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Healing of Guinea Pig Injures Contaminated with *Pseudomonas aeruginosa* by using 0.7Hz Square Pulsed Magnetic Field (New Method)

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Abstract

Pseudomonas aeruginosa is considered one of the main dangerous causes of burn wound infection and known as highly resistant to antibiotics. In this work the effect of Pulsed Magnetic Field (PMF) on the growth activity of *P. aeruginosa* is studied. Recently on our lab it is reported that exposure of *P. aeruginosa* to 0.7 Hz (at resonance frequency) magnetic impulses for 1h caused 24% inhibition of bacterial cellular growth relative to the unexposed one. Dermal burns in the thigh of 25 guinea pigs were done by thermal conduction through the transfer of 1.080 kJ to circular cross sectional end of (0.760+0.0002) cm in diameter. The animals were divided into 5 equal groups (5 guinea pigs per each) namely A, B, C, D and E. Animals of group A were injured and contaminated with a swap of $1x10^8$ CFU/ml of *P. aeruginosa*. Animals of group B were injured and contaminated with a swap of $1x10^8$ CFU/ml of *P. aeruginosa* and treated by exposure to 0.7 Hz PMF for 3 hours at a rate of 1 h/day. Animals of group C were injured and contaminated with a swap of $1x10^8$ CFU/ml of *P. aeruginosa* previously exposed to 0.7 Hz PMF for 1 hour. Animals of group D were injured without contamination and treated by exposure to 0.7 Hz PMF for 3 hours at a rate of 1 h/day. Animals of group E was left as control (injured without contamination or exposure to PMF). The results indicated significant progress in wound healing for group B and almost same healing progress for groups C, D. It was concluded that the use of 0.7 Hz PMF significantly accelerates healing of wounds contaminated with *P. aeruginosa*. The new method is promising and need of further work to use such fields for sterilization and/or treating contaminated wounds is a must.

Keywords: wound, *P. Aeruginos*, Pulsed magnetic field, Resonance frequency.

Introduction

P. aeruginosa which is a gram-negative, motile, rod shaped bacterium that belongs to the family Pseudomonadaceae. *P. aeruginosa* is a common nosocomial contaminant so this opportunistic pathogen is commonly associated with hospital-acquired infection especially prevalent among patients with burn wounds [1], cystic fibrosis [2], acute leukemia, organ transplants, and intravenous-drug addiction. The most threaten problem of *P. aeruginosa* is infecting wounds of patient with severe burns, since severe thermal injury destroys the barrier function of the skin and this enables bacteria to gain easy access to the injured tissue. The denatured protein of the burn scar provides a good environment for microbial

growth and the avascularity of the burn wound partly shields the micro-organisms from host defense mechanisms [3]. Treatment of *P. aeruginosa* by antibiotics proved ineffectiveness since it is notorious for its resistance to antibiotics and this intrinsic resistant to many antibiotics due to the permeability barrier afforded by its gram-negative outer membrane. Also, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic antibiotics also arises from the combination of unusually restricted outer-membrane permeability and secondary resistance mechanisms such as energy-dependent multidrug efflux and chromosomally encoded periplasmic-lactamase [4]. Therefore β considerable efforts have been made towards the development of alternative methods for the treatment of bacterial growth; one of the promising methods is exposing the microbe to the electromagnetic field. Strasak L. et al. in (2005)[5] observed the ability of Escherichia coli to form colonies decreased with exposing to 2.7-10 mT, 50 Hz with time of exposure in the range 0-12 minutes and this reduction increase with increasing magnetic field intensity and with increasing time of exposure. Novak et al. (2007) [6] showed exposure of yeasts to magnetic field of induction 10 mT, 50 Hz with varied exposure time in the range 0–24 min caused higher reduction in microbial counts at longer exposure time. However the medical applicability of this technique is limited due to the need of very high field strength of several kV/cm and very high temperature. Fadel et al. (2005) succeeded to control the growth of Ehrlich tumours in mice[7], while in (2010) succeeded to control the liver and spleen tumour metastasis[8] ,and Fadel et al. (2009) also control the growth of fungi using Amplitude Modulated waves at resonance frequency with bioelectric signals generated during a particular metabolic activity[9]. In more recent work, Fadel et al. (2013) showed that exposure of *P. aeruginosa* to 0.7 Hz PMF could control the activity of the bacteria and cause changes in the structure of the DNA. Therefore the aim of this study is to find out the applicability of treatment of injuries infected with *P. aeruginosa* by 0.7Hz-PMF.

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Materials and Methods

- Animal Housing:

Each animal was caged separately with free access of water and diet and all animals were subjected to a normal day, night rhythm. The room temperature was about 22 ± 2 ^{o}C.

- Experimental animals:

The experiment of the present study was carried out on 25 male (275±25) gm guinea pigs. They were divided into 5 equal groups namely A, B, C, D, E, each group composed of 5 animals.

Group A: Burns' wounds of this group were culture of *P. aeruginosa* containing 1x10^{8} CFU/ml and did not receive any treatment.

Group B: Burn's wound contaminated of by a surface this swab with group were a culture of *P. aeruginosa* containing 1x10^{8} CFU/ml and then treated by 0.7Hz-PMF for 3 h at a rate of 1 h/day.

Group C: The wound of each animal from group C was contaminated with a swap of 1x10^{8} CFU/ml of *P. aeurginosa* previously irradiated with 0.7Hz-PMF for 1h.

Group D: The wound of each animal of this group was not contaminated by bacteria and treated by exposure to 0.7Hz-PMF for 3 h at a rate of 1 h/day.

Group E: The animals of this group were injured and left without contamination and did not receive any treatment.

- Inducing wound burn injury:

The hair of the upper part of the right hind limb was shaved by electrical hair shaving machine. The Guinea pig was anaestheszied (by using ether). The skin segment was cleaned with alcohol. A partial skin thickness burn wound of approximately 0.76 cm diameter was induced on the shaved hind limb of all animals by thermal conduction through the transfer of 1.080 kJ using

round aluminium stamp with a contact circular of (0.50+0.0002) cm in diameter

- Contamination procedure

Fresh culture of the tested strains were grown on MacConkey Broth medium overnight at 37^{o}C in a shaking water bath, and enough culture was added to the test medium to reach a final concentration of 1x10^{8} CFU/ml. One ml of culture that had been growing overnight was inoculated to 100 ml of the medium Mac broth and then incubated and allowed to grow to reach to mid-to-late exponential phase. Immediately after introducing the burn wound, Burn's wound were culture of *P. aeruginosa* containing 1x10^{8} CFU/ml.

- Treatment procedure and measurements:

Recently on our lab it is reported that exposure of *P. aeruginosa* to 0.7 Hz (at resonance frequency) magnetic impulses for 1h caused 24% inhibition of bacterial cellular growth relative to the unexposed one. Treatment by 0.7 Hz-PMF was applied to the burn wound area by using magnetic gun. The gun placed at a distance of 0.5 cm apart from the treated area. The field strength at this distance is 0.5x10^{-4} tesla. Figure-1 illustrates the treatment system using PMF. Automatic Vernier caliper is used to measure the wound surface area diameter. Measurements were conducted 24 hours post burn for each animal daily up to 10 days. The wound diameter was taken 3 times and average value was taken for each animal in each group.

- Statistical Analysis:

The statistical analysis *t*-was *test* with performed a minimal using confidence level of 0.05 for statistical significance. Each experiment was performed at least three times with a minimum of three samples per termination point.

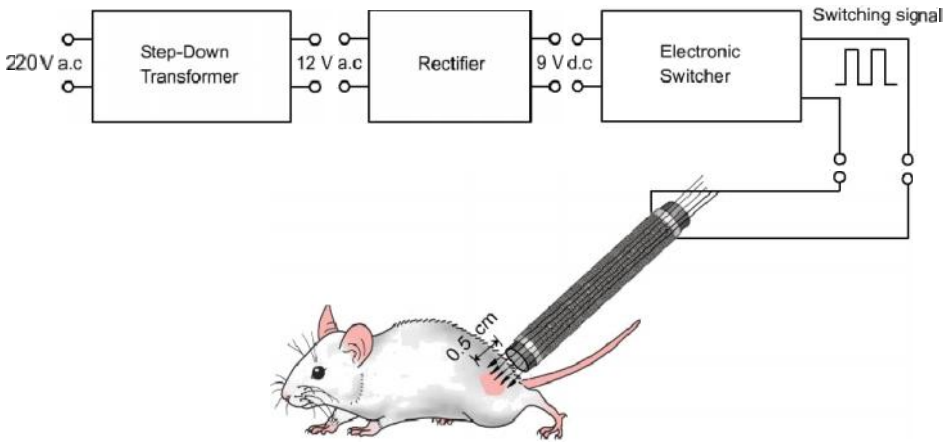

Figure-1: Treatment of the burn wound of the guinea pig by exposing to PMF using Magnetic gun 0.5 cm apart.

Results and Discussion

The in vivo study has been carried out on guinea pig skin, in which a burn wound of a diameter (1.44+ 0.0002) cm was injured. The Diameter of the wound of the different experimental groups was measured at day 10 for each group (5 animal/group) and the average value was considered which represented by the histogram in Figure-2. The results show remarkable decrease in the diameter of the wound received treatment by 0.7 Hz PMF (Group B) in comparison with un-treated one (Group A). Also, the data show that the wound diameter in group C and D was approximately equal with a significant decrease than that of group E, which indicates the efficiency of using PMF as therapeutic tool in wound burn healing mechanisms. The Photographs 1-6 show the progress of the wound diameter at day 1 and day 10 post wound injury.

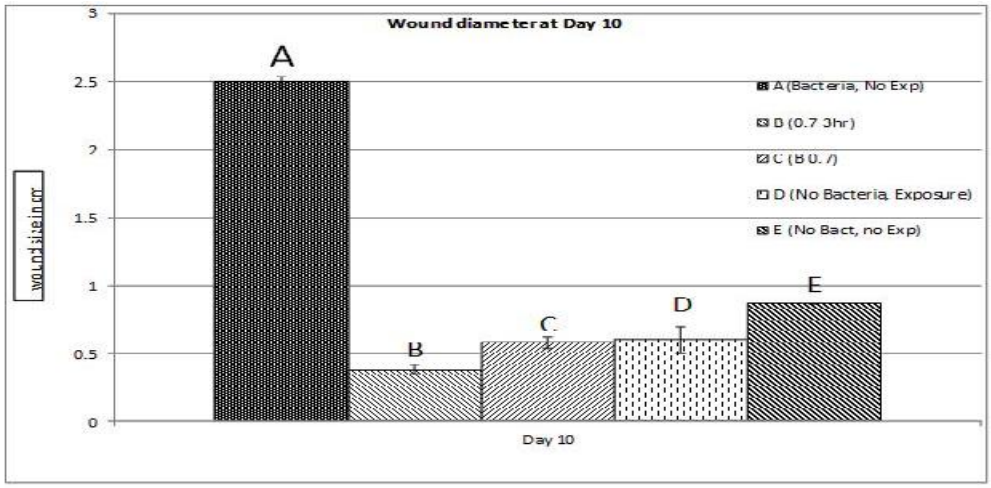

Figure:-2 Histogram expresses the results of the wound diameter progress in the different test groups

Photo-1: Shows the wound diameter 24 hours post injury.

Photo-2: Shows the wound infected by *P. aeurginosa* 10 days post infection with no further treatment

Photo-3: Shows the wound infected by *P. aeurginosa* 10 days post infection and after treatement by 0.7 Hz PMF for 3 hours.

Photo-4: Shows the wound infected by pre exposed *P. aeurginosa* to 0.7 Hz PMF 10 days post infection and no treatments is received

Photo-5: Shows for the clean wound at day10 after treatment by 0.7 Hz PMF for 3 hours.

Photo-6: Shows the clean wound at day 10 without infection or treatment with PMF.

It is reported previously that *P.aerguinosa* infects wounds of patients with severe burns, since severe thermal injury destroys the barrier function of the skin and this enables bacteria to gain easy access to the injured tissue. The denatured protein of the burn eschar provides a good environment for microbial growth and the avascularity of the burn wound partly shields the micro-organisms from host defense mechanisms [5]. Here appears the effectiveness of the PMF at resonance frequency (0.7 Hz) as the mention field could alter the electrostatic balance of the membrane components in a manner that producing loss of integrity and/or disorganization and thus triggering growth arrest or death of bacterial cells which lead to minimizing the toxins secreted by bacteria. This gives a good explanation to the increasing in the wound diameter in the groups didn't receive any treatment throughout the received PMF 0.7 Hz treatment. The remarkable decrease in wound diameter for groups received treatment but not infected by bacteria is due to that PMF has a vital role in healing of the wounds as studies show that EM field improve tissue repair and wound healing [10] also several authors showed effectiveness of PEMFs in reducing the wound-healing duration [11]. It has been shown that in vitro PEMF exposures favors collagen production by fibroblasts and also increases the tensile strength of scar tissue rat [12]. The increased tensile of the scar tissue is due to the increase in synthesis, alignment, and maturation of collagen [13]. Pulsed electromagnetic fields (PEMF) have been shown to enhance the tensile strength of diabetic wounds [14]. Tepper et al. [15] reported that PEMF promotes angiogenesis primarily by stimulation endothelial release of FGF-2 inducing paracrine and autocrine changes in the surrounding tissue and as result improve O_2 and nutrition supply to the wound. Also, PEMF prevent tissue necrosis and electrical stimulation results in restart of inflammatory and proliferative phases. There are reports that electrical stimulation increases fibroblast functional capacity, collagen synthesis and induction of wound reepithelialization and accelerating wound healing.

By comparing the progress in wound healing for groups infected by previously irradiated microbes with that infected by healthy bacteria, we found remarkable increase in the wound surface area in the second group other than the first group which is mainly due the interaction of toxins secreted by microbes with the protein contents of the infected area [16] while the pre-exposed microbes is unable to secret toxins or due to that the amount of toxins secreted by the bacteria diminished. These findings may be due to the decrease in the number of cell division of microorganisms in the infected area and / or the decrease of activity of the microorganism to secret toxins.

Conclusion

The results presented in this work indicated that treatment of *P. aerguinosa* infected wounds by 0.7 Hz pulsed magnetic field accelerate the healing process as indicated by wound diameter. The new method is promising and need of further work to use such fields for sterilization and/or treating contaminated wounds is a must.

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