International Journal of Engineering & Technology, 7 (3.36) (2018) 45-47

International Journal of Engineering & Technology

Website: www.sciencepubco.com/index.php/IJET

Research paper

Investigation of Pulse Electric Field Influence on Cell Trypsinization Assay

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Abstract

This study is aimed at investigating the effect of pulse electric field on cell attachment properties towards the enhancement of wound healing process. The Human Colorectal Adenocarcinoma cell line (HT29) was use in this study. The HT29 cells were treated with an electric field of 600V/cm amplitude for 500µs pulse duration in suspension and seeded for 24 hours. A time-lapse live imaging of the trypsinization assay of the HT29 cell was carried out using integrated devices for live cell imaging that was equipped with Charge Coupled Device (CCD) Camera, Temperature and Carbon IV Oxide (CO_2) Controllers as well as, an Inverted Microscope. The electric treatment was found to decrease the adhesiveness of the cell by 41% where the treated cells detached from the substrate in 340 seconds as compared to control group that took 480 seconds to completely detached from the substrate immediately after trypsinization. Hence, the study suggested that the application of appropriate electric field, can affect the cell signalling pathways which in turn decrease the degree of cell adhesion on the substrate. The decrement in the cell adhesion could facilitate wound healing process via increased cellular migration, since the speed of cell migration is inversely proportional to the strength of cell adhesion.

Keywords: *Attachment; pulse electric field; trypsinization; wound healing.*

1. Introduction

Even though successes have been recorded in cell suspension culture, many applications in biology and biotechnology required cells that adhere onto a substrate. Adhesion of cells to each other and to their extracellular matrix (ECM) is also important in creating cell shape and organization in tissue engineering. Furthermore, comprehending how cells adhere is significant in knowing disease development like cancer and muscular dystrophies which mainly comprise of a failure in cell adhesion. Additionally, cell adhesion is an essential process in numerous physiological processes such as wound healing, malignancy as well as embryogenesis [1-2].

In anchorage –dependent cells, cell adhesion plays a vital role in cell survival and growth, because it supports tissue and organ organization [1]. For example, the inhibition of early cell attachment events, like cell spreading, triggers rapid apoptosis [3] or lack of cell colonization and differentiation [4]. By contrast, in cancer cells, the presence of adhesion contacts is not a prerequisite for survival and growth [5]. The key features of cancer cell are cell polarization and down regulation of integrin expression attributable to breakdown of cell-cell contacts and ECM contacts [5]. The alteration in adhesive behaviour of tumour cells decides their modified morphology while their invasive properties are determined by their migration ability [6]. The change in cell adhesion plays a crucial role in all stages of tumour genesis. Further changes in cell adhesiveness are essential for a cell to attach to a particular target organ, to grow independently and to form metastasis [7]. Electroporation has been exploited in cancer treatment for increasing transport of chemotherapy drugs through the plasma membrane of cancer or malignant cells. The applied electric field in electrochemotherapy increases the uptake and accumulation of anti-tumour drugs into the malignant cells, thereby increasing the cytotoxic effect of the drugs [8]. Furthermore, a number of effects of applied external electric field have revealed to include altering the cellular functions and also cell surface redistribution and cytoskeletal reorganization [9]. Thus, manipulation of the cell adhesion ability is a very significant precondition for inhibition of the cancer cell ability to grow and invade [5]. Hence, this paper focuses on the influence of applied external electric pulses on HT29 cell line adhesion, so as to explore the effect of electric field on cancer cell development in addition to wound healing process.

2. Material and method

2.1. Cell culture

The HT29 cell line is used and grown in a 25cm^2 culture flask as a monolayer in RPMI 1640 medium that is enhanced with 10% Fetal bovine serum (FBS) and 1% antibiotic (penicillin-streptomycin). The cells are then incubated in an environment that

is humidified containing 5% CO_2 and temperature of 37° C. [10-11]. As soon as the Cells reach 80-90% confluence, Tryple Express Solution (TES) is employ for their harvest and passage, but if confluence is not reach after two (2) to three (3) days the spent medium is changed. However, for a subculture process, the following procedure was adopted: First, the old medium was discarded through aspiration and 2ml of phosphate buffer saline and PBS (without calcium and magnesium chloride) is used for washing the cells while 2ml of TES is added for cells detachment. This is then incubated for 5-10 minutes at 5% CO_2 and temperature of 37°C until all cells become round and fully detach from the substrate. At this point, an equal volume of complete growth medium is added to stop the effect of the detachment enzyme (TES). Finally, the cells are re-suspended to form a uniform suspension which are then utilized for further experimentation or seeded in new flasks for culturing.

2.2 Electroporation

The electroporation process of the HT29 cell line in suspension was carried out using ECM 830 a commercial electroporator made from BTX Harvard apparatus. This apparatus operates mainly in two modes: (i) the low voltage (LV) mode (5V to 500V and Pulse Duration: 10ms to 999ms (1ms resolution)). (ii) The high voltage (HV) mode (501V to 3000V and Pulse Duration: 10µs to 600µs (1µs resolution)).
In this study, the following were considered as the LV and HV modes parameters used: (i) LV Mode: voltage is 240V with a 4mm electrode gap to achieve a 600V/cm electric field strength, for cell proliferation assay, cell length analysis and trypsinization assay. (ii) HV Mode: voltage of 600V with a 10mm electrode gap to achieve 600V/cm electric field strength for cell attachment analysis.

2.2.1 Electroporation protocol for cell trypsinization assay

The procedure for cells trypsinization is as describe in section 2.1. Also, the effect of the added TES is neutralized by adding an equal volume of complete growth medium and 0.8ml portion of 43000 cells/ml concentrated cells suspension are poured into a 0.4 cm cuvette which is placed in an electroporator (BTX ECM 830) chamber. The process of electroporation was done using an electric field of 600V/cm intensity for 500µs duration and the cuvette is transfered to a bio-safety hood. After that, 0.6 ml each of the electroporated cells and non-eletroporated cell suspensions (as Control) are seeded respectively in two new 25 cm^2 flasks containing 7ml of an earlier warmed complete growth medium and are incubated at 37°C and
5% CO_2. The cells were harvested after 48H in culture at room temperature [10].

2.3 Cell trypsinization assay

After 48H in culture, spent media was drained and discarded, 3ml of PBS was used in washing the cells and 2ml of tryple express solution (detaching enzyme) was added to the flask. Cell was immediately place on a top stage of Nikon Ti-series inverted microscope. Imaged are acquired every 10 second for a duration of 780 seconds (78 frames in total) using 20X phase-contrast microscopy and MetaMorph software (with time-lapse multidimensional acquisition). The process was carried out for both electrically treated cells and cells in the control group. The investigation is aimed at the effect of electric field (600V/cm for 500µs) on HT29 cell line adhesion properties. Cell detaching from a substrate assume a spherical morphology [12]. Therefore, the amount of cell detachment was computed by taking ratio of the number of spherical or rounded cells at each time point to the total number of cell in that field of view (both rounded and unrounded) multiply by 100%. This gives the percentage of cell detachment at that time point. The experiments were repeated 3 times each and the average percentage of detach cells was calculated at each time point.

3. Results and discussions

3.1. Cell trypsinization assay

The Figure 1 shows the trypsinization progression for the electrically treated HT29 cell line and the control group over a period of 480 seconds. The electrically treated cells begin to detach from the substrate at about 180 seconds after the application of TES and completely detach after 340 seconds. In the other hand, the control group starts dissociating from the surface of the flask at about 280 seconds after trypsinization and completely detached after 480 seconds. The detachment in the treated group was 85.6% after 240 seconds while the detachment in the control group was 68.7% after 240 seconds. This shows that the electrically treated cells detached relatively faster during trypsinization process. Thus, the decrease in the adhesion properties could beneficial to cellular behaviour during wound healing process [13-14]. The result suggests that the use of 600V/cm and 500µs decrease the degree of cell adhesion which could influence cell migration in wound healing process.

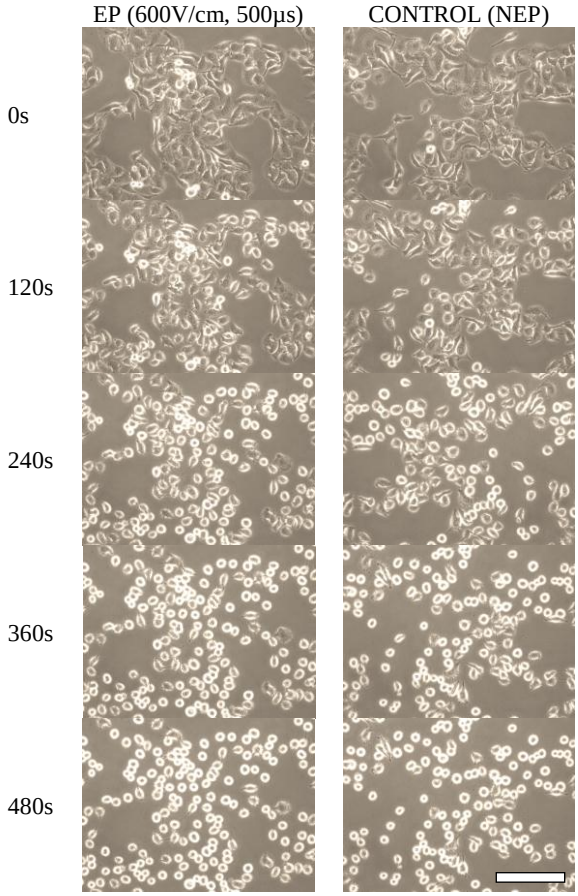

Fig. 1: Trypsinization process of HT-29 cell line under pulse electric treatment and control group over a period of 480 seconds. (Scale bar = 50µm).

4. Conclusion

The investigation revealed that, exposing HT29 cell line to 600V/cm electric field strength and 500µs pulse duration has greatly reduced the adhesion strength of the cells line by 41% as compared to the non-electroporated cells counterpart under the same condition. This could be that the electric field up-regulate the signalling pathway of cell adhesion molecule such as integrin

and cadherin and facilitated cell attachment. Therefore, the study could be useful in facilitating cell migration during wound healing process, since cell adhesion properties forms the basis of cell migration and other physiological processes.

Acknowledgement

The authors wish to thank the Tertiary Education Trust Fund (TETFund) for sponsoring the paper publication through Ababakar Tafawa Balewa University Bauchi, Nigeria. We also wish to thank the University Tun Hussein Onn Malaysia for providing the equipment and materials used in conducting the research.

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