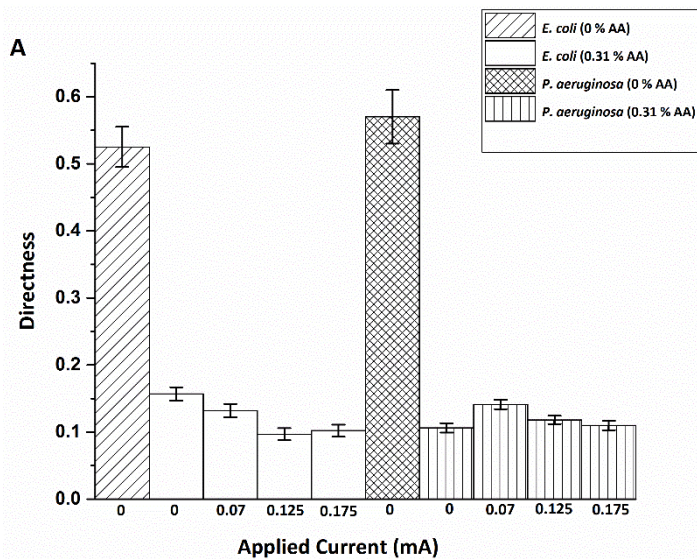
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 210 **Fig. 2.** *E. coli* (panel A) and *P. aeruginosa* (panel B) cellular speed distribution in  $\mu\text{m/s}$  in  
 211 response to ES alone at applied current settings listed in mA. The vertical axis indicates resulting  
 212 scaled Gaussian of distributed cell speeds,  $F(|\mathbf{v}|)$ . *E. coli* shows the greatest response to ES alone  
 213 at 0.07 and 0.175 mA, while *P. aeruginosa* shows the greatest response at 0.07 mA within the  
 214 standard error. The average forward migration index (FMI) in the x and y directions for *E. coli*  
 215 and *P. aeruginosa* in response to the application of current is plotted in panel C. Results denote  
 216 increased migration along the electrotactic gradient (y axis) with the application of current  
 217 (relative to 0 mA baseline).

### 218 Single-cue Chemotactic Experimental Metrics

219 Both species experience a reduction in their FMI (Figure 4), average cellular speed  
 220 (Figure 3 A, B), and directness (Figure 3 C, D) with the introduction of 0.31 % AA alone,  
 221 relative to baseline (no AA). These results indicate that treatment with AA significantly impairs  
 222 bacterial motility, even when used alone. Furthermore, treatment with AA has equivalent effects  
 223 on both species, pointing to a broader, more conserved mechanism of action.

224 Studies have found that bacterial cellular ATP processes are disrupted by exposure to  
225 AA<sup>23</sup>, which is one reason why AA has great potential as an antimicrobial agent. Weak acids can  
226 cross bacterial membranes more readily than strong acids, because of the equilibrium between  
227 their ionized and non-ionized forms, the latter of which can freely diffuse across hydrophobic  
228 membranes. This ultimately collapses the proton gradients necessary for ATP synthesis. When  
229 acetic acid dissociates, it acidifies the cytoplasm, causing acid-induced proton unfolding and  
230 membrane and DNA damage<sup>23</sup>. This effect is specific to bacteria, because host somatic cells  
231 contain cholesterol, which controls cell permeability; the interior of the phospholipid bilayer is  
232 occupied by hydrophobic fatty acid chains, such that the membrane is impermeable to water-  
233 soluble molecules and most biological molecules, including ions<sup>31</sup>.

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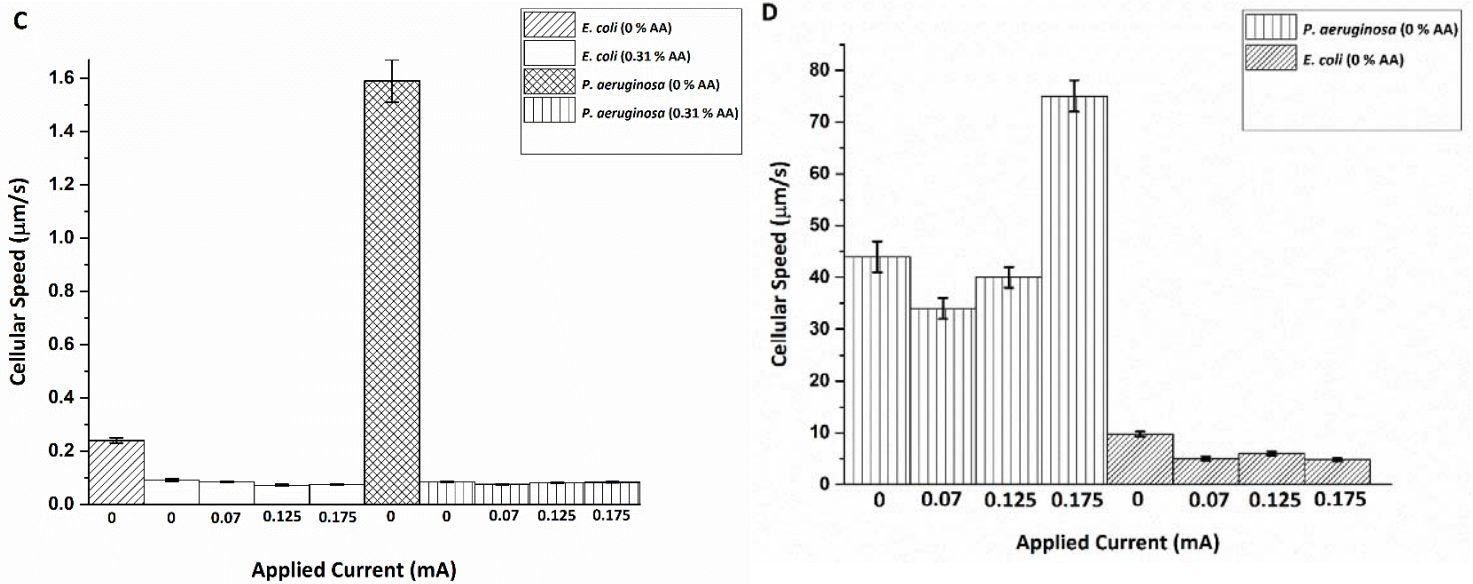
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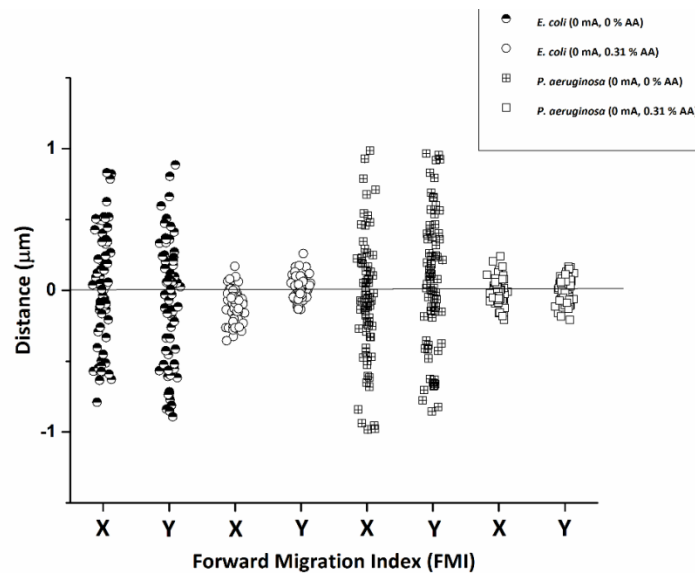
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254 **Fig. 3.** Average directness resulting from applied current and AA concentrations listed for *E. coli*  
 255 (panel A) and *P. aeruginosa* (panel B); average cellular speed for *E. coli* (panel C) and *P.*  
 256 *aeruginosa* (panel D). Results indicate an increase in directness relative to baseline with the  
 257 application of current alone, but a decrease in directness with the application of AA alone or with  
 258 current. Results also indicate a reduction in cellular speed with 0.31% AA (without or without  
 259 current) or with current alone, except for *P. aeruginosa* at a current of 0.175 mA (no AA).



260  
 261 **Fig. 4.** Vertical scatter indicating forward migration index (FMI) distribution in x and y  
 262 directions for *E. coli* and *P. aeruginosa* comparing the effect of 0.31% AA alone on cells.

263 Results indicate a drastic reduction in migration in both the x and y directions with the  
264 application of AA.

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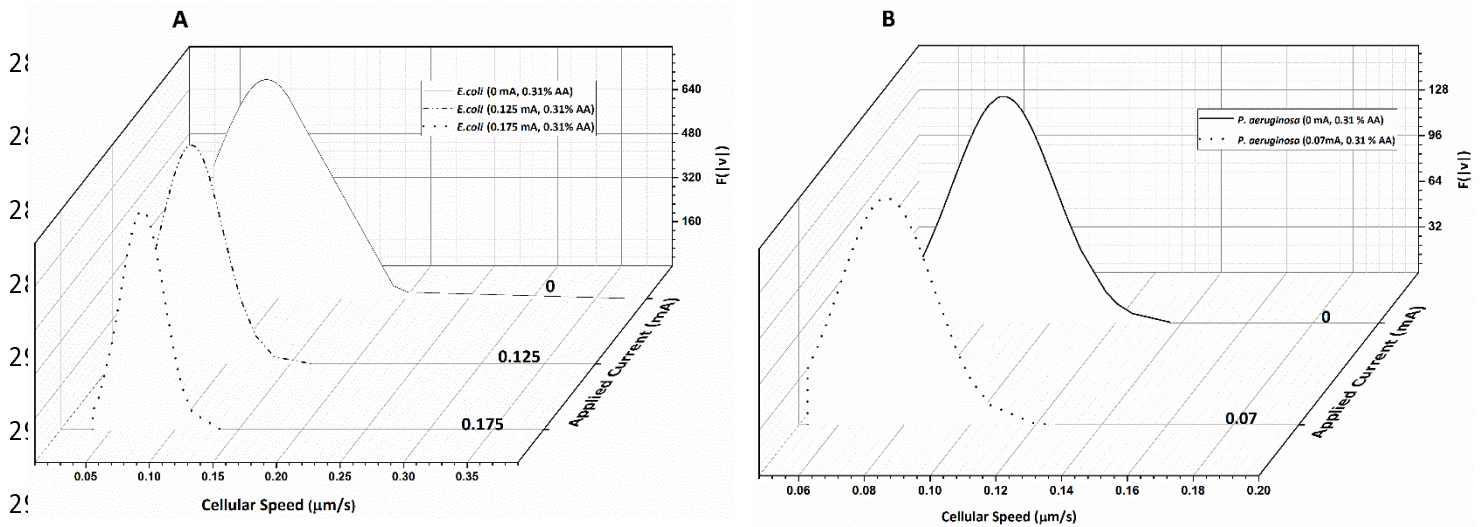
### 266 **Multi-cue Electroceutical Experimental Metrics**

267 Resulting average cellular speeds for *E. coli* in the presence of 0.31% AA were  $0.091 \pm$   
268  $0.005$ ,  $0.085 \pm 0.002$ ,  $0.072 \pm 0.003$ , and  $0.074 \pm 0.002$   $\mu\text{m/s}$  for 0 mA, 0.07 mA, 0.125 mA, and  
269 0.175 mA DC, respectively (Figure 3C). Resulting average cellular speeds for *P. aeruginosa* in  
270 the presence of 0.31% AA were  $0.085 \pm 0.002$ ,  $0.075 \pm 0.002$ ,  $0.081 \pm 0.001$ , and  $0.083 \pm 0.002$   
271  $\mu\text{m/s}$  for 0 mA, 0.07 mA, 0.125 mA, and 0.175 mA DC, respectively (Figure 3D).

272 A reduction in cellular speed relative to baseline (0.31% AA, no current) (Figure 3C, D  
273 and Figure 5) was pronounced for both *E. coli* and *P. aeruginosa* upon electroceutical  
274 application. The most dramatic reductions in speed occurred at 0.070 mA DC for *P. aeruginosa*,  
275 while *E. coli* was equally impaired at higher currents (0.125 and 0.175 mA DC). These results  
276 suggest that there is an ideal current range in an electroceutical setting for *E. coli*, which would  
277 be upwards of 125  $\mu\text{A}$ , while the ideal range for *P. aeruginosa* would be less than 0.125 mA for  
278 both single and multi-cue situations (namely 0.07 mA). However, a current of approximately  
279 0.07 mA may be effective across a broader range of strains, meaning that a single application of  
280 current (with AA, especially) could provide powerful disinfection in the context of a wound  
281 infection. A very pronounced reduction in FMI was also observed at 0.175 mA DC for *E. coli*  
282 and at 0.07 mA for *P. aeruginosa* when single-cue ES was compared to our electroceutical  
283 approach (AA + current) (Figure 6).

284

285



293 **Fig. 5.** *E. coli* cellular speed distribution in  $\mu\text{m/s}$  in response to ES and 0.31% AA specific  
294 currents. Results indicate a great reduction in cellular speed upon electrochemical treatment (panel  
295 A). *E. coli* was equally responsive to both 0.125 mA and 0.175 mA settings *P. aeruginosa*  
296 cellular speed distribution in  $\mu\text{m/s}$  (panel B). Results indicate a great reduction in cellular speed  
297 with electrochemical treatment of *P. aeruginosa* at the 0.07 mA setting.

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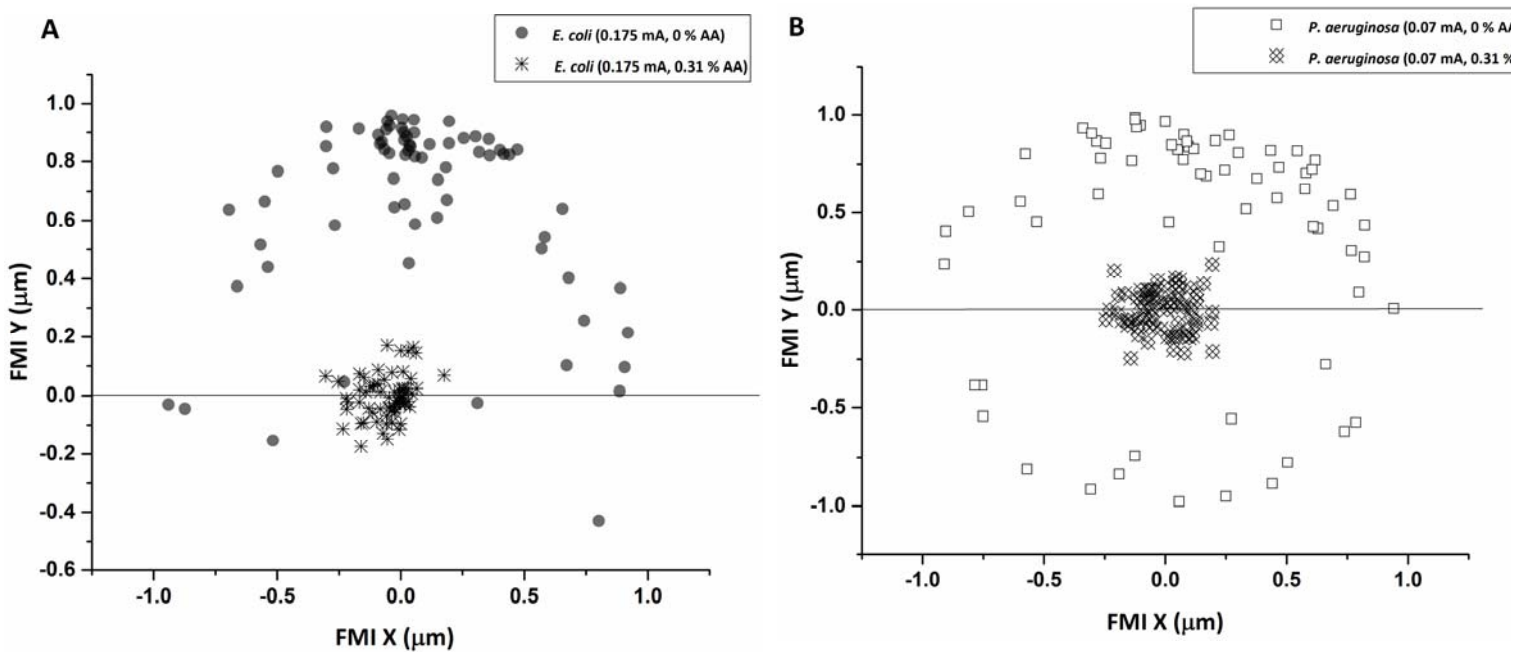
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317 **Fig. 6.** The forward migration index (FMI) distribution for *E. coli* at the 0.175 mA setting in the  
318 presence and absence of 0.31% AA (panel A). Results indicate a drastic reduction in overall  
319 migration when 0.31% AA was applied in combination with ES at 0.175 mA. The FMI  
320 distribution for *P. aeruginosa* at a 0.07 mA setting in the presence and absence of 0.31% AA  
321 (panel B). Results indicate a drastic reduction in overall migration when 0.31% AA was applied  
322 in combination with ES at 0.07 mA.

323

## 324 Conclusions

325 Clearly, *P. aeruginosa* responds well to both ES and our electroceutical approach at 0.70  
326 mA DC, while *E. coli* is more responsive at higher current (0.175 mA DC). It is not surprising  
327 that *E. coli* and *P. aeruginosa* respond differently to ES settings, as their chemotactic sensing  
328 systems differ. *P. aeruginosa* has a more complex sensing system than most microbes with  
329 multiple chemotaxis genes that constitute several chemotaxis systems with defined functions<sup>32</sup>.  
330 *E. coli* uses a two-component regulatory system consisting of an extracellular sensor and  
331 response regulator<sup>32</sup> that is more susceptible to oxidative stress than *P. aeruginosa*<sup>33</sup>. Hence,



332 electroceutical device design should take into account the differing impacts that such disinfection  
333 strategies may have on several relevant wound pathogens. In the case of *P. aeruginosa*, quorum  
334 sensing (QS) plays a prominent role in its virulence and biofilm formation, which may offer an  
335 ideal target for anti-biofilm therapies.

336         Oxidative and nitrosative stresses, which can be augmented by electroceutical  
337 approaches, play important roles in bacterial inhibition/elimination *in vivo*. In particular, *E. coli*  
338 can be toxified by as little as 5  $\mu\text{M}$  extracellular hydrogen peroxide<sup>34-35</sup>, and because it is a small  
339 uncharged molecule, hydrogen peroxide diffuses across membranes rapidly and has the ability to  
340 cause profuse DNA damage when the intracellular concentration rises to 1  $\mu\text{M}$ <sup>27</sup>. *P. aeruginosa*,  
341 on the other hand, has developed a multitude of defense mechanisms to tolerate stress conditions,  
342 even  $\text{H}_2\text{O}_2$  at relatively high levels, but remains susceptible to other oxidative stressors<sup>33</sup>.  
343 Because the buildup of electrochemical oxidative products at the anode and cathode would occur  
344 with the application of ES, while ES alone may be a successful strategy for disinfection, but in  
345 combination with AA these effects are likely to be augmented. Combining AA with ES should  
346 enhance disinfection, as AA has the ability to disrupt essential ATP processes and cause cellular  
347 DNA damage, thereby impairing both the ability to communicate and initiate defense  
348 mechanisms.

349         As motility is one microbial defense against opsonization by the host immune defenses,  
350 locomotive impairment would effectively trap bacterial cells, allowing for their clearance by  
351 migrating phagocytic leukocytes. Since migratory cell behavior in the presence of electrical cues  
352 is a naturally existing process within the human body, and if we take the 0.1  $\mu\text{A}/\text{mm}^2$  DC current  
353 density generated as a lower limit for directing leukocyte migration for wound healing, the  
354 corresponding current density settings tested here are likely to induce leukocyte migration that

355 would accelerate the natural wound healing process while simultaneously inhibiting  
356 opportunistic pathogens. An applied electric field within the physiological range can also induce  
357 the directional electrotaxis of epithelial cells and fibroblasts, along with neutrophils and  
358 endothelial cells, suggesting a potential role in cellular positioning during wound healing<sup>36</sup>.  
359 Because phagocytic neutrophils and monocytes are up to 20  $\mu\text{m}$  in diameter<sup>37</sup>, have cell walls  
360 that actively prohibit entry of water soluble molecules<sup>31</sup>, and have a negative surface potential,  
361 unlike invading pathogens, they can migrate to the wound site unaffected. As a consequence, our  
362 electroceutical approach could create a bacterial trap that would accelerate the natural wound  
363 healing process, while augmenting bacterial clearance.

364

## 365 **Acknowledgements**

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367 The authors sincerely thank the Natural Sciences and Engineering Research Council of Canada  
368 (NSERC), the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), and the  
369 Ontario Ministry of Research and Innovation (MRI) for funding this study.

370

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