

Full Length Research Paper

## Effect of alternating and direct currents on *Pseudomonas aeruginosa* growth *in vitro*

Hamid Maadi<sup>1,2\*</sup>, Morteza Haghi<sup>1,2</sup>, Reza Delshad<sup>1</sup>, Haleh Kangarloo<sup>3</sup>, Mohammad Ali Mohammadnezhady<sup>1,2</sup> and Gholam Reza Hemmatyar<sup>1</sup>

<sup>1</sup>Microbiology Department of Islamic Azad University of Urmia, Iran.

<sup>2</sup>Young Research Club.

<sup>3</sup>Physic Department of Islamic Azad University of Urmia, Iran.

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Eradication of pathogenic bacteria from important part of our life such as dental tools, foods and wounds is necessary. Based on the effect of natural selection, these bacteria become resistant to antibiotics. In some cases such as the section where burnt are treated in the hospital, we observe high rate of mortality as well as high numbers of resistant bacteria. In order to solve these problems, electrical stimulation (ES) is proposed. This has being shown to be an effective method. One of the reasons why it works could be due to the bacteria static property of electrical stimulation. So, more studies must be done so as to reach optimum voltage and currents. The test media were Muller-Hinton agar and eosin methylene blue (EMB) agar. In this research *Pseudomonas aeruginosa* which was isolated from patients' wounds was examined with levels of alternating and direct current (AC and DC) electrical stimulation (1.5V, 3.5V, 5.5V and 10V) to see if these currents could inhibit *P. aeruginosa* growth *in vitro*. The experiment was performed in two forms: The first was carried out immediately while the second was carried out 19 h after being cultured. Different patterns of zone of inhibition were observed in the two forms of our research. AC current had low inhibitive effect on *P. aeruginosa*'s growth. Anode and cathode showed different zone of inhibition, in each of the forms and media. The maximum inhibition zone (22 mm) was observed around cathode in 3.5 V direct current which was immediately used in the media. Direct current significantly inhibits growth of *P. aeruginosa*. Based on other studies on different bacterial species, ES can be applied to sterilization and controlling of superficial infections like in burnt patients.

**Key words:** Electrical stimulation, *Pseudomonas aeruginosa*, wound healing.

### INTRODUCTION

Electrical stimulation (ES) has been used for hundreds of years for a variety of purposes including muscle strength training and wound healing. Promoting wound healing is possible with some mechanisms including increased circulation (Petrofsky et al., 2005; Kloth, 2002), increased angiogenesis (Bai et al., 2004; Zhao et al., 2004; Ojingwa and Isseroff, 2003) increased proliferation of epidermal

tissue and antibacterial effect of ES (Rowley et al., 1974). Bacteria static effect of electrical stimulation was for the first time reported over 30 years ago by Rowley et al. (1974). Inhibition of bacterial growth has been reported by other researchers who used different types of electrical stimulation (Merriman et al., 2004; Kincaid and Lavoie, 1989; Petrofsky et al., 2008). Two main types of electrical stimulation were used in these studies: High-voltage pulsed current which is extremely painful for the patients and in which the duration of treatment with this ES is short and the second, low intensity direct current with opposite properties. Based on the effect of natural selection, bacteria become resistant to antibiotics. In some cases such as the section where burnt are treated in the hospital, we observe high rate of mortality as well

\*Corresponding author. E-mail: hamid.maadi@gmail.com. Tel: +989149397057. Fax: +984413451372.

**Abbreviations:** AC, Alternative current; DC, direct current, EMB, eosin methylene blue; ES, electrical stimulation.



**Figure 1.** Experimental setup, glass petri dish with stainless-steel electrodes connected to DC power supply before placement into incubator

as high number of resistant bacteria. So, we can use ES as a common antibacterial against these resistant bacteria. We can also use ES as a common sterilizer to sterile dental tools, food, etc.

ES with different waveforms, voltages, etc have different effects on bacteria growth. So, it provides a vast field for research. Some authors showed wound healing with DC micro currents while others showed only healing with strong AC currents (Feedar et al., 1992; Franek et al., 1999; Houghton et al., 2003). Report has being made to show that in the early stages of wound healing, the polarity needs to be positive around the wound and negative away from the wound, but there are other reports contrary to this (that is, the polarity needs to be negative around the wound and positive away from the wound) (Yarkony, 1994; Bogie et al., 2000; Demir et al., 2004). Due to differences in results and types of ES, more research needs to be done to reach an optimum and standard methods. In addition, for various purposes, we need to find different standard methods.

In this study, we used different voltages and media culture. We did comparable studies of both AC and DC effect on bacteria growth. We also gave ES once immediately after culture and in the other form, 19 h after culture to investigate their different effects on bacterial growth.

## MATERIALS AND METHODS

### Organisms tested

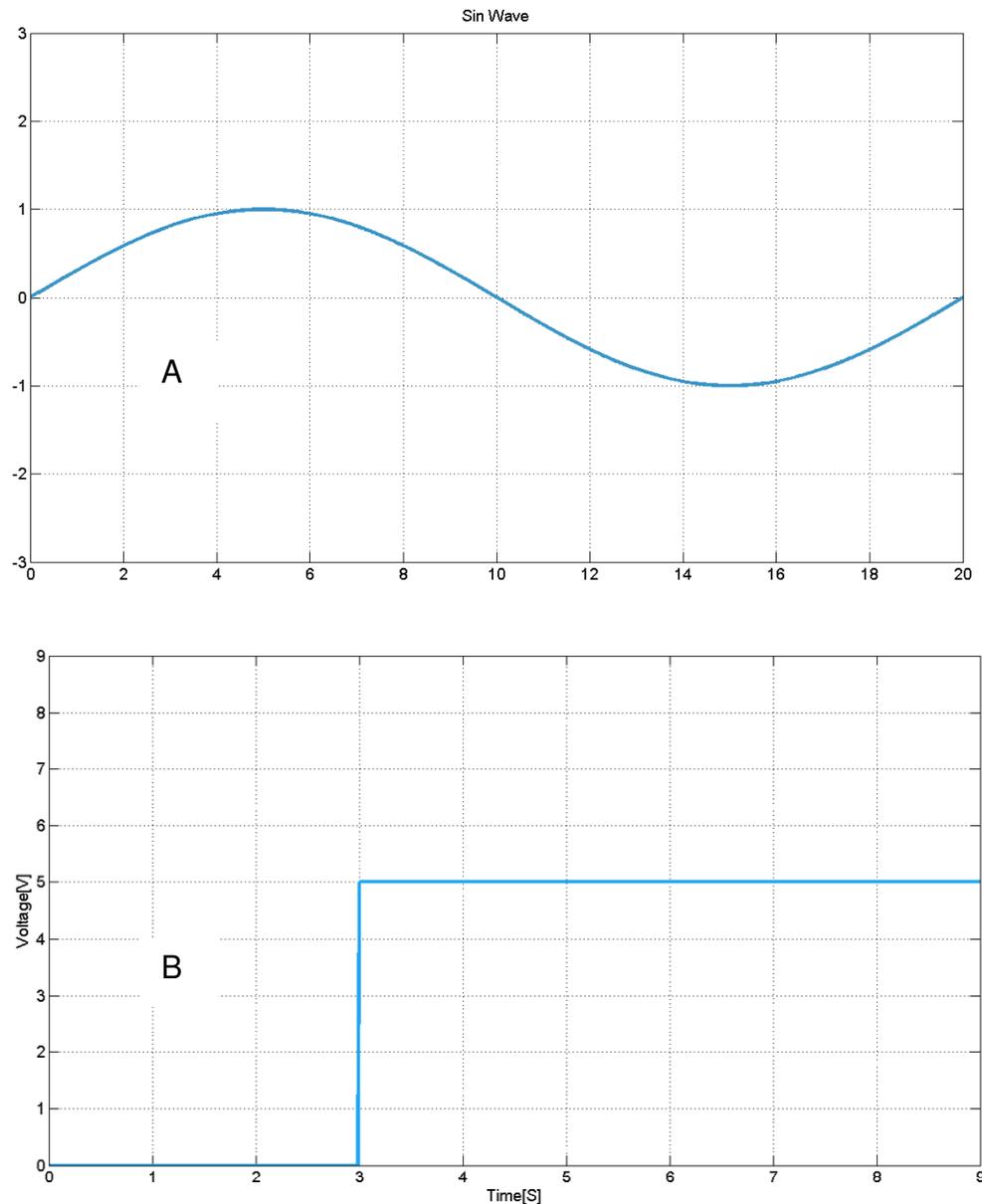
*Pseudomonas aeruginosa* (gram-negative rod) which were used in this study were isolated from patients in Motahari hospital laboratory,

Iran.

### Procedures and instrumentation

Sterile glass Petri dishes were used throughout the experiment. Two holes were bored through each glass 100 mm Petri dishes (7 mm diameter and 45 mm apart). Stainless-steel wires were used as electrodes (0.44 mm gauge and 23.20 mm length). Sterile electrodes were covered by plastic to prevent the wires from rolling and breaking contact with the medium. All tools were sterilized by autoclaving before using. We used DAZHENG DC power supply (PS-303D) and ALFA step-down transformer as the ES devices.

We studied antibacterial effect by Kirby-Bauer technique (Bauer et al., 1996) which was standardized by the National Committee for Clinical Laboratory Standards (NCCLS) instruction with one difference; instead of using standard antibiotic discs, we used ES. The test media selected were Muller-Hinton agar and eosin methylene blue (EMB) agar. Muller-Hinton agar was used in the semi quantitative Kirby-Bauer technique for determining effectiveness of antibiotics due to the consistency of the widths of zones indicating inhibition of bacterial growth (Kincaid and Lavoie, 1989). To run the test, wires in the Petri dishes were connected by alligator-clip leading to ES devices. The experimental setup is shown in Figure 1. Voltages of 1.5, 3.5, 5.5 and 10 were applied to the test organisms. In the first type of our study, each plate was incubated at 37°C for 19 h following exposure to ES. In the second type, each plate was incubated at 37°C for 19 h and then was exposed to ES for 19 h at 37°C. The width of the zone of inhibition parallel with the wire electrodes where no bacterial growth occurred was measured with a millimeter ruler. Test results represent the average of two measurements per zone of inhibition in each plate. Changes in the pH of the media were determined by using pH paper touched to the surface of the media. By measuring the voltage and the resistance of the medium, we found out that by increasing the voltage, the resistance of the medium decreases, so the conductivity of the medium would increase. Two kinds of currents were used in this study. The first current which was used in the first stage is the DC signal. The frequencies of these signals are zero and the amplitude of DC



**Figure 2.** Comparison of waveforms. (a) AC signals with 50 HZ frequency and 20 ms period (b) DC signals with zero frequency.

signals is variable. The second current used in the second stage is AC signals. There are many types of AC signals, but in this study sine waves were used as AC signals with 50 HZ frequency and 20 ms period (Figure 2).

### Controls

Current was sent through sterile media without organisms and then bacteria were cultured on media to determine the presence of potential toxic electrochemical products from the interaction of current, wire and media. Seeded plates with the wire electrodes in place were incubated without exposure to ES to determine whether the wires themselves, the media, or a combination of both would be inhibitory to bacterial growth. Seeded plates with the wire elect-

rodes in place were incubated without exposure to ES and organisms to ensure sterility.

### Data analysis

Results were analyzed by two-way analysis of variance (ANOVA). Regression analysis was performed by using the Microsoft Excel 2007 and MATLAB 2009B.

### RESULTS

We have four variables: (1) Type of ES (AC and DC)

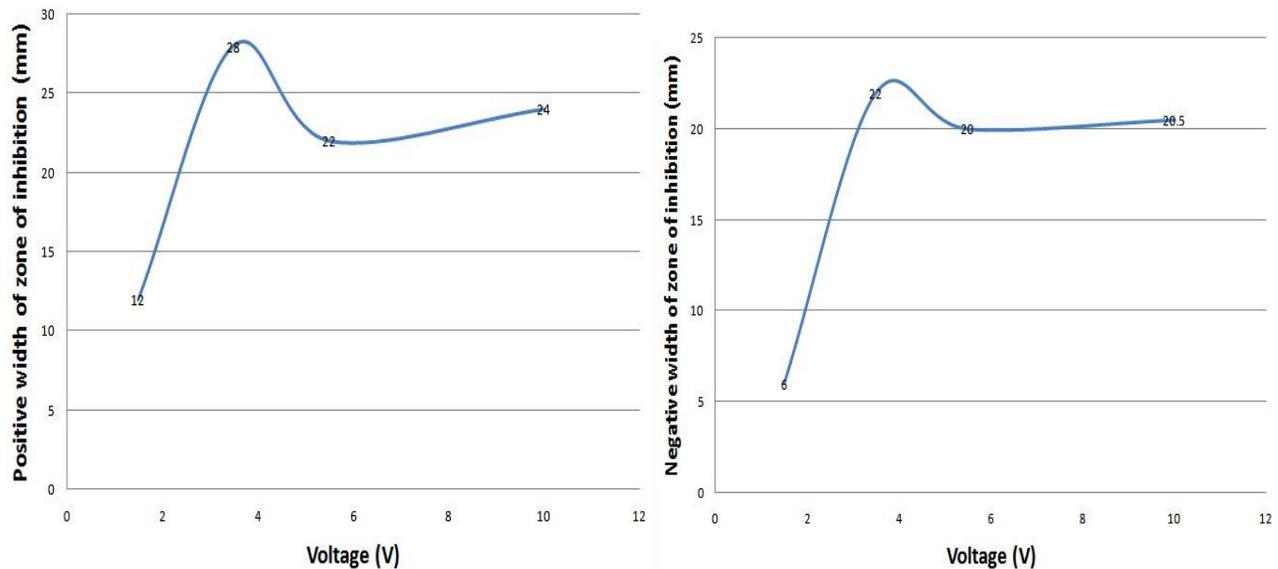


Figure 3. Inhibitory zone of Mueller-Hinton agar with DC (type 1).

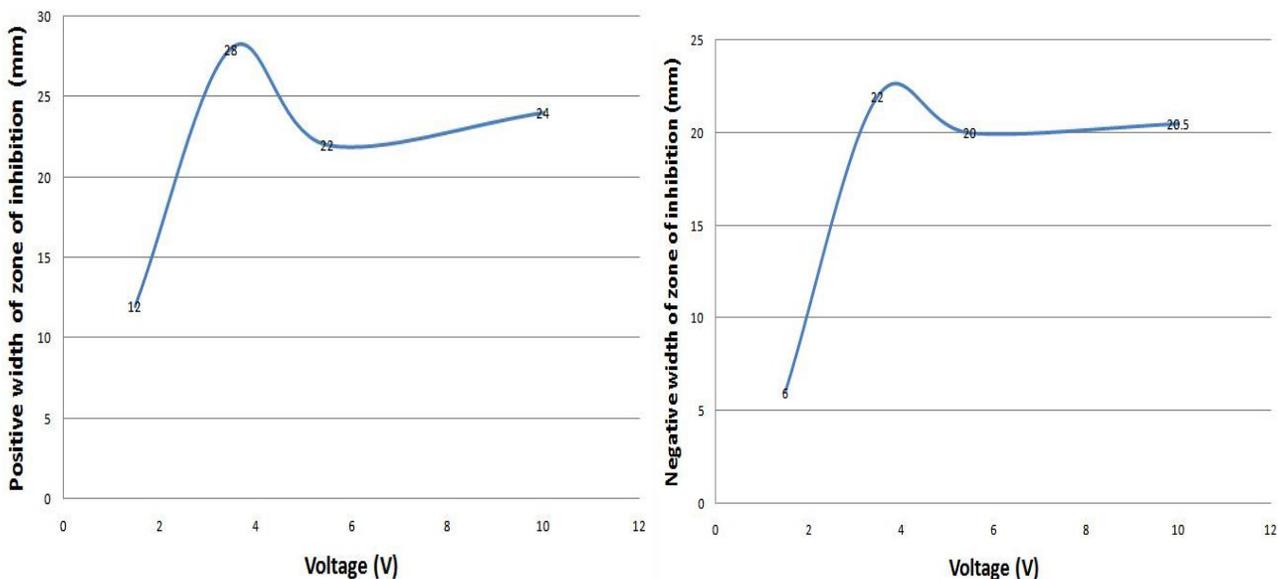


Figure 4. Inhibitory zone of Mueller-Hinton agar with DC (type 2).

treatment; (2) electrode polarity; (3) type of media culture; (4) different voltages. ANOVA results revealed that there are significant statistical differences with the type of ES (DC and AC), ( $p < 0.05$ ), media culture and different voltages ( $p < 0.05$  and  $p < 0.01$ ). Statistical analysis revealed no significant differences with electrode polarity. Several control plates established that exposure to the current was the cause of the bactericidal effect on the test organisms. Sending current through sterile media without organisms and then culturing bacteria on media showed no inhibition of growth around the cathode, but the test organisms would not grow around the anode

because of the presence of toxic electrochemical products. Plates containing seeded medium that were not exposed to ES showed no inhibition of growth, indicating that the wires themselves, the medium, or both, had no inhibitory effect. Seeded plates with the wires electrodes in place without exposure to ES and organisms displayed that the sterling method was perfect and no bacteria growth was observed. Growth inhibition of *P. aeruginosa*, represented by the measurements of the zones of inhibition, is shown in Figures 3, 4, 5, 6 and 7. In other cases with AC stimulation, we did not see any inhibitory effect on bacterial growth.

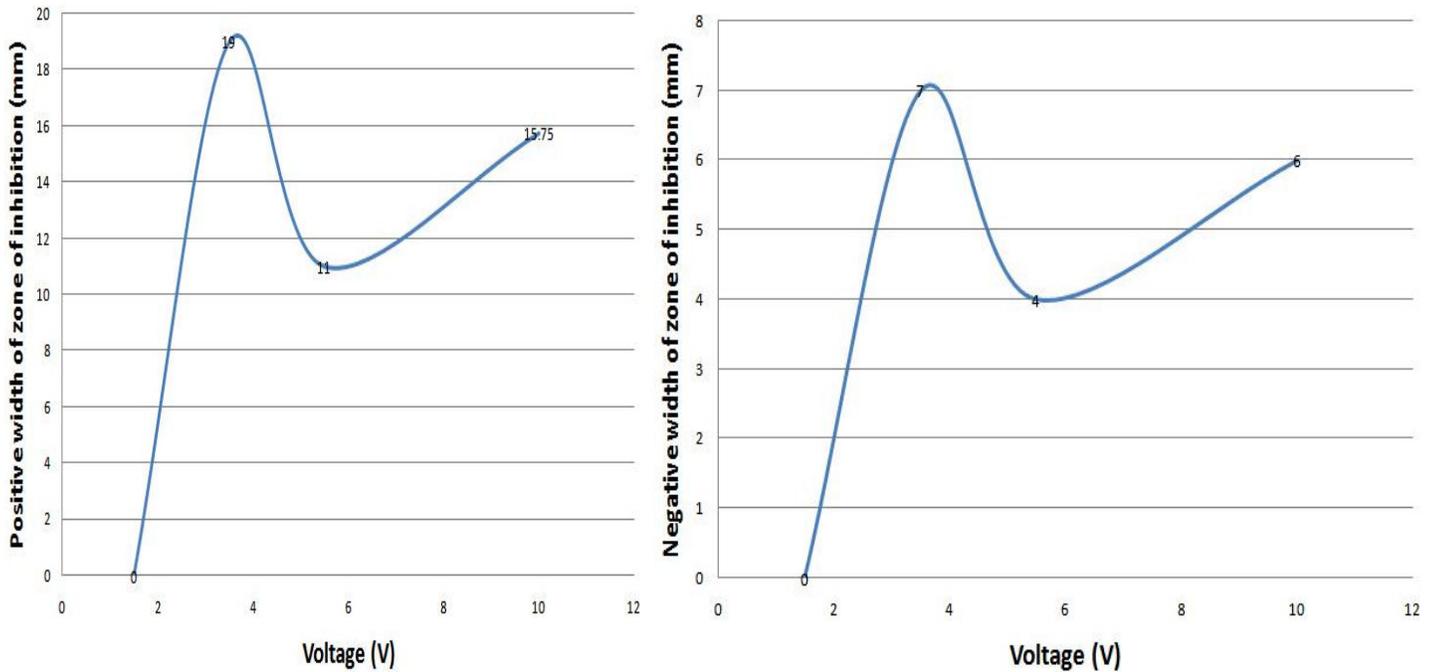


Figure 5. Inhibitory zone of EMB agar with DC (type 1).

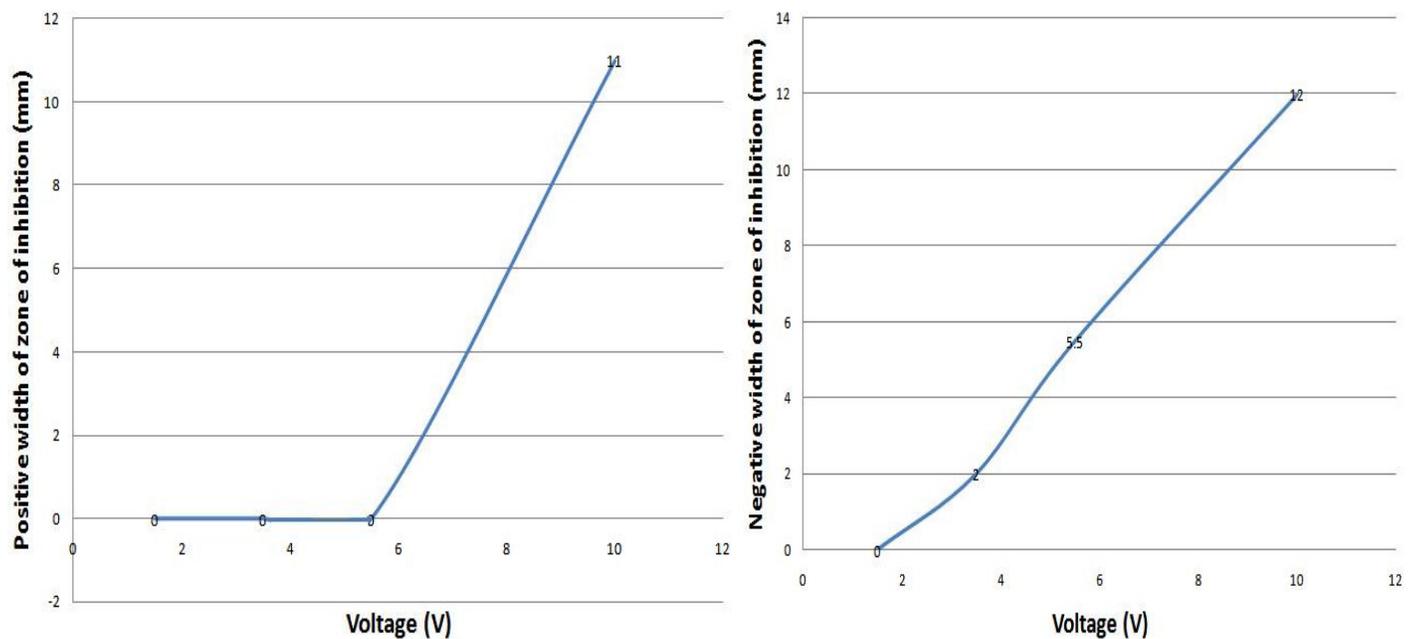


Figure 6. Inhibitory zone of EMB agar with DC (type 2).

**DISCUSSION**

Direct current can be effective in killing common wound infecting bacteria *in vitro*. One of the advantages of this study compared with other studies is that we did comparable studies of both AC and DC effect on bacteria

growth. The data analysis revealed that there are statistically significant differences between the effect of DC and AC on bacteria growth. According to the averages obtained, DC has better antibacterial effect than AC.

Data analysis revealed that there are no significant statistical differences between cathode and anode. Effects

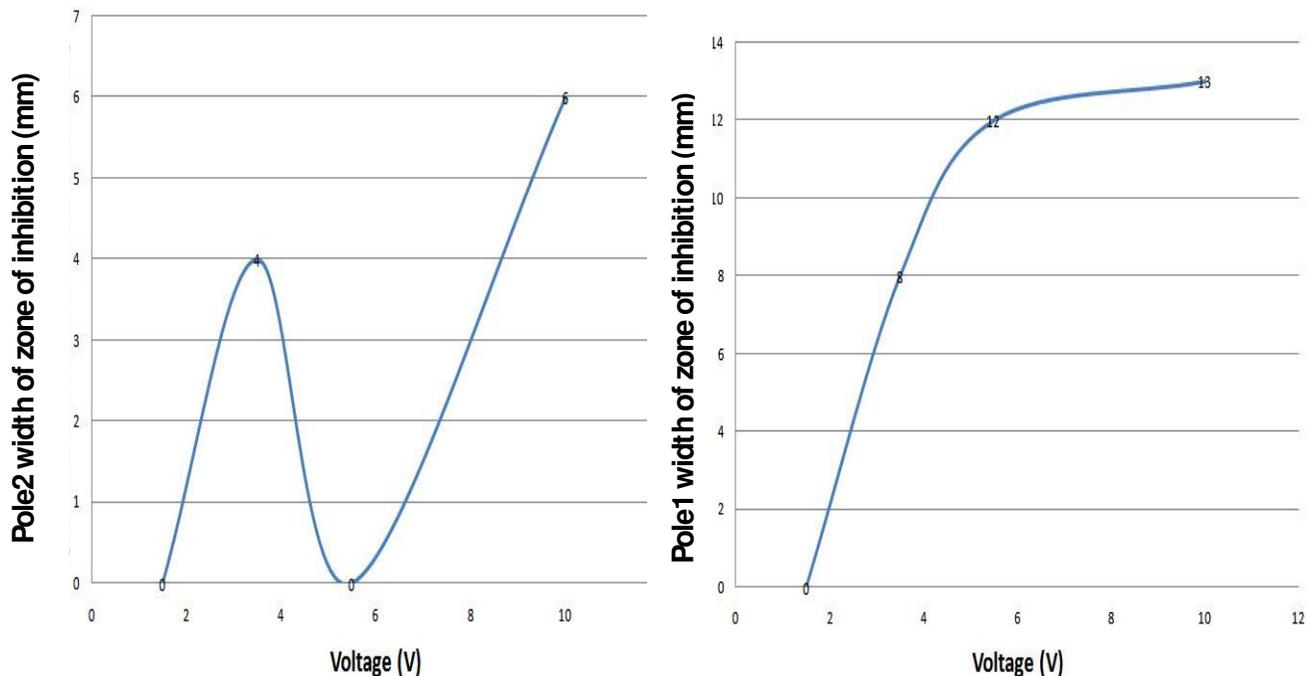


Figure 7. Inhibitory zone of Mueller-Hinton agar with AC (type 1).

of ES around the anode were complicated by the production of some toxic electrochemical end products created by passing current through the wire. These results are similar to those reported by Kincaid and Lavoie, (1989). But, we must notice that at 1.5 and 3.5 voltages in EMB agar we did not observe any electrochemical toxic products around the anode. Growing bacteria at the cathode end after sending current through sterile media without organisms suggest that no permanent change had occurred there. No bacteria growth at the anode end was observed after sending current through sterile media without organisms suggesting that lethal end products had accumulated and persisted. On the other hand, anode is painful to the patient if use for a long time unlike cathode. The other advantages of this study in comparison with other studies is that in this study we use two different media culture to see if there is differences in results by changing the media culture. The data analysis revealed that there are significant statistical differences between two media culture. So, we observed that with changes in the media, results would change because of different properties of media culture. So, this effect must be shown *in vivo* or in clinical wounds. In this study we used different voltages to reach an optimum voltage. The data analysis revealed that there are significant statistical differences between voltages. According to the averages obtained in method one of our study (that is, immediate exposure to ES), 3.5 V DC is the best voltages for bacteria growth inhibition (in both media cultures and around both electrodes). In method two of our study (that is, exposure to ES 19 h after culture) the averages obtained, 5.5 V

DC is the best in Mueller-Hinton agar (around the both electrodes) and 10 V DC is the best in EMB agar (around the both electrodes). If we consider method one as prevention and method two as treatment, we can use ES for both purposes.

Exposure to ES at the cathode has sufficient inhibitory effect on bacteria growth. Since cathode does not induce pain to the patient, it can be use for a long time. In this study, we suggest that treatment duration of 19 h or less than 19 h a day with 3.5 V DC and negative polarity around the wound and positive polarity away from the wound have better inhibitory effect on bacteria growth than other forms.

Alternatively, the actual current flow through the Petri plates was very low because of resistance from the test medium. If there is less resistance to current flow in human skin, then lower settings might have bactericidal effect in a clinical setting (Merriman et al., 2004). Further investigations can be done *in vivo* on the application of DC with different voltages at both polarity and AC with different waveforms when their inhibitory effect on bacteria growth had been proved *in vitro*. Other investigation can include the effect of other type of electrical current on microorganisms' growth *in vitro*.

## Conclusion

The purpose of this investigation is to study the effect of different AC and DC voltages with different conditions on bacterial growth. A statistically significant bacterial inhibi-

tory effect was found for direct current with different voltages. Because of, low intensity direct current inhibitory effect on bacterial growth according to these findings, we can practically use low intensity direct current to promote wound healing. These findings must be shown *in vivo* or in clinical wounds.

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