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ARTOCARPUS ALTILIS COMPOUND BASED STUDY FOR ANTI-CANCER PROLIFERATION ON HELA CELL VIA ELECTROPORATION METHOD

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ABSTRACT

Survivors of cancer often have multiple permanent side effects due to the usage of drugs during the therapy. This study combining the compound artocarpus altilis and the optimized electroporation method for maximum HeLa cancer cell anti-proliferation activity. Artocarpus altilis or also known as sukun, is a natural compound, which has the capability to treat cancer as it has anti-proliferation effect in cancerous cells. Hence, in this current work, voltage amplitude of 200V/cm, 400V/cm, 600V/cm, 800V/cm, 100V/cm with 30µs of pulse duration used in electroporating HeLa cancer cell to look at the effects on cell's extension, cell size and growth rate. However, this study requires further investigation to identify the optimal electroporation conditions that can be applied to effectively deliver the artocarpus altilis extracts to the HeLa cancer cells in order to inhibit its proliferation.

Keywords: electroporation; irreversible electroporation; artocarpus altilis.

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1. INTRODUCTION

This review elucidates technique of Irreversible Electroporation with the combination of Sukun (Artocarpus Altilis) extract on HeLa cancer cell anti-proliferation activity. Electroporation or also known as electropermeabilization is a process where cells are exposed to an electric field to form nanoscale pores through a cell membrane [1-2]. This process is used to deliver impermeable particles into cells such as genes (DNA) and drugs [3-4]. Electroporation technique can be applied to almost all types of cells that cannot be accessible through other methods. Electroporation is a process where structural rearrangement done within the cell membrane by using short intense electric pulse [5-6]. Permanent or temporary pores in the cell membrane is depending on the voltage parameter applied to the cell. Irreversible Electroporation happens when electric field causes permanent pores of the membrane, while Reversible Electroporation is the temporary permeabilization of cell membrane after which the cells sustained [7-8]. Previous study has proven that Sukun extract has the ability to decrease the cancer cell viability and has potential as an anti-cancer agent. This is because of its contain called artocarpin which reduced cell viability by inducing apoptosis [9]. Since the natural properties of the Artocarpus Altilis inhibit the proliferation of cancer cells, electroporation can be applied to deliver this plant extracts to the cells, thereby opposing the proliferation of the HeLa cancer cells. Thus, research on this Electroporation blending of Natural extract must be a vital improvement of biomedical application. By date, there is no research by combining this method.

1.1. Electroporation

Numerous studies have proved that proper electric pulse could permeabilize cell membrane and it is thought to produce aqueous-filled pores [11-12]. Electroporation is an extensively known technique to deliver impermeable particles to the cell, like drugs, antibodies and deoxyribonucleic acid [13-14]. According to [21], electroporation is effective with nearly all cell and species types which shows its versatility. Electroporation is also recognized as a non-toxic and non-viral method. Moreover, EP process does not modify the function and biological morphology of focused cell, safer, less immunologic and highly efficient. Electroporation is a phenomenon to generate permeabilization of cell membrane in both

reversible (RE) mode and cell irreversible (IRE) mode. Electrogenetheraphy; method for gene insertion into cells (15-16) and Electrochemotherapy; uptake of potent and non-permeable anti-cancer drugs to kill cancerous cells (17-18) are the important application of reversible electroporation at present. Irreversible electroporation however has been investigated comprehensively in cell engineering. Previous research on liver cancer study has proven that IRE has the ability to kill the cancerous cells completely in non-thermal system (19-20). IRE is not only being practiced in medical field, however it is also being used in food industry for sterilization purpose and food pre-processing since 1961 [8].

1.2. Artocarpus Altilis Extract

To date, there are plenty of researches focusing on the findings of new types of natural chemotherapeutic agents derived from plants. This is proved to be excellent sources of new compounds. Herbal medicines have been used in medication over the past decade. Approximately 75% of the world's population has therapeutic experience over herbal remedies according to World Health Organization. Artocarpus Altilis is one of the plants that have potential in curing cancer. Artocarpus Altilis is a member of the genus Moraceae that contains about 50 species of trees. It is widely used in traditional medication to heal inflammation, infection, diabetes, cardiovascular problems and so on. It is proven by Enos Tangke Arung that Sukun wood extract has the capability to treat cancer. Human breast cells viability decreases after being induced with Sukun extract, which primarily contains artocarpin [9-10].

2. METHODOLOGY

2.1. Cell Lines and Culture

In this research, experiments are conducted entirely using HeLa cancer cell. HeLa cell cultured in 25cm^2 flask kept in CO_2 incubator. Several aspects must take into consideration such as accurate temperature control, humidity, CO_2, reliability and sterility. Firstly, old culture media aspirated from the cell culture flask will be transferred into conical flask. Cells are washed by using 3ml of PBS. PBS will then be aspirated from the flask. Then, 2ml of trypsin added into the culture and culture flask is stored in CO_2 incubator for 10 minutes. After 10 minutes of incubation, cells are observed under a microscope. Fully trypsinized cells

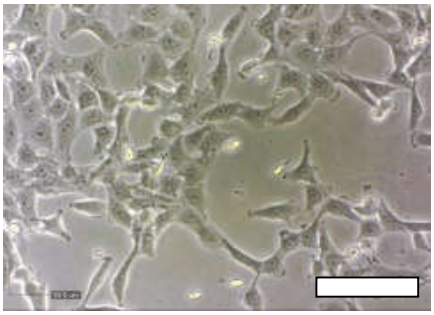
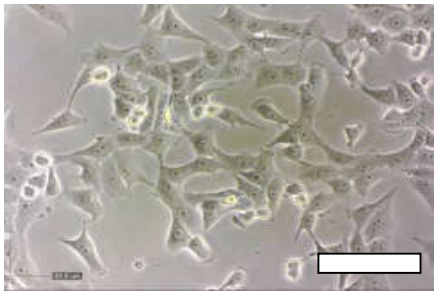
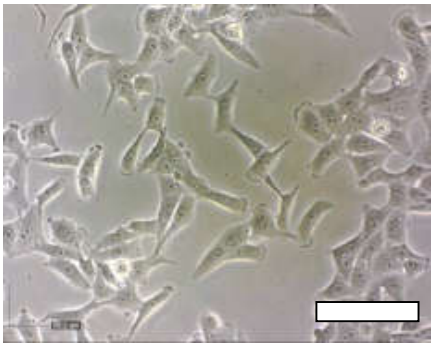
should appear in rounded shape and no longer attached to the surface of the flask. 1ml of media will be added to the old culture flask and 5ml to the new flask. Next, about 0.2ml of cells are aspirated and dispensed into new flask and the availability of the cells in the new flask are examined. Then, HeLa cell will be stored in an incubator at 37°C and 5% CO_2 gas. Cells will undergo sub-culture process every 3 to 5 days whenever they meet 80% to 90% of confluency.

2.2. Electroporation

Electroporation setup is to extensively electroporate the cells, which means create pores that can be either temporary irreversible electroporation or reversible electroporation. Purpose of this technique is to deliver biological elements into cells for further live investigations. ECM830 electroporator was used for electroporating the cells in this experiment. There are two modes of operation in ECM830 electroporator, 5V to 500V will be the low voltage mode and pulse length of 10ms to 999ms. And high voltage mode with output voltage ranging from 501V to 3KV and pulse duration of 10μs to 600μs. Electroporation method comes with cuvette (BTX Harvard Apparatus) with electrode gaps of 1, 2 and 4mm and volume of 100μl, 200 μl, 400 μl accordingly to the exposure of large suspension volumes. In this experiment, cuvette with 4mm is used as it is suitable for mammalian cell. Cell must reach confluency approximately 80-90% on the day of electroporating the cell. Next, cells will be mixed with triple express and neutralized with same amount of volume of growth medium. Then, 800μl of cell transferred into a 4mm electrode gap cuvette (BTX Harvard Apparatus). After that, cuvette placed into the safety stand. Once done, safety stand is connected to BTX 830 Electroporator. Finally, ECM 830 Electroporator is set up accordingly (parameter involved: voltage and pulse duration) and induced. The electroporated cells were then seeded into 6-wells plates containing 2ml of complete growth medium. At last, it is incubated at 37°C and 5% of CO_2 prior to harvesting.

3. RESULTS AND DISCUSSION

Table 1. Image of HeLa cell after electroporation in 25cm^2 flask (scale bar 50µm)

Time (Hour) / **Voltage**	**48**
NEP	
200 V/cm Pulse Duration 30µs	
400 V/cm Pulse Duration 30µs	
600 V/cm Pulse Duration 30µs	

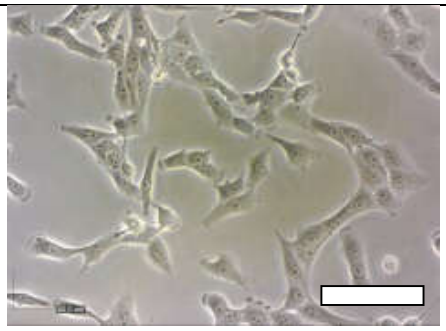
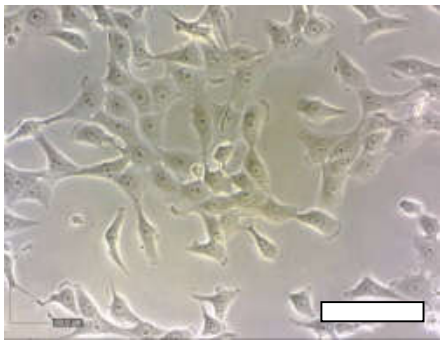
800 V/cm Pulse Duration 30µs	
1000 V/cm Pulse Duration 30µs	

Table 2. Electroporated and non electroporated cell parameter

Voltage (V/cm)	Average Cell Length (µm)
NEP	59.13
200	59.38
400	56.62
600	53.13
800	62.25
1000	59

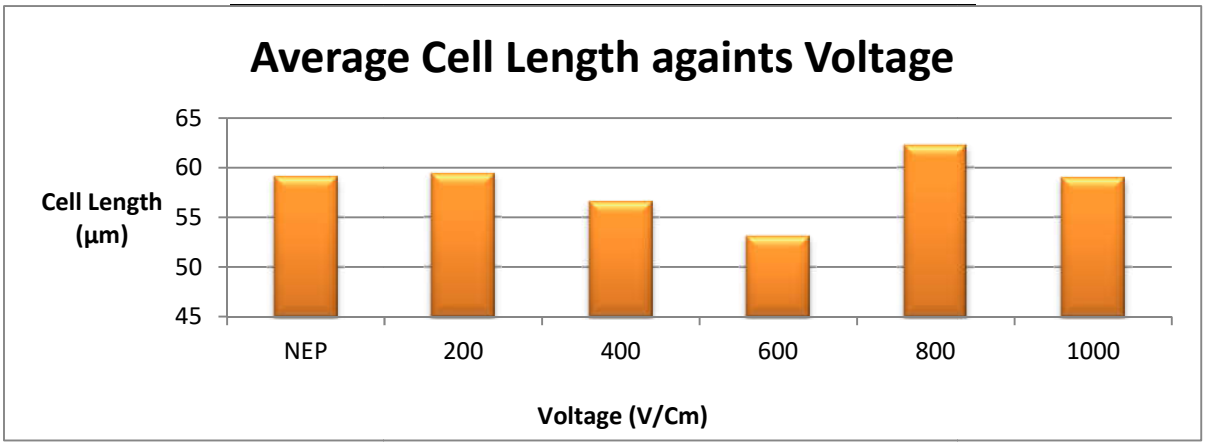


Fig.1. Average cell length (µm) against voltage (V/cm)

Referring to Table 1, the electric field application does not contribute much effects on the HeLa cell in the first 6th hour. However, when compared to the control cell, the experimental

data shows electric field does has effect on the growth of the cell after it has been incubated for 12 hours and more. The result shown in Table 2 shows the comparison of the HeLa cell length with electric pulse and without electric pulse. At the same time, electroporation also enhances the proliferation of HeLa cells. This is due to the significant increase in the electrical permeability when subjected to electroporation. Electroporation cause temporal membrane defects, where the electrical impedance of the plasma membrane began to fluctuate after the application of electroporation. As proven in the previous study, the use of electric field alters the permeabilization of a cell membrane in order to form nanoscale pores in the cell membrane. This leads to absorption of surrounded medium and nutrients and changes the size of the cell and proliferation of HeLa cell.

Fig. 1 shows the graph of the cell length average versus voltage (non-electroporation, 200v/ cm, 400V/ cm, 600V/ cm, 800V/ cm and 1000V/ cm) with the pulse duration of 30μs. The EP cell spread to a maximum length of 62.25μm with 800V/cm and minimum length of 53.13 μm with 600V/cm. Overall, the experiment demonstrated that various voltage parameters do effects on HeLa cell's extension, cell size and growth rate. However, this study requires further investigation to identify the optimal electroporation conditions that can be applied to effectively deliver the Artocarpus Altilis extracts to the HeLa cancer cells in order to inhibit its proliferation.

4. CONCLUSION

This proposed method of using electroporation with Sukun extract were experimented and discussed in detail. Investigation on both EP and Sukun extract proven that these methods can be combined in in-vivo studies to demonstrate the cell response towards the treatment. Currently, we are at the stage of establishing the Electroporation parameter. Next stage is to investigate on the compound extract (Artocarpus Altilis) and the anti-cancer mechanism by combining the compound Artocarpus Altilis and the optimized Eletroporation method for maximum HeLa cancer cell anti-proliferation activity. It is hoped that the result of this research will lead to the development of a better cancer treatment.

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