The Effect of Low Frequency Alternating Electric Current on Human Skin Cancer cells

Mohammed.Hasan.Al-Eghwairyeen*

The Institute of Biomedical Technology / Royal Medical Services
Amman-Jordan

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Abstract- This papers aims to investigating the effect of low frequency alternating electric current (AC) on the growth of human skin cancer cells in vitro. Results of this experiment showed a clear positive effect of low frequency alternating electric current on the proliferation of adherent skin cancer cells.
It was concluded that low frequency alternating electric current significantly induces the growth of human cancer cells. Subsequently, cancer patients must avoid any contact with electric current or be in the vicinity of such electric fields as in the case of physiotherapy treatment.

Index Terms, Class II clinical laboratory, adherent, proliferation, haemocytometer, Smart View software, mA peak-peak.

I. INTRODUCTION

Cutaneous wound healing is a dynamic biological process that begins with tissue injury and aims at discontinuation of further injury, recruitment of injured cells, formation and remodelling of the new tissue to resemble the function and form of the original tissue (Cukjati and Savrin, 2004). When traditional methods of wound management are unable to promote wound healing, the wound is considered to be chronic. Typical chronic wounds are pressure ulcers in spinal cord injuries, ischemic ulcers in lower extremities of patients with peripheral vascular disease and ulcers in diabetic patients (Cukjati and Savrin, 2004). Regeneration of skin wounds requires epithelial cell migration in order for the re-epithelisation of the wound to take place (Farboud et al, 1988). The first stimulus to the migration of these cells is the electric field produced inside the skin as soon as it is wounded (Farboud et al, 1988; Nuccitelli, 2003; Jennifer et al, 2003). This finding has triggered the possibility that an externally applied electric field may assist in the stimulation of wound healing (Nuccitelli, 2003; Sillman et al, 2003). In 1968, the first report on the use of direct electric field for stimulation of wound healing was published (Karba et al, 1997; Jercinovic et al, 1995). The mechanism of cancer cell motility is possibly similar to normal cells but the regulation of their motility could be very different. Several studies have shown that cell transformation also changes their response to electrical current stimulation (Mycielska and Dajmgoz, 2004).
The aim of this research was to investigate the theory that electric field can assist in wound healing and at the same time study the behaviour of cancer cells when exposed to such conditions. This was done via an in vitro experiment in which skin cancer cells were exposed to a constant alternating electric current of magnitudes 5 and 10 m A peak-peak respectively and a frequency of 60 Hz for a period of two days followed by the testing of the growth and proliferation of the cells after 24 and 48 hours.

II. METHODOLOGY

The intention of the experimental work was to provide a constant alternating current to a cell culture for a defined period of time after which the effect of this current on the growth and proliferation of the cell culture would be examined and any changes recorded and analysed. All of the cell culture experimental work performed in this project took place at the facilities provided by The University of Surrey in a class II clinical laboratory at the department of Biomedical and Biomolecular Sciences at the School of Engineering. Human squamous cell carcinoma cell line (scc-15) was purchased from ATCC (LGC Promochem, Teddington, UK). These cells were grown in a 1:1 mixture of Dulbecco's HEPES modified medium and Hams F12 with 2.5 mM L-glutamine, and 1.2 g/L sodium bicarbonate. The medium was supplemented with 10% foetal bovine serum, 400ng/mL hydrocortisone and 100 units/ mL penicillin-

streptomycin-neomycin. The cells were cultured in a humidified incubator with 5% CO2 / 95% air at 37C°. The cells were passaged regularly 2-3 times a week. This was done by removing and discarding the culture medium, briefly incubating the cells in the presence of 4 mL (for a 25 cm2 flask) Trypsin-0.03% (w/v) EDTA solution. An equal volume of culture medium was added and the cell suspension was centrifuged at 13,000 revolutions per minute (rpm) (Centaur, Newport Pagnell, UK). The media was then discarded and fresh medium was used to re-suspend the pellet and a 1:4 subculture ration was used. A suitable cell culture container for use in this experiment was chosen to be a sterile disposable 6-well multi-dish from Nunc Inc. An electrode assembly was used to apply the electric field to the cell culture used in the experiment. A set of two electrodes was placed in four of the 6 wells of the multi-dish. The dimensions of each electrode was made to be 23x15 mm and the size of the well allowed for a maximum distance of 20 mm between the electrodes. Electrodes were made from a readily available gold plated pin array found in the form of a 1 row 36-way gold plated header rows were cut into sections of 10 pins each to individual electrodes.

Electrodes were then soldered on SRBP strip boards each of dimensions 115 x 80 mm which would fit exactly on top of the 6-well multi-dish plate. Ten-pin array electrodes were then soldered on the strip board with a distance of 20 mm separating each two electrodes. Special wires which can withstand sterilisation were soldered to each strip board so as to convey electric current to the electrodes. The four strip boards were then autoclaved in a steam sterilizer provided in the clinical laboratory. This strip board-pin array assembly will now fit into the 6-well multi-dish so that each pin array extends into specified well of the multi-dish as.

A constant-current source circuit was designed and constructed to provide the required alternating current necessary to perform the experiment.

A total number of four multi-dishes were used in the experiment and five of the six wells of each multi-dish were seeded with 3 × 104 cells/ml after counting them using the haemocytometer. Two ml of culture medium was then added to each well. The strip boards with the mounted electrodes were then placed on top each multi-dish and covered with the multi-dish cover. The experimental preparation was performed in a standard Class II Advanced laminar flow Bio-Safety Cabinet from BIOUELL and the actual experimental procedure was then performed in a CO2 Incubator from New Brunswick Scientific.

The wells of the multi-dishes were divided as shown in table 1.

Table I: Showing the content of the wells in each multi-dish and their corresponding symbols.

Number of well	Name of the test	Contents of the well	Symbols used in the results
A1	Control-1	(cell culture & medium)+ Electrodes	C = Average of control-1 and control-2
B1	Control-2	(cell culture & medium)+ Electrodes	
A2	Test-1	(cell culture & medium)+ Electrodes+ Electric field	T= Average of Test-1 and Test-2
B2	Test-2	(cell culture & medium)+ Electrodes+ Electric field	
C1	Control-3	(cell culture & medium) only	C1
C2	Medium only	Medium only	M

The experiment was divided into two parts according to the current used and the effect of each current will discussed separately. Two multi-dishes were used for each experiment. At the end of each day of incubation, the specific multi-dish was taken out of the incubator, images of each well were taken prior to counting, and then the number of the cells in the different wells of the multi-dish was counted via the haemocytometer. One chamber of the haemocytometer was used for counting the suspension cells in the specific well and the other chamber used for counting the adherent cells after treating them with trypsin. The trypsinisation process was performed as follows: after the end of each day, the specific multi-dish was taken out of the incubator, each well was then emptied and 2 ml of trypsin added to it and the multi-dish was then placed back in the incubator for 3 minutes and taken out after that and agitated to remove all the remaining cells from the bottom of the well. Images of the wells were taken prior to the trypsinisation process using Smart View for WDM software linked with the Eclipse TS100 Inverted Microscope from Nikon under which the cells were examined.

III. RESULTS

For each experimental current (5 mA and 10 mA), a total number of three types of graphs were plotted:

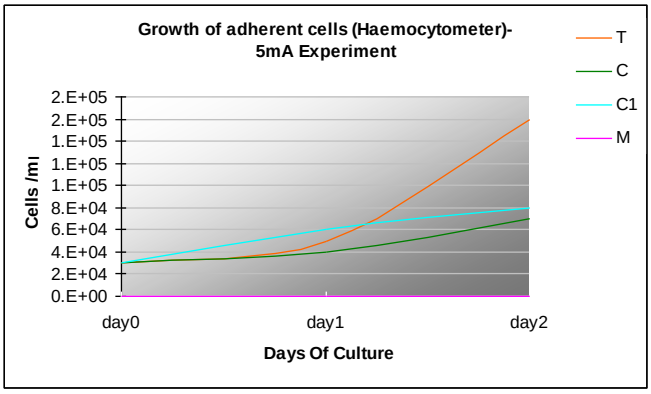
1- Graph A shows the trend in the growth of adherent cells throughout the experiment as counted using the haemocytometer.

2- Graph B shows the change in the growth of suspension cells as counted using the haemocytometer.

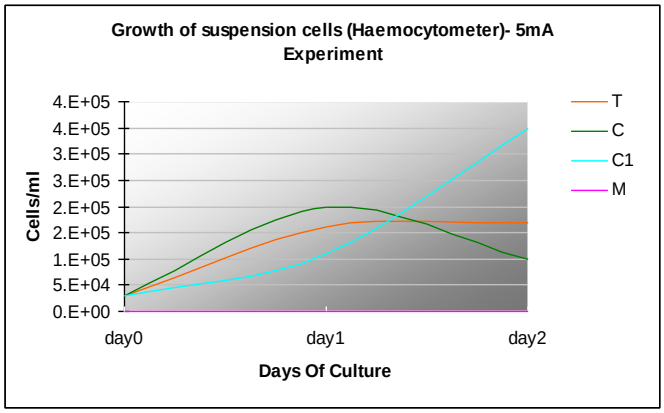
3- Graph C shows the change in the growth of adherent cells as illustrated by the number of cells that have adhered to the bottom of the well of each multi-dish. This was achieved by comparing the number of cells in the images obtained for the centre of the well.

Results of the 5 mA experiment:

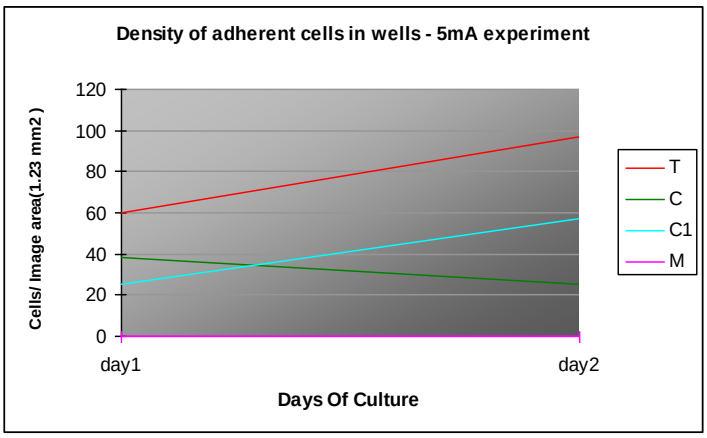
This section is divided into three parts according to the number of graphs that were made for each current as mentioned earlier.



Graph 1-A: Growth of adherent cells using 5mA-(Haemocytometermethod) over a 48 hour incubation period (2 days)



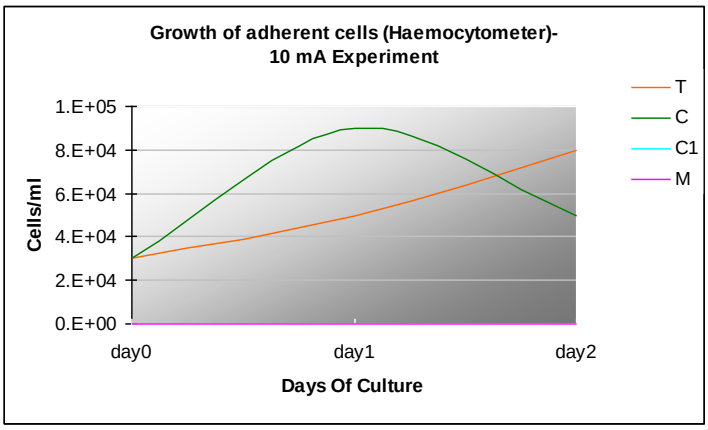
Graph 1-B: Growth of suspension cells using 5mA-(Haemocytometer method) over 48 hour incubation period (2 days)



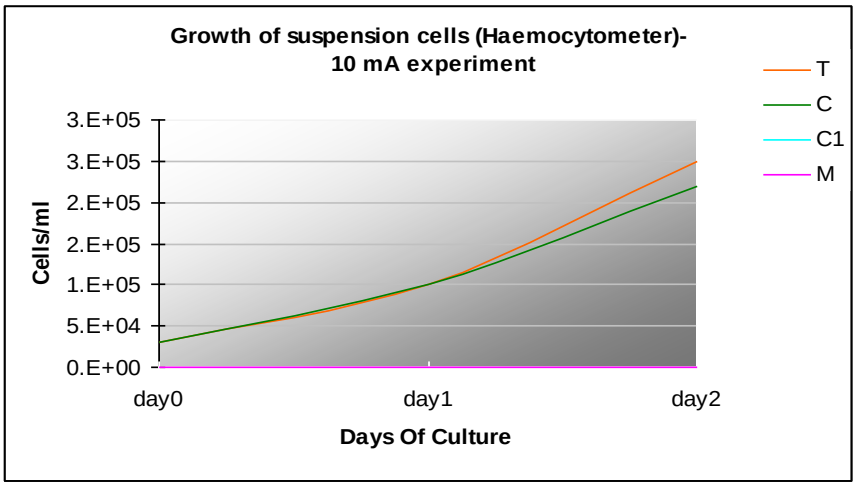
Graph 1-C: Shows the trend in the growth of the adherent cells in the bottom of each well as calculated from images taken to the well.

Results of the 10 mA experiment :

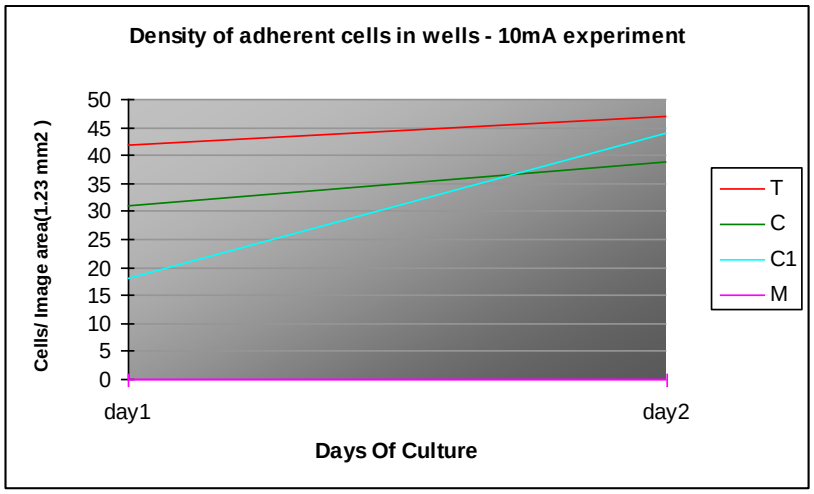
This section is also divided into three parts according to the number of graphs that were made for each current as mentioned earlier.



Graph 2-A: Growth of adherent cells using 10 mA-(Haemocytometer method) over 48 hour incubation period



Graph 2-B: Growth of suspension cells using 10 mA-(Haemocytometer method) over 48 hour incubation period



Graph 2-C: Shows the trend in the growth of the adherent cells in the bottom of each well as calculated from images taken to the well.

IV. Discussion

In this study it was confirmed that skin cancer cells respond positively to electric fields of 5 and 10 mA at 60 Hz if applied constantly for a 24 hour period. The experiment was successful as the cells have continued to grow throughout the experiment and were their growth was influenced significantly by the presence of electric field compared to control cells. Results could be noted immediately from images taken of the wells of the multi-dishes at the end of the experiment Fig 8. and were confirmed when cells were counted via the haemocytometer.

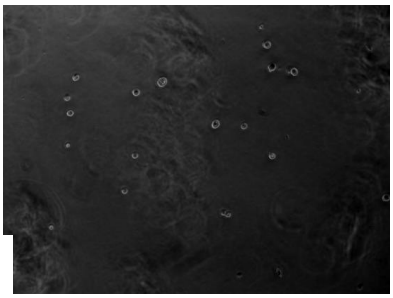
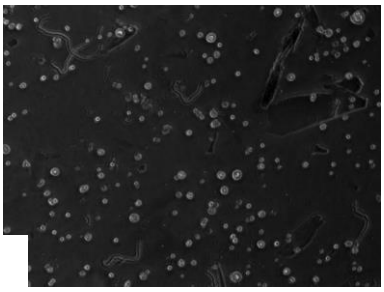
a  b 

Fig 8 Images of the wells of the multi-dish for a control sample (a) and a test sample (b).

The advantage of this finding is that it can be used as the basis of designing a system for use with treatment of chronic wounds which has not yet been introduced to healthcare. Furthermore, In vitro experiments on such currents have not been investigated before which makes the findings of this work a new insight to wound healing.

Moreover, during the course of the experiment an observation not reported previously in literature was noted which is the aggregation of cells on the electrodes used in the experiment Fig 9. This was more prominent in electrodes used to apply electric field to the cell culture than those which did not convey electric current. This type of aggregation could provide a valuable insight to the effect of the electrode material on the direction of migration of skin cells and therefore help in determining the site for placing such electrodes during treatment,

An electrode used to provide current to the cell culture 

An electrode used in the experiment without 

Fig 9 Images of electrodes used in experiment taken by a digital camera fitted on an inverted microscope via Smart View software (magnification x 4).

V. CONCLUSION AND FURTHER WORK:

The result of the experiment showed a clear effect of the 5mA alternating current on the growth of adherent cells compared with the control cells. However, the effect of The 5 m A alternating current on the suspension cells seems to be negative.
The 10mA alternating current had a similar effect on the adherent cells as the 5mA alternating current in that it increased their growth compared to the control. However this effect was with a lesser degree than the 5mA current. A slight increase in the growth of suspension cells was also noted with this current.
Furthermore this experiment has shown clearly the affinity of cells to inert material by growing on the electrodes that were used in the experiment.
From this work we conclude that alternating electric field with a frequency of 60 Hz and magnitude of 5–10mA peak to peak seems to have a clear affect on promoting the growth of skin cancer cells and subsequently normal skin cells when compared with control cells.
This emphasises the fact that dedicated medical equipment for wound healing can be designed and built on this basis and can be introduced to healthcare to help in the treatment of chronic wounds.
Moreover this study emphasises the fact that cancer patients must avoid any contact or be in the vicinity of such electric fields or undergo any treatment that involve electric fields such as physiotherapy treatment as this will dramatically worsen their condition and lead to the fast development of the disease.

The accuracy of visualising the wells and capturing their images can be greatly improved if a fixed frame was designed and mounted on the stage of the microscope on which the 6 well multi-dish can only fit in one fixed position. This will enable images of the same spot in the well to be taken every time. Moreover, the use of a computer program to count the cells form well images will greatly enhanced the accuracy of measuring the growth of adherent cells.
Furthermore, a more accurate method of calculating the total number of cells is needed, one of which is a traditional blood cell counter specially re-calibrated to count skin cells rather than blood cells.

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AUTHOR

Mohammed Hasan Al-Eghwairyeen, Jordanian Royal Medical Services, The Institute of Biomedical Technology, ghwairy@gmail.com.