Effect of Pulsed Electromagnetic Fields on Healing of Infected Burn Wound

Haytham Mohamed El-Hafez*, Ali Osman Ali Selim, Adel Abdel Hamid Nosseir**, Fadel Mohamed Ali*****

* Basic Science Department, Faculty of Physical Therapy, Cairo University
** Physical Therapy Department for Surgery, Faculty of Physical Therapy, Cairo University
*** Biophysics Department, Faculty of Science, Cairo University

ABSTRACT

Background. *Electromagnetic fields are used for promotion of healing in various conditions such as bone, cartilage, ligaments and nerve injuries. However, there are still controverseries about the use of these fields for promoting skin burn wound healing either in infected or non infected burn wounds.* ***Objective.*** *The aim of this study was to investigate the effect of electromagnetic fields in the form of square amplitude modulated wave "QAMW" on the healing of infected or non-infected burn wounds in guinea pigs.* ***Methods.*** *Fifty male guinea pigs were used in this study. The animals were divided into five equal groups (N=10) namely three control groups (A, B, and C) and two study groups (D and E). Partial skin thickness burn wound of approximately 4 cm^2 was induced on the hind limb of each animal.Pseudomonas aeuroginosa bacteria (surface swab with culture containing 10^7 CFU/ml) infected the burn wounds of group A after one day of burn and left without treatment. The burn wounds of group B left without either infection or treatment. The burn wounds of group C were infected by the same bacteria and treated for one hour only (by 0.5 Hz QAMW), then left without treatment. The burn wounds of group D were infected by the same bacteria and treated by 0.5 Hz QAMW for a period of one hour (for 15 days). The burn wounds of group E were not infected by bacteria and treated by 0.5 Hz QAMW for a period of one hour (for 15 days). Wound healing was evaluated by measuring the wound surface area "WSA" before exposure to electromagnetic fields and every 5 days. In addition, the survival percentage of animals of each group was calculated.* ***Results.*** *Results showed that the WSA of the treatment group (D & E) were significantly less than that of the control groups and of significantly high survival percentage than that of the control groups.* ***Conclusion.*** *According to the results of this work, it can be concluded that EMF in form of 0.5 Hz QAMW is an effective method in enhancing healing in both infected and non-infected burn wounds. This is due to enhancement of the cellular activity in the injured area of the wound that accelerated wound healing*

Key words: *electromagnetic fields, burn, infected wound, amplitude modulated waves*

INTRODUCTION

Electromagnetic fields are used for promotion of healing in various conditions such as bone, cartilage, ligaments, and nerve injuries. However, still there are controverseries about the use of these fields for promoting skin burn wound healing either in infected or non-infected burn wounds.

The skin is the largest organ in the body. It is composed of epidermis, dermis, hair follicles, sweat and sebaceous glands and a wide variety of specialized glandular structures[2]. The skin has many functions as protection against injuries, secretion of sebum and sweat, regulation of heat exchange, cosmic coverage, water barrier, and acts as a part of the immune system[1]. On the other hand the anatomical analysis of guinea pig skin suggest that the guinea pig's skin is more similar to

human skin than to the skin of other rodents. So, guinea pig is a useful animal for studying cutaneous biology, experimental pathology, pharmacology and toxicology[11].

Burn is the coagulative necrosis of skin and underlying tissue caused by contact with heat[9]. There are four degrees of burn injuries, which are; superficial burn, partial thickness burn, full thickness burn, and Char burn[5]. Wound healing comprises two essential components; regeneration and repair. The difference between these two phases is based on the resultant tissue. In regeneration, specialized tissue is replaced by the proliferation of surrounding undamaged specialized cells. In repair, lost tissue is replaced by granulation tissues that mature to form scar tissue[10].

Biological effects of electromagnetic fields on different systems of the body depend on their frequencies and amplitude. One of the most recent theories is the "metabolic biomagnetic resonance model theory"[4]. This theory explains that most of living cells maintain an electric potential across their plasma membrane, in which many ions pump and voltage-dependant ion channels are located. Many cell types generate local ion current loops through selectively metabolic processes. These naturally occurring flows of electric currents through cellular membranes produce change in biomagnetic fields. The amplitude and waveform of these biomagnetic fields depend on the density and direction of the ionic charge flowing in the metabolic process. A magnetic field resulting from a certain metabolic process may stimulate ionic motion in the neighboring cell and cause action potential with a common stimulating field. So, metabolic phenomenon will be energized. If the amplitude of the applied field is much greater than the amplitude of the "metabolic biomagnetic frequency"; damage to this metabolic function could occur.

According to the above-mentioned theory, this study was designed to detect the suitable frequencies of electromagnetic field that can stop the growth of bacteria. El-Hag, (2003)[3] studied the effect of extreme low frequency magnetic fields on the growth characteristics of Pseudomonas. He found that square amplitude modulated wave (QAMW) has enhancing and inhibiting effects on the growth of the microorganism at the frequencies 0.3 Hz and 0.5 Hz respectively. Inhibition of growth of microorganism was due to changes in the structural properties of plasmid DNA[3]. The aim of this study was to investigate the effect of electromagnetic fields in the form of square amplitude modulated wave "QAMW" on the healing of infected or non-infected burn wounds in guinea pigs.

MATERIAL AND METHODS

Fifty male guinea pigs were used in this study. The mean age of all animals was 12±2 months. The mean weight was 400±20 gm. Partial skin thickness burn wound of approximately 4 cm^2 was induced on the hind limb of each animal. The animals were divided into five equal groups (N=10) namely three control groups (A, B, and C) and two study groups (D and E).

Fresh microbial cell culture of the tested strains were grown on trypticase soy broth "TSB" medium overnight at 37 °C in a shaking water bath. Enough culture was added to the test medium to reach final concentration of $1x10^7$ CFU/ml. (CFU is Colony Forming Unit which is the minimum number of separable cells on the surface or in semi-solid agar medium which gives rise to a visible colony of progeny on the order of tens of millions of cells in number). CFUs may consist of pairs,

chains and clusters as well as single cells. This "Microbial Cell Culture" sample is prepared in microbiology laboratory as follow:- Fresh culture of the tested strains were grown on trypticase soy broth "TSB" medium overnight at 37 degrees Centegrade in a shaking water bath, and enough culture was added to the test medium to reach a final concentration of $1x10^7$ CFU/ml. One millimeter of culture that had been growing overnight was inoculated to 100 ml of the medium TSB. Then this culture incubated and allowed to grow to reach the mid-to-late exponential phase.

One ml of culture that had been growing overnight was inoculated to 100 ml of the medium TSB and then incubated and allowed to grow to reach the mid-to-late exponential phase.

Pseudomonas aeuroginosa bacteria (surface swab with culture containing 10^7 CFU/m) infected the burn wounds of group A after one day of burn and left without treatment. The burn wounds of group B were left without either infection or treatment. The same bacteria (surface swab with culture containing 10^7 CFU/m) infected the burn wounds of group C. This group was treated for one hour only (by 0.5 Hz QAMW), then left without treatment.

The same bacteria (surface swab with culture containing 10^7 CFU/m) infected the burn wounds of group D. This group was treated by 0.5 Hz QAMW for a period of one hour (for 15 days). The burn wounds of group E were not infected by bacteria and treated by 0.5 Hz QAMW for a period of one hour (for 15 days).

The survival percentage of the animals of each group was calculated according to the following equation

$$\text{Survival Percentage} = \frac{\text{After exposure survived animals - Pre exposure survived animals}}{\text{Pre exposure survived animals}} \text{ x } 100$$

Therapeutic equipment

Square amplitude modulated waves "QAMW" were generated using two generators. The wave carrier was of 10 MHz generated from an arbitrary wave generator "type TTi TGa 1230" manufactured by TTi (Thurby Thunder Instruments Limited) in UK. Waves from these generators were amplitude modulated by another generator model AFG 310 manufactured by Sony, Tektronics in Japan.

Evaluation equipment

Wound healing was evaluated by measuring the wound surface area "WSA" before exposure to electromagnetic fields and every 5 days. The following tools were used; sterilized transparency film, tipped transparency markers by Schwon Stadilo, Germany, carbon paper, metric graph paper (1 mm^2) and white paper. In addition, the survival percentage of animals of each group was calculated.

Procedures of the study

Guinea pigs are lifted by one hand placed under the trunk while supporting the rear quarters with the other hand. Injury of the lung or liver may result from grasping the animal around the thorax and abdomen[6].

The hair of the upper part of the right hind limb was shaved by electrical hair shaving machine. The guinea pigs were anaesthetized "by using ether". The skin segment was cleaned with alcohol. A partial skin thickness burn wound of approximately 4 cm^2 surface area was induced on the shaved hind limb of all animals. This burn wound was

done by using a round aluminium stamp with a contact area of 4 cm^2 and weight of 85 gm. This stamp was heated to 80 °C and applied for 40 seconds without pressure on the shaved skin[8]. Each animal was caged separately with free access of water and diet. All animals were subjected to a normal day-night rhythm. The room temperature was at 22±2 °C.

Square amplitude modulated waves "QAMW" of 0.5 Hz was applied to the treated area using two electrically insulated parallel plate cupper electrodes. The total field strength at the treated area was 270 V/m with QAMW field of 70 V/m.

The measurement of the burn surface area was carried out by tracing the burn wound perimeter. In this stage, the measurement of burn surface area conducted 24 hours post the infection. Then re-measure the wound surface area (WSA) at the sixth, eleventh, and the sixteenth days post burn. The plastic sheet was placed over the burn wound. The burn wound perimeter was traced with a fine tipped transparency marker. The tracing process was repeated three times to establish measurement reliability. A carbon paper was placed over the metric graph paper. Then the traced transparency film was placed over carbon paper with a white paper in between. Then the number of square millimeters were counted on the metric graph paper. This process was repeated three times with every burn wound by the same investigator[7].

RESULTS

Table (1) and figure (1) show the mean values of the measured WSA in cm^2 for all studied groups. As shown from the table and figure the mean value of WSA of group A was 3.54±0.10 cm^2 after 24 hours. There was an increase in WSA after 5 days and after 10 days with no significant difference. While after 15 days, there was remarkable increase in WSA of the animals, so the guinea pigs of this group died.

In the second control group, "group B" the mean value of WSA was 3.57±0.09 cm^2 after 24 hours. After 5 days, 10 days and 15 days, the WSA decreased significantly. The mean values of WSA at those intervals were 2.86±0.32 cm^2, 1.97±0.48 cm^2, and 1.12±0.38 cm^2 respectively.

In the third control group, "group C" the mean value of WSA was 3.54±0.16 cm^2 after 24 hours. This group treated by one hour application of 0.5 Hz QAMW, then left without treatment. The WSA mean values after 5 days and 10 days increased with a high significant difference than before. The mean values were 3.67±0.21 cm^2 after 5 days ($t = -3.545$ and $p<0.006$) and 4.8±0.61 cm^2 after 10 days ($t = -4.208$ and $p<0.008$). All animals died after 15 days.

The mean value of WSA for group D was 3.5±0.2 cm^2 after 24 hours post burn. Group D was treated by 0.5 Hz QAMW for a period of one hour (for 15 days). After 5 days the mean value of WSA was 2.64±0.32 cm^2 with high significant decrease ($t = 8.67$ and $p <0.0001$). This improvement continued at the 10^{th} day and 15^{th} day, where the WSAs mean values were 1.26±0.17 cm^2 (t-test = 36.33 and $p<0.0001$) and 0.64±0.47 cm^2 ($t = 35.25$ and $p<0.0001$) respectively.

Table (1): Comparative analysis of WSA mean values (cm^2) after 24 hours post induced burn, and after five, ten and fifteen days of post burn for studied groups (Control groups A, B and C and experimental groups D and E).

	Group		After 24 hours	After 5 days	After 10 days	After 15 days
Control groups	Group A	*Mean ± SD*	3.54±0.10	3.7±0.13	5±0.83	0 *
		t-test		-1.172	-2.54	0 *
		p (<0.05)		0.271	0.064	0 *
		Significance		NS	NS	0 *
	Group B	*Mean ± SD*	3.57±0.09	2.86±0.32	1.97±0.48	1.12±0.38
		t-test		7.144	8.882	9.716
		p (<0.05)		0.0001	0.0001	0.002
		Significance		HS	HS	HS
	Group C	*Mean ± SD*	3.54±0.16	3.67±0.21	4.8±0.61	0 *
		t-test		-3.545	-4.208	0 *
		p (<0.05)		0.006	0.008	0 *
		Significance		HS	HS	0 *
Experimental groups	Group D	*Mean ± SD*	3.5±0.2	2.64±0.32	1.26±0.17	0.64±0.47
		t-test		8.67	36.33	35.25
		p (<0.05)		0.0001	0.0001	0.0001
		Significance		HS	HS	HS
	Group E	*Mean ± SD*	3.5±0.13	2.25±0.22	1.17±0.14	0.53±0.82
		t-test		18.82	43.31	65.25
		p (<0.05)		0.0001	0.0001	0.0001
		Significance		HS	HS	HS

* = died

The mean value of WSA for group E was 3.5±0.13 cm^2 after 24 hours post burn. Bacteria did not infect group E. This group treated by 0.5 Hz QAMW for a period of one hour (for 15 days). After 5 days of application of QAMW, the mean value of WSA decreased significantly. It was 2.25±0.22 cm^2 with t-value of 18.82 and $p<0.0001$. In addition, the mean value decreased significantly after 10 days and after 15 days. The mean values were 1.17±0.17 cm^2 ($t= 43.31$ and $p<0.0001$) and 0.53±0.82 cm^2 ($t=35.25$ and $p<0.0001$) respectively. In summary, the results showed that the WSA of the treatment group D & E were significantly less than that of the control groups.

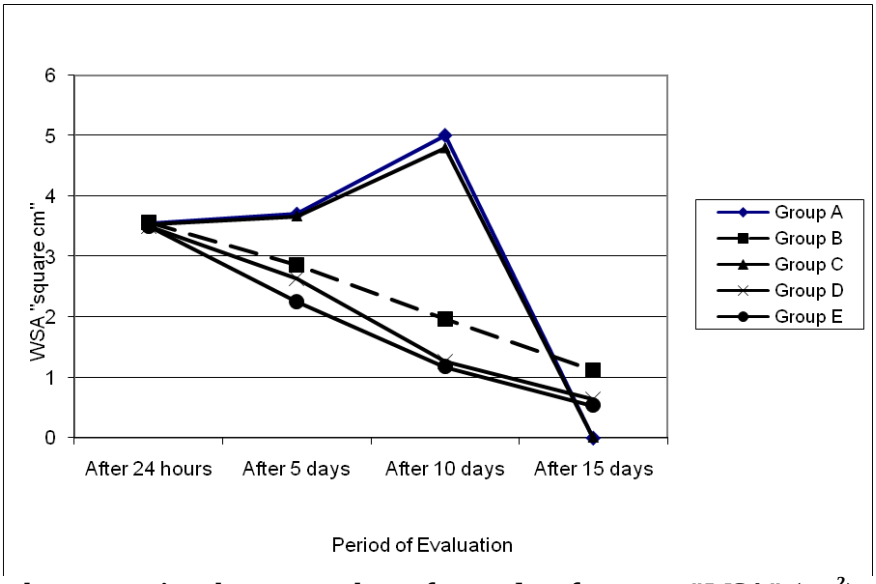

Fig. (1): Line graph representing the mean values of wound surface area "WSA" (cm^2) post induced burn after 24 hours, 5 days, 10 days and 15 days for all studied groups.

Table (2) and figure (2) show the survival rate percentage of guinea pigs in the studied groups. Survival percentage was measured daily from day one post-burn to the end of the second week. As shown from figure (2), the animals of groups "A" and "C" died from skin infection and septicemia at the 10^{th} day. While there was a three guinea pigs survived until the end of second week in group "B". In addition, there was a significant high survival percentage of group "D" and group "E" than that of other studied groups. At the end of the second week there was 9 animals survived in group "D", while the survived animals in group "E" were eight. These results indicate that the survival period of the animals increased as they exposed to 0.5 Hz QAMW.

Table (2): Survival percentage rate (%) of the animals of studied groups (Control groups A, B and C and experimental groups D and E) from Day One "D 1" to day 14 "D 14" post burn.

	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14
Group A	100 %	100 %	70 %	60 %	50 %	40 %	40 %	30 %	30 %	0 %	0 %	0 %	0 %	0 %
Group B	100 %	10 %0	90 %	90 %	80 %	80 %	80 %	70 %	50 %	40 %	30 %	30 %	30 %	30 %
Group C	100 %	100 %	70 %	60 %	60 %	50 %	40 %	30 %	20 %	0 %	0 %	0 %	0 %	0 %
Group D	100 %	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	90%	90%
Group E	100 %	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	90%	90%	80%

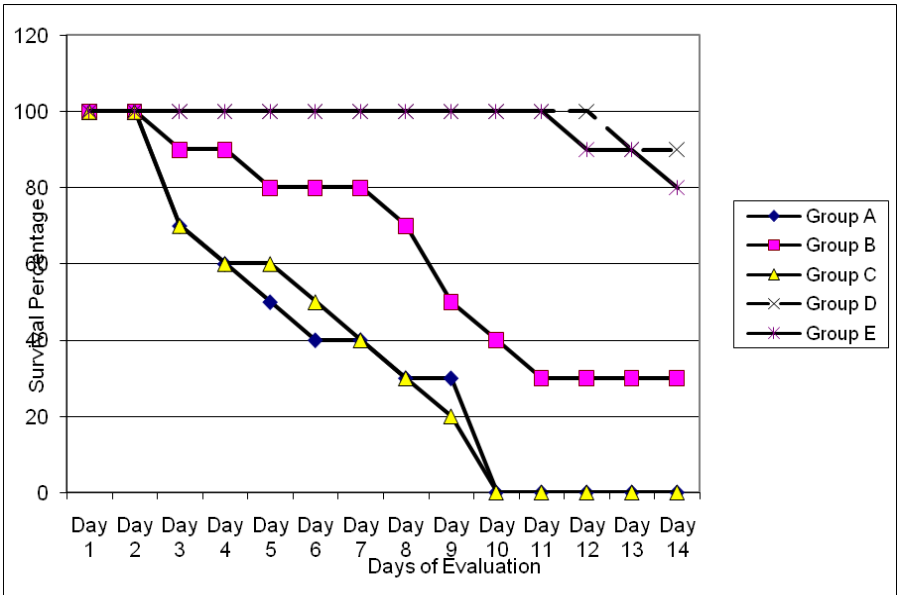

Fig. (2): Line graph representing the relationship between survival percentage and days following burn induction for all studied groups.

DISCUSSION

The exposure of Pseudomonas aeuroginosa bacteria to 0.5 Hz QAMW inhibits the microorganism growth in addition to the change of the molecular structure of plasmid DNA[3]. Exposure of the infected wounds to these waves resulted in inhibition of the biological activity of the microorganism and caused genetic changes in the pseudomonas bacteria. These genetic changes rendered the microorganism's ability to excrete toxins and carry on cellular division. All these changes in molecular structure of the microorganism and activity were of great assistance to the immune system of the animal to be capable of getting rid of the microorganism.

There is one more new finding in the data of group E animals. The wounds in this group were not infected by the microorganism and received treatment with 0.5 Hz QAMW. The wound healing of the animals in this group indicated that the treatment with these waves enhanced the cellular activity in the injured area of the wound that accelerated wound healing.

One more point worthy for discussion here is the data from group C. In this group the animal wounds were infected with previously exposed microorganism to 0.5 Hz QAMW. One may state here that the media where the PS was exposed before infecting the wound contained toxins previously secreted. Therefore, some toxins were transferred to the wound together with weak microorganism causing its destructive effect on the infected wound. However further studies are needed to evaluate these data which may be used in the future for production of vaccines to pseudomonas bacteria.

Summary and Conclusion

The biological effects of the electric and magnetic fields have become a topic of increasing attention. Electromagnetic fields are

used for promotion of healing in various cases such as bone, cartilage, ligament, and nerve injuries. However, there are controversies about the use of these fields. The results of this study indicated that the wound surface area of the experimental groups reduced significantly more than that of the control groups. Electromagnetic field in the form of 0.5 Hz QAMW is an effective and useful approach to accelerate healing time in either infected or non-infected burn wound and to control bacterial infection in burn injuries. In addition, electromagnetic field is a simple and valuable method of treatment procedure for physical therapists. According to the results of this work, it can be concluded that EMF in form of 0.5 Hz QAMW is an effective method in enhancing healing in both infected and non-infected burn wounds.

Recommendations

The results of this study have indicated a need to consider the following recommendations:

- Add EMF in form of 0.5 Hz QAMW to routine infected wound management in patients with thermal injuries.
- Further investigations are needed to compare between different forms of EMF waves for promotion of wound healing infected by other types of bacteria.
- Further researches should be undertaken to evaluate the data obtained after applying different lines of treatment to accelerate wound healing.

REFERENCES

1. Cartner, L.P. and Hiatt, J.: "Integument, Color Textbook of Histology" WB Saunders, 264-283, 1997.
2. Dellmann, H.D. and Brown, E.M.: "Textbook of Veterinary Histology", 1st Edition, Lee and Feigner, Philadelphia, Chapter 18: 459-493, 1976.
3. Elhag, M.A.: "Effects of Extremely Low Frequency Electric Fields on Some Bioelectrical Properties and Biological Activities of Some Microorganisms". PhD Thesis, Biophysics Department, Faculty of Science, Cairo University, 2003.
4. Fadel M.A.: "Stimulation and Control of Cellular Functions by Electromagnetic Signals; New Theory" in Fadel MA "Principles of Biophysics" Academic Press 4th Edition, pp: 180-187, 2006.
5. Feller, I., Tholen, D. and Cornller, G.: "Improvement in Burn Care" Am J. Association 244: 2074-2078, 1980.
6. Harkness J.E. and Wagner, E.J.: "The Biology and Medicine of Rabbits and Rodents". Williams and Wilkins, Baltimore, Philadelphia, 34-85, 1995.
7. Kloth, L.C. and Feeder, J.A.: "Acceleration of Wound Healing with High Voltage Monophasic Pulsed Current". Phys Ther, 68(4): 503-508, 1988.
8. Knabl, J.S., Bayer, G.S., Bauer, W.A. Schwendenwein, I., Dado, P.F., Kucher, C., Horvat, R., Turkof, E., Schossmann, B. and Meissl, G.: "Controlled Partial Skin Thickness Burns; An Animal Model for Studies of Burn Wound Progression". Burns, 25(3): 229-235, 1999.
9. Richard, R.L. and Staley, M.J.: "Burn Care Rehabilitation; Pathologic Manifestations of Burn Injury", Eds 1, FA Davis Company, Philadelphia, 30-47, 1994.
10. Walter, J.B. and Israel, M.S.: "General Pathology". Churchill, Livingstone, 1987.
11. Woodward, D.F., Nieves, A.L., Williams, L.S., Spada, C.S., Hawley, S.B. and Duenes, J.L.: "A New Hairless Strain of Guinea Pig: Characterization of the Cutaneous Morphology and Pharmacology", In Maibach H.I., Lowe N.J. Eds, Models in Dermatology, 4: 71-78, 1989.

الملخص العربي

تأثير المجالات الكهرومغناطيسية المتقطعة على إلتئام جروح الحروق المصابة بالعدوى

تستخدم المجالات الكهرومغناطيسية فى علاج حالات عديدة كإصابات العظام والغضاريف والأربطة وكذلك إصابات الأعصاب. ومع ذلك يوجد إختلافات كثيرة فى استخدام هذه المجالات للمساعدة فى إلتئام جروح حروق الجلد المصابة بالعدوى والغير مصابة بالعدوى.
الهدف: تهدف هذه الدراسة لبحث تأثير المجالات الكهرومغناطيسية المتقطعة ذات التردد 0.5 هيرتز على إلتئام جروح حروق الجلد المصابة والغير مصابة بالعدوى فى الخنازير الغينية. **الطريقة:** أجرى هذا البحث على خمسين من ذكور الخنازير الغينية، وقد تم احداث حروق من الدرجة الثانية السطحية فى فخذ الطرف الخلفي لكل منهم مساحته 4 سم^2. وقد تم تقسيم الحيوانات عشوائياً إلى خمسة مجموعات متساوية؛ ثلاث مجموعات ضابطة "أ، ب، ج" ومجموعتين تجريبتين "د، هـ". وتم تعريض جروح المجموعة "أ" لبكتيريا "سودوموناس أوريجينوزا" (عن طريق مسحة سطحية للجرح بالبكتيريا بتركيز 10^7 لكل مليميتر) وذلك بعد يوم من الإصابة وتركت الحيوانات بدون علاج. أما المجموعة "ب" فلم تصاب بالبكتيريا وتركت بدون علاج. والمجموعة "ج" تمت اصابتها بنفس البكتيريا وتم تعريضها للمجالات الكهرومغناطيسية ذات التردد 0.5 هيرتز لمدة ساعة واحدة فقط وتركت بعد ذلك بدون علاج. وحيوانات المجموعة التجريبية الأولى "د" تم اصابة الجروح فيهم بالعدوى البكتيرية وتعريض الجروح للمجالات الكهرومغناطيسية ذات التردد 0.5 هيرتز لمدة ساعة واحدة يومياً ولمدة 15 يوم. والمجموعة التجريبية الثانية "هـ" لم تصاب بالبكتيريا وانما تم تعريض الجروح للمجالات الكهرومغناطيسية ذات التردد 0.5 هيرتز لمدة ساعة واحدة يومياً ولمدة 15 يوم. وقد تم قياس مساحة الحروق قبل بداية التعريض وبعد الحرق بأربعة وعشرون ساعة لكل حيوان وبعد بداية العلاج بخمسة أيام وعشرة أيام وخمسة عشر يوماً. كما تم حساب النسبة المئوية لوفيات الحيوانات فى كل مجموعة خلال فترة الدراسة.
النتائج: أظهرت النتائج وجود فروق ذات دلالة إحصائية عالية بالنسبة لمجموعات الدراسة "د، هـ" بعد مدة التجربة بمقارنتها بالنتائج قبل بدء التجربة حيث قلت مساحة الحروق بشكل ملحوظ. كما أظهرت أيضاً قلة عدد الوفيات فى مجموعات الدراسة بمقارنتها بعدد الوفيات فى المجموعات الضابطة. **الخلاصة:** من الممكن أن نستخلص من هذا البحث أن الموجات الكهرومغناطيسية المتقطعة ذات التردد 0.5 هيرتز ساعدت على نشاط الخلايا في منطقة الجلد المصاب بالجرح مما ساعد على التئام الجرح وتعتبر طريقة فعالة فى علاج جروح حروق الجلد المصابة بالعدوى والغير مصابة بالعدوى .

الكلمات الدالة: المجالات الكهرومغناطيسية، الحروق، الجروح الملوثة، الموجات المعدلة .