Effect of Electrical Stimulation on Bacterial Growth

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Abstract

Background; Electrical stimulation has been used as a modality for many years for wound healing. One of the mechanisms proposed for why it works is that electrical stimulation is believed to be bacteria static . However, early studies making this claim used much greater than normal stimulation voltages and currents. **Methods and procedures**; In the present investigation, 3 types of bacteria, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus were examined with physiological levels of AC (5 and 20 ma) and DC electrical stimulation to see if these same currents that traverse through tissue during normal electrical stimulation for wound treatment could impair bacterial growth. **Results**; DC micro current did not alter bacteria growth for any of the three bacteria studied. Pseudomonas aeruginosa showed significantly reduced growth with both (5 and 20 ma) AC current intensities ($p<0.05$) whereas the other bacteria showed no consistent reductions in growth with AC current; each showing some sensitivity to AC stimulation. While there were significant differences between the pH of the cultures with AC stimulation in the experimental vs. the control groups in some conditions, there was no consistency in any correlation between pH and growth. Thus, while pseudomonas was inhibited with AC stimulation, there was no evidence from these studies that electrical stimulation had a consistent bacteria static effect on the other bacteria. Even in pseudomonas, bacteria growth was only slowed and not stopped with 30 minutes of stimulation. **Conclusions**; while there was some sensitivity at least in one bacterium to AC stimulation, largely the bacteria static effect of electrical stimulation under the conditions used here was de minimis.

Key words: wounds, electrical stimulation, bacteria, healing

Introduction

Wound healing, especially in people with diabetic neuropathies, can be extremely difficult to accomplish.[1] For example, of people who have diabetic ulcers on their feet, inability of wounds to heal causes an average amputation rate to be about 25%[2,3]. Many diabetic ulcers and even decubitus ulcers can take months, years, or never heal at all.[2, 3, 4]

This inability of conventional medical practice to heal wounds has been termed "*a failure of the health care system*".[5] Even newer therapies such as myocutaneous flaps, water debridement, and other techniques have not increased the healing rate appreciably in the past fifty years or the rate of reoccurrence.[5]

One modality that has been studied is electrical stimulation.[6] Many papers point to the use of electrical stimulation across wounds to increase wound healing.[7, 8] However, even this technology does not always work. Some papers show wound healing with DC micro currents while others show only healing with strong AC currents.[9, 10, 11] Some papers report that in the early stages of wound healing the polarity needs to be positive near the wound and negative away from the wound and other papers report the opposite.[12, 13, 14] In fact, in a summary of different studies on wound healing, Yarkony reported nothing conclusive in setting the intensity of currents, whether it was DC or AC stimulation or the frequency of electrical stimulation in the ability of electrical stimulation to heal wounds.[12] Recent evidence, however, links this disparity to possibly being linked to room temperature. Vasoconstriction of the circulation during treatment due to a cool or cold examination room blocks the blood flow increase due to electrical stimulation of the wound. If the room is warmed, blood flow increases and wounds heal well with electrical stimulation.[15, 16]

Numerous mechanisms have been suggested on why electrical stimulation promotes wound healing including increased circulation,[1,17] increased angiogenesis[18, 19, 20] increased proliferation of epidermal tissue, and a bacteria static effect of electrical stimulation.[21]

The possible bacteria static effect of electrical stimulation was first reported over 30 years ago by Rowley & McKenna.[22, 23] Using high voltage electrical stimulation (300 volts), bacteria (E. coli) died after a brief session of electrical stimulation. While a few studies have used similar voltages for clinical electrical stimulation of wounds[11, 24] most studies that report healing use a fraction of these voltages and currents. [12, 13, 25, 17] The stimulation voltages used by Rowley et al. would be extremely painful for the patient. Further, since DC micro current has been shown, in some studies, to aid in healing of wounds, when the voltages used by Rowley and colleagues are compared to DC micro currents, they are thousands of times higher. Thus, the currents used in these studies were not clinically relevant and, therefore, the application of these studies to real life situations is questionable.

The present investigation was conducted to study the effect of electrical stimulation on bacterial growth in three common bacteria that are seen in wounds, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus Aureus.[23, 26] These bacteria were grown in culture and then exposed to either DC microcurrent (100 μamps) or biphasic sine wave stimulation at 5 or 20 ma for 30 minutes. These currents are normal currents used in wound healing.[16, 15] Bacterial growth was then assessed after 24 hours of incubation to see if the normal currents that are used with electrical stimulation clinically to heal wounds can alter bacterial growth.

Methods

Bacteria-

Bacteria were grown in a soy broth (tryptic soy broth, soybean- casein digest) from Hardy Diagnostics (Cat # C7141, Santa Monica, CA). The bacteria used were Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. These bacteria were obtained from stock concentrations (Micro biologics, ST Cloud, Minnesota). Bacteria were grown in broth at a temperature of 37^{o} C until the concentration of bacteria was between 4-5 on the McFarland Scale.[27] All bacteria were grown and tested for activity by standard microbiological light microscopy. The broth was tested for growth in an absorption spectrometer at a wavelength of 580 nanometers; the relationship between bacterial density and broth absorption was established. Once this was established, stock broth cultures were placed between two 2 X 2 cm carbonized rubber electrodes, so that they could be subjected to electrical stimulation for a period of thirty minutes. The separation distance between the electrodes was 10.3cm. The covered culture size was 10.3 X 8.5 cm and 6.75 cm deep. The volume was 400ml. Using these electrodes and electrode sizes, the impedance of the broth was 1280 ohms during electrical stimulation, a valve similar to that recorded across wounds.[15] The size of the broth holder was selected so that if current was measured over a 0.5 cm distance in the broth, the recorded current was similar to that recorded in human wounds.[15, 16]

Electrical Stimulation- AC electrical stimulation was provided by a challenge 8000 powered muscle stimulator (MPTS Tustin, CA). This stimulator provided 4 channels of current controlled output with a biphasic sine wave at 250 μsec pulse width and a frequency of 30 Hz. DC stimulation was provided at a current of 1 ma by a battery with a regulated current controlled output.

pH- The pH was measured with an Accruement Basic AB15 pH meter (Fisher Scientific, Singapore).

Procedures

Using the stock broth bacterial cultures described above, each of the 3 bacteria were placed in a bacterial broth in the rectangular cultures. After the broth had been cultured, electrical stimulation was applied for thirty minutes. AC current was delivered with biphasic electrical stimulation (sine wave) at a frequency of 30 Hz and a pulse width of 250 microseconds at intensities of either 5 or 20 ma for a period of 30 minutes. The stimulation output was current controlled. DC stimulation was also applied for 30 minutes. The bacterial culture along with cultures where no stimulation was applied, were then placed back into the incubator for another 24 hours and growth was assessed. The process was repeated on 8 cultures for each bacterium.

Statistical Analysis-

Statistical analysis involved the calculation of means, standard deviations, and t tests. Values in the text are shown as the mean+/-SD. The level of significance was $p < 0.05$.

Results

Initially, the McFarland Scale was calculated at a frequency of 580 nanometers for each of the bacteria. Absorbency at this frequency was plotted against set concentrations of bacteria to obtain a calibration curve. The correlation between the concentrations of bacteria to the absorbency was 0.98. This yielded an R^2 of 0.978 and, the equation related concentration to absorbency was Y = 260.87x. Given the calibration on the McFarland Scale, broth was mixed and the studies were completed as described under procedures. The results for each bacterium are listed below.

Escherichia coli - The results showing growth in Escherichia coli are shown in figure 1, panel A and B. Panel A, in figure 1, shows the bacterial colony growth from the beginning to the end of the study (24 hours after the initial incubation). As shown in panel A of figure 1, with a current of 5 milliamps, the increase in bacterial colony growth over 24 hours was 365 +/- 57 x 10^6 bacteria per milliliter whereas for 20 milliamps stimulation the increase in growth was 366 +/- 16 x 10^6 bacteria per milliliter. For the controls, where no stimulation was accomplished, bacterial colony growth increased by 401 +/- 7.5 X 10^6 bacteria per milliliter over the 24 hours. There was no statistical difference between growth in the 5 and 20 ma and control groups ($p < 0.01$). Bacterial colony growth for DC stimulation was 460 +/- 74 x 10^6 bacteria per milliliter. The only significant growth reduction compared to the controls was for stimulation at 20 ma ($p<0.01$). Here, however, the reduction in growth was only 9.8%.

pH was also measured at the beginning and at the end of studies. For the control bacteria colonies (no stimulation) pH started at 7.38 +/- 0.04. At the end of the 24 hour period, pH averaged 6.16 +/- 0.18, a reduction of an average of 1.22 pH units. For bacterial stimulation at 5 and 20 milliamps, there was a significantly greater reduction in pH over the 24 hours compared to the control colonies ($p > 0.01$). The change in pH was small, averaging 0.4 pH units. For DC stimulation, the change in pH, over the 24 hour period was not significantly different than that of the controls ($p > 0.05$). There was no correlation for any group in pH and bacterial growth ($p>0.05$).

Pseudomonas aeruginosa- As shown in the top panel of figure 2, under all four conditions, there was an increase in bacterial growth over the 24 hour period for these colonies. As shown in panel A of figure 2, the increase in bacterial growth with 5 milliamps of stimulation averaged 204 +/- 116 x 10^6 per milliliter, for 20 milliamps stimulation averaged 228 +/- 121 x 10^6 bacteria per milliliter and after DC stimulation averaged 331 +/- 161 x 10^6 bacteria per milliliter over the 24 hour period. The growth for all conditions was significantly lower than growth for E. coli ($p< 0.05$). Compared to the control group, the bacterial growth over the 24 hour period was significantly less for the AC stimulation than for the control group ($p<0.05$). Bacterial growth averaged a 38% reduction in growth with AC stimulation. For DC stimulation, there was no significant difference to the growth of the control group ($p>0.05$).

As shown in the bottom panel of figure 2, the overall change in pH was less than that observed for E. coli ($p < 0.01$). However, the reduction in pH, which averaged 1.25 +/- 0.5 in the control group, was only statistically less with stimulation at DC and 20 ma

AC ($p<0.05$). There was a significant correlation between pH and growth only for stimulation at 20ma. ($p<0.01$)

Staphylococcus aureus- Figure 3 shows the results of the Staphylococcus experiments. As shown in the top panel of figure 3, the average change in growth for these bacteria was 358 +/- 8 x 10^6 bacteria per milliliter after stimulation with currents of 5 milliamps, 376 +/- 20 x 10^6 bacteria per milliliter after stimulation at 20 milliamps and 405 +/- 69 x 10^6 after DC stimulation. Only the growth after 5ma AC stimulation was different than that of the controls ($p < 0.01$). However, the reduction in growth was only 3.9% compared to the control group.

As shown in the bottom of the panel, the pH was different in the 5ma group compared to the control group ($p<0.01$). There was no difference between the DC and AC 20 ma groups compared to the control groups ($p>0.05$). There was a significant correlation between pH and growth in the 20ma and DC groups ($p<0.01$).

Discussion

One of the mechanisms causing wounds to not heal is bacterial invasion.[21] Bacteria rupture cell membranes and maintain chronic inflammation to prevent wound healing[27] Many studies show that either DC microcurrent or low current AC stimulation has the ability to increase wound healing.[6, 9, 10, 17] In earlier studies, Rowley and colleagues [22, 23] published data showing that electrical stimulation has a bacteria static effect on Escherichia coli and several other bacteria. Barranco et al. [28] showed inhibition in Staphylococcus aureus growth with 400μA DC stimulation. But the electrodes were metal and at 400 μamps, corrosion was noted. Other in vitro studies with 100 μA DC stimulation also showed a bacteria static effect but here silver electrodes were also used. [29, 30] Other studies showed a bacteria static effect of DC at 1, 5, or 10 ma but not AC stimulation. [31] These studies showed large changes in pH with DC stimulation, probably due to the fact that, unlike clinical situations where stimulation is a 30 minute modality, the current was left on for hours.[32] This pH change was seen also with AC stimulation at 500 volts. [33] But these studies were conducted in vitro with a small separation distance between the electrodes. Thus the current per mm of distance on the bacterial cultures were large. Further, metal ions like silver can kill bacteria in themselves.

In addition, these studies were conducted at very high stimulation voltages (over 300 volts). In wounds, nerve endings are sensitized by the release of cytokines.[34] Even mild electrical stimulation can therefore be painful. In previous studies, [16, 1, 15] the current used to heal wounds was less than 20 ma. Therefore this was the current used in these studies.

In the present investigations, the density of the broth, size of the chamber and size of electrodes were adjusted so that the electrodes were separated by the distance seen in a typical wound treatment.[15, 16] The impedance of the broth matched that of a human wound, about 1200 ohms between the electrodes. This, in itself, is different than previous studies where large current densities were seen due to high voltages being applied to small bacteria cultures. While we used a large chamber for the broth, the current density may have still been somewhat higher then in a limb due to the higher volume in a limb. However, while current density may have been somewhat higher than in a wound here, earlier in vitro studies used a fraction of the volume used here between the electrodes and electrodes were closer together making current density in e.g. Rowley et al [22, 23] much higher than in wounds.

When currents were applied in the present experiments for a 30 minute stimulation period, similar to a standard treatment period for wound healing, DC stimulation had no effect on bacteria growth. AC had some effect on each of the bacteria but the only real effect on growth with electrical stimulation was on pseudomonas. Any effect seen on Escherichia coli and Staphylococcus aureus was small. For pseudomonas, the growth reduction at either AC current was large.

However, bacteria can still be destroyed by electrical stimulation through another mechanism in wounds. Electrical stimulation and, electrical stimulation of wounds causes the release of prostaglandins and other cytokines.[35, 36, 37, 38] These prostaglandins and other cytokines attract macrophages to the area where electrical stimulation is applied.[31] Therefore, although electrical stimulation may not have a direct effect on bacteria in wounds, there is some evidence that white blood cells such as macrophages

are attracted as a secondary effect of the electrical stimulation, this may explain why electrical stimulation may be associated with bacterial death.

The present studies still remain in direct contradiction to the studies by Rowley et al [22, 23] showing a direct effect of electrical stimulation on inhibiting growth in Escherichia coli. However, while Escherichia coli and Staphylococcus aureus showed either a statistically insignificant change in growth or an insignificant clinical change in growth with electrical stimulation, this is not to say that electrical current does not alter bacterial behavior. Bacterial motility and attachment to substrates appears to be impaired by stimulation with electric currents (39). This is especially true for pseudomonas where flagella are needed for mobility (40). The fact that pseudomonas here did have some impaired growth may be due to the fact that there are two flagella proteins involved in its mobility and attachment- if either is impaired, growth is reduced [41]. Here, these flagella, since they use contractile proteins and ion channels for movement, may be impaired with electric current. This makes these bacteria different from the other 2 which do not move by this mechanism.

However, there are conditions where electrical stimulation will inhibit growth in all 3 bacteria. While currents of up to 20ma in themselves with these same bacteria did not reduce bacterial growth [39,42], in combination with antibiotics, the reduced ability of the bacteria to attach to substrates made them more susceptible to attack by the antibiotics. Thus while antibiotics alone or electrical stimulation alone did not kill these bacteria, the combination of the two did [39,42]. Thus, electrical stimulation with either DC or weak AC currents may have an unseen effect which may kill bacteria in vivo but not under these circumstances in vitro. Further investigation is warranted. Here, for example, current

was used for 30 minutes, a standard treatment modality. Perhaps longer exposure to current would have a more dramatic effect in vivo.

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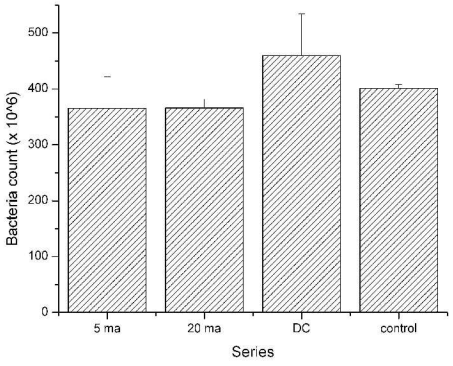
Figure 1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300
200
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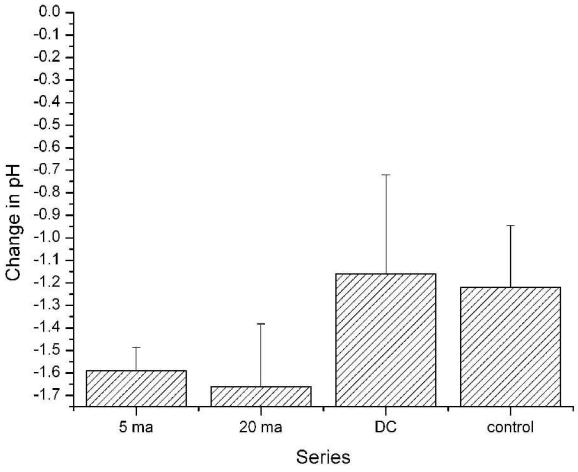
0
5 ma
20 ma
DC
control
Series

A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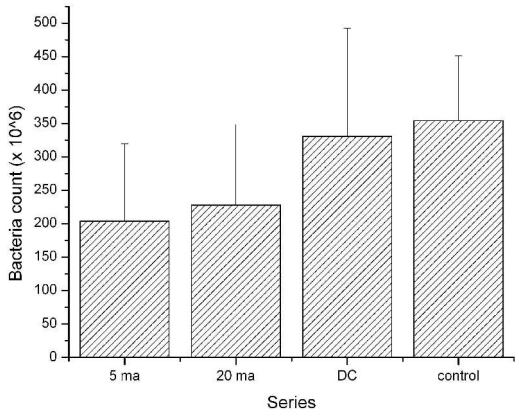
Series

B

Figure 2


Bacteria count (x 10^6)
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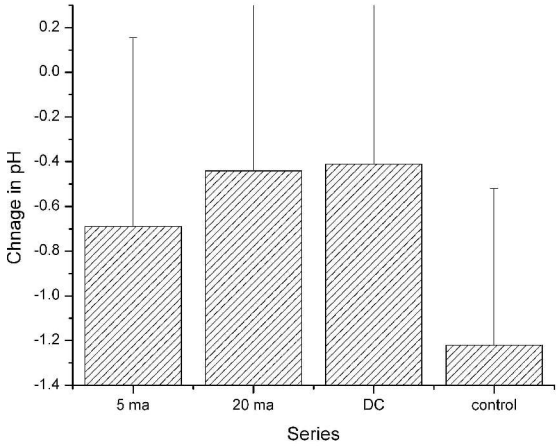
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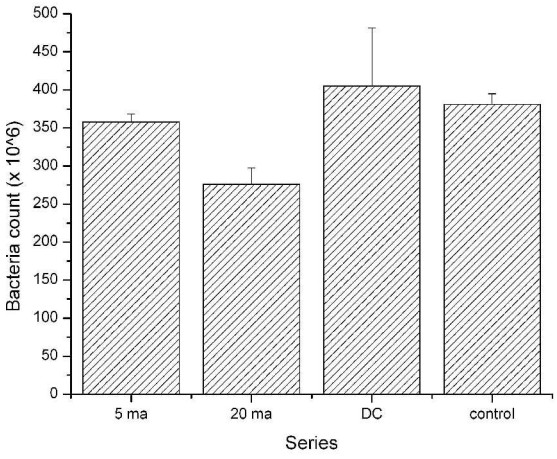
Figure 3


Bacteria count (x 10^6)
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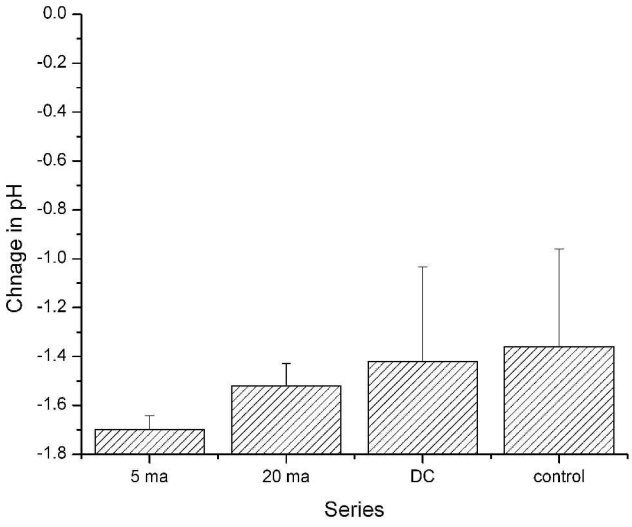
Chnage in pH
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