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Enhancement of MCF-7 and HeLa Cell Interfacial Interactions using Pulse Electric Field and Natural Sustainable Resources

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Abstract

Electroporation plays a part in chemotherapy, providing a promising strategy to increase the potency of anticancer medications and enhance treatment outcomes in some types of malignancies. Electroporation is a biological technique in which short electrical impulses are applied to cells or tissues, which temporarily destroying their cell membranes. This disruption creates temporary pores or channels in the cell membrane, allowing for the introduction of membrane that allow foreign substances such as DNA, RNA, proteins, proteins, or drugs into to enter the cells. The effectiveness of the technique will aid in the development of new cancer cell therapies and wound healing approaches. This review addresses the effects of electroporation techniques on breast cancer cells (MCF-7) and cervical cancer cell (Hela) using electric pulse ranging from 100-100V/cm. On top of that, honey extracts will be injected into breast cancer cells and cervical cancer cells using electrical pulses or the electroporation technique to examine the anti-cancer mechanism. This research finding may lead to fewer adverse effects of cancer treatment while maintaining patients' health and enhancing overall life expectancy.© 2024 The Authors. Published by ELSEVIER B.V.

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1. Introduction

When cells are subjected to an electric field with appropriate amplitude, a phenomenon known as electroporation takes place, increasing the permeability of their plasma membrane [1]. Electroporation continues to be an actively researched technique with ongoing developments and applications. CRISPR-Cas9 components are frequently delivered into cells by electroporation for gene editing. Researchers can target genes and instigate exact alterations in the genome by introducing the Cas9 protein and guide RNA molecules. In order to successfully carry out gene editing, electroporation enables effective distribution of these ingredients [2]. Investigations into electroporation as a strategy to improve vaccine effectiveness being studied. Researchers want to promote robust immune responses and boost vaccine efficacy by electroporating cells to release DNA or RNA encoding antigens. This strategy has shown potential outcome in vaccine development [3]. While in tissue engineering, cells within tissue constructions can be electroporated to inject genetic material or components. Researchers can regulate tissue development, improve functionality, or promote cell behaviors in synthetic tissues by precisely delivering genes or signaling molecules [4]. Other than that, electroporation technique being applied in microbial research where DNA are inserted into bacteria, yeast, or other microorganisms using the said method. This enables genetic engineering techniques including gene knockouts, gene expression research, and the creation of recombinant proteins. In synthetic biology and microbial genetics, electroporation is said to be a useful method [5].

Several clinical and experimental studies have investigated the potential anticancer properties of honey. Consequently, honey has been chosen for this study due to its potential underlying mechanism. Honey is a natural sweet viscous fluid made by bees [6] that comprises almost 200 ingredients [7]. Honey is rich in a variety of elements, including minerals like calcium, potassium, and iron, enzymes, amino acids, vitamins like vitamin C and B-complex vitamins, enzymes, and carbs like fructose and glucose. Depending on the type of honey, the levels may vary, but it can offer trace amounts of these healthy substances [8]. Since ancient times, honey has been utilized as a home treatment for wound healing. It has a low pH and antibacterial qualities, which together provide an environment that prevents bacterial growth and aids in wound healing. Minor burns, cuts, and abrasions can benefit from topically applying honey to lessen swelling, stop infection, and fasten recovery. In addition, antioxidants contain in honey, such as flavonoids and phenolic compounds, can help protect the body from the detrimental effects of oxidative stress caused by free radicals. Antioxidants have been associated with several health advantages, such as a lower risk of chronic illnesses like heart disease and several forms of cancer. It could possess antibacterial qualities which could aid in the fight against some bacteria, including Helicobacter pylori, which is linked to gastric ulcers [8]. This research will concentrate on three various types of honey: Tualang honey, Kelulut honey, and Natural honey. These honey kinds are currently riding a wave of market appeal, making them accessible and widely available. It would be much easier for people with cancer to get a treatment for their condition if it were available as a chemotherapeutic drug [1].

This research study will focus on breast cancer cell MCF-7 as well as cervical cancer cell HeLa. A well-known and extensively researched cell line generated from human breast cancer is called MCF-7. Over several decades, MCF-7 and HeLa have been the subject of in-depth research and thorough characterization. Their traits, developmental patterns, genetic changes, and reactions to diverse stimuli have all been extensively studied. This vast body of knowledge makes comparative research, repeatability, and experimental result interpretation easier [10]. Researchers from all around the world have access to MCF-7 and HeLa cells since they are extensively distributed through cell banks and research organizations. They are widely used and well-known in the scientific community, which encourages cooperation, data sharing, and study comparison. These cells develop more slowly than certain other cancer cell lines, which may be helpful for some experimental plans. Researchers can analyze cell behavior for extended periods of time thanks to their slower growth without experiencing substantial overgrowth. Furthermore, these types of cells can divide endlessly in culture, enabling lengthy research and a constant supply of cells, they are regarded as immortal. They are excellent for a variety of experimental applications due to their rapid proliferation [11].

2. Literature Review

2.1. Reversible and Irreversible Electroporation

Alternation of cell permeability which occurs in the lipid bilayer of the membranes of all prokaryotic and eukaryotic cells is defined as electroporation [12]. There are two types of electroporation: reversible electroporation and irreversible electroporation. Reversible electroporation is a phenomenon that causes transitory hole openings in the cell membrane, allowing cell survival when the electric pulse is removed [13]. Reversible EP usually used to upload impermeable compounds into cells [14]. Chemicals such as dyes, medications, nucleic acids, and proteins are commonly introduced into cells through Reversible electroporation [15]. Irreversible types of electroporation result in permanent pore creation in the cell membrane. These permanent pores caused by electrical pulse will lead to cell death by lysis [16]. When the amplitude of the produced transmembrane potential exceeds a specific threshold, the cell membrane is torn, and the cell dies as a result of a loss of homeostasis [17].

2.2. Honey

Honey has been used therapeutically for 8000 years, which has significantly raised interest in its potential health advantages. The outgrowth of floral nectar that rich in variety of chemical constitutions which aids in nurture benefits in human diseases from acute to chronic and deadly diseases [2]. Numerous verified investigations have shown that the health advantages of these honeys have recently become more popular and in-demand. According to a prior study, honey contains 80% carbohydrates, 35% of which are glucose, 40% of which are fructose, 5% of which are sucrose, and the remaining 20% is water. It also contains active biomolecules such as vitamins, minerals, amino acids, vitamins, minerals, enzymes, organic acids, flavonoids, and phenolic compounds [3]. Honey is classified according to its floral source or bee species. The two primary categories of bee species are Apis (stinging bee) and Meliponine (stingless bee). Apis dorsata produces the polyfloral honey known as Tualang honey, while Kelulut honey is produced by Meliponine [4]. Based on numerous documented studies, Tualang and Kelulut honey said to have all these properties like anti-stress, antidepressant, antioxidant, anxiolytic, antibacterial, anti-cancer, anti-inflammatory and physicochemical [4-6].

Table 2.2: Honey effects studies on cancer cells

Author	Type of Honey	Cell	Result
Ghashm et al., 2010	Tualang	OSCC And HOS	Early apoptosis has an antiproliferative effect on oscc and hos cell lines. [2]
Samarghandian et al., 2011	Not stated	ACHN (Human Renal cancer cell line)	Concentration and time decrease malignant cell viability [7]
Sefat et al., 2016	Kelulut	Colon Cancer	KH has chemopreventive properties
Yazan et al., 2016	Tualang	Breast Carcinogenesis	Tualang honey reduces breast cancer risk by modulating hematologic, estrogenic, and apoptotic actions.
Afrin et al., 2018	Manuka	Human colon cancer	By reducing cell proliferation and promoting apoptosis, MH enhanced 5-FU's chemotherapeutic effects [8]

Al-koshab et al., 2020	Tualang	Oral Squamous Cell Carcinoma	The substantially decreased the occurrence of oral squamous cell carcinoma (OSCC) and inhibited cancer cell proliferation. [9]
Cianciosi et al., 2020	Manuka	CSCs-like from human colorectal carcinoma (HCT-116 cell line)	MH reduced the volume of CSC-like spheroids, affected their morphology, and induced apoptosis and ROS accumulation.
Sarfraz ahmed et al., 2022	Honey Sugar	Breast cancer (in vivo) rat model	HAS showed significant % reduction in size of tumor
Danila cianciosi et al., 2022	Manuka	Colon cancer	MH reduction in survival ability of cancer cells

3. Methodology

3.1. Cell Culture

MCF-7 breast cancer cells and cervical cancer cells are studied using in vitro techniques. Cells were cultured in RPMI1640 media supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic (penicillin and streptomycin) from Gibco USA. In an incubator set at 37 °C with 5% CO2, cells were kept developing and keeping their ideal humidity and temperature levels. Every 3 to 5 days, cells will go through a sub-culture procedure, at which point they will be 80–90% confluent. Old medium will be aspirated and discarded from the culture flask for the sub-culture process. Cells were then washed three times in 2 ml of phosphate buffered saline (PBS) to remove medium serum and allow trypsin to detach the cell from the flask surface. The cells were then rinsed three times with 2ml of phosphate buffered saline (PBS) to remove the media's serum to ensure that trypsin could successfully separate the cell from the flask surface. Trypsin was added to 2 ml of cells, and they were allowed to digest for 5 to 10 minutes. To counteract the effects of trypsin, the flask was filled with a twofold volume of complete growth media and centrifuged at 1000 rpm for five minutes. The cell pellet will be resuspended in full growth media once the supernatant has been removed [10].

3.2. Electroporation

In research on molecular biology and biotechnology, the ECM830 Electroporator is a frequently used electroporation machine. It is created by the electroporation and electrofusion technology business BTX Harvard Apparatus. The ECM830 Electroporator's precise electrical pulses are intended to transfer substances efficiently and precisely into cells, such as proteins, nucleic acids, or other compounds [11]. Electric pulses required to electroporate the cells were produced using an ECM 830 electroporator. The voltage range for this device is from 5 to 3,000 V, and the pulse length ranges from 10 to 10 seconds. Additionally, it has a digital display that shows the supplied voltage and pulse length. The cells were electroporated using a single pulse electric field with an intensity of 200 V/cm with a constant pulse duration of 200s. Then, 2ml of complete growth media was added culture flask after the cell suspension seeded. In a separate well with 2ml of media, 800µl cells from the same starting flask were likewise planted without electroporation. Later, cells will be kept at 37 degrees and 5% CO2. Cells were observed after 48 hours of incubation.

Fig. 1. ECM830 Electroporator

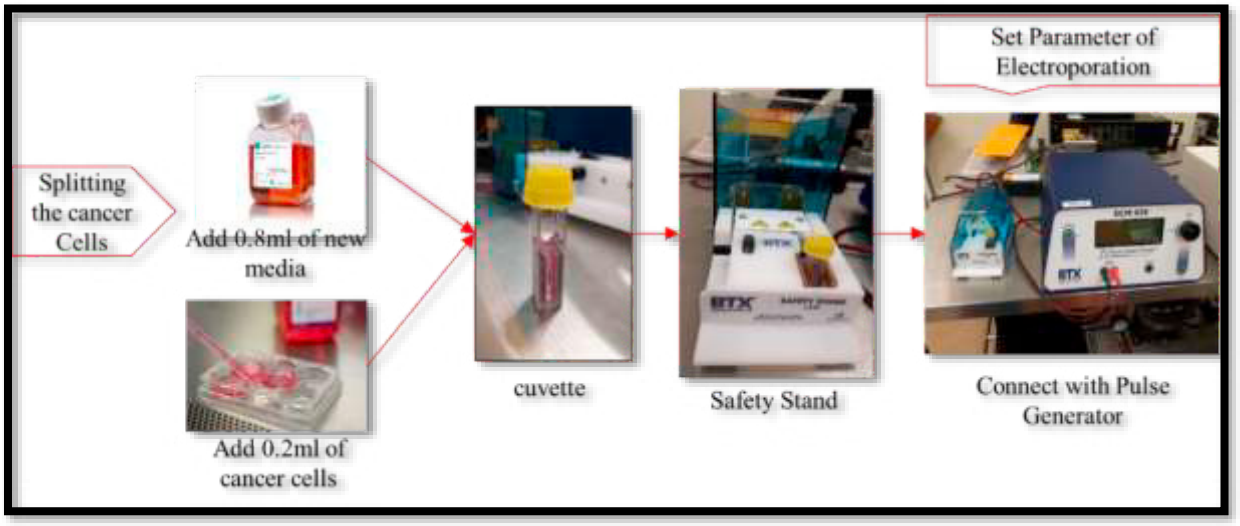

Fig. 2. Electroporation Setup

4. Experimental Results

4.1. Cell Culture

Figures 3 and 4 show the proliferation of MCF-7 and HeLa cells over the course of 36 hours. Cell culture is the basic technique in the field of research where proper method must be applied to accomplish further investigation. The outcome of culture process, growth of both types of cancer cell shown in above image. Cells can grow rapidly if the right amount of nutrients (growth medium, amino acids, carbohydrates, mineral, salts vitamins, serum) and space given [28]. Maintaining a controlled cell culture method, reagent procedure, and a sterile work environment are all key components in determining the growth rate of cancer cells. According to the results reported by K Sato [28], cells took 24 hours to proliferate in line with the result gained in this study. After the 5th day of cell culture process, it will reach confluency at 80-90%.

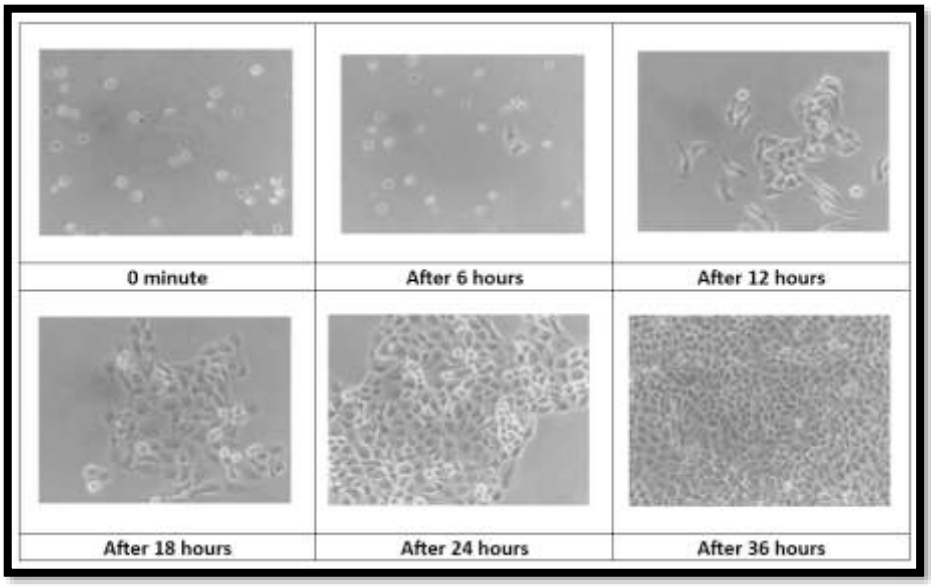

Fig. 3. Breast Cancer Cell Proliferation in 36 hours

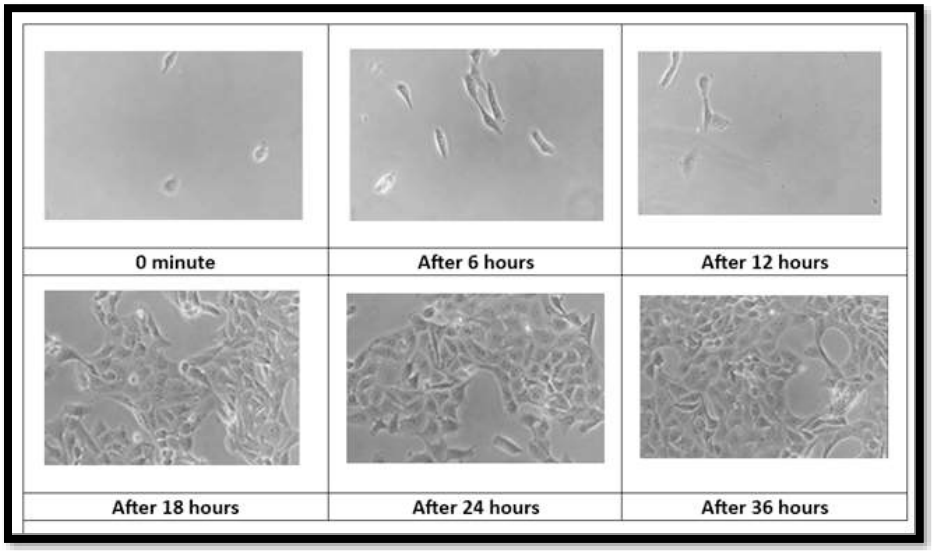

Fig. 4. Cervical Cancer Cell Proliferation in 36 hours

4.2. Electroporation

The result clearly showed that electroporation or pulse electric field influences cell morphology. After being induced by electric field, the cell appears to be swelled and expanded and continue to proliferate. This is a phenomenon of Reversible electroporation. It is cell permeabilization with the application moderate electric pulse to the cell which created temporary pores in the cell membrane allow the entry and exit of molecules and ions, upsetting the normal osmotic balance. Water and ions may consequently enter the cells, causing them to swell and grow larger [29]. Electric field parameter also assisted the cells in uptake of more nutrients containing in complete growth media as more aqueous pores formed resulting from the electrical pulses which agreement with the result obtained by Mamman, Hassan Buhari,

et al. 2021 [30]. Electroporation technique will be continued in this study with different parameters and upload the natural extract honey to investigate the anti-cancer mechanism. The study's primary goal is to investigate the anti-cancer mechanism by electroporating honey extracts into breast cancer (MCF-7) and cervical cancer (HeLa) cells. The cancer cells are subjected to short electrical pulses ranging from 100-100V/cm, causing temporary disruption to their cell membranes via the electroporation process. This disturbance causes transitory pores or channels to form in the cell membrane, increasing cell permeability in both cervical and breast cancer cells. The overarching goal is to improve honey uptake, which is renowned for its potential anticancer effects, by utilizing the electroporation approach. The transient pores formed in the cell membrane allow external chemicals to enter, increasing cell permeability. This method of targeted delivery is intended to boost the efficiency of anti-cancer chemicals within breast and cervical cancer cells.

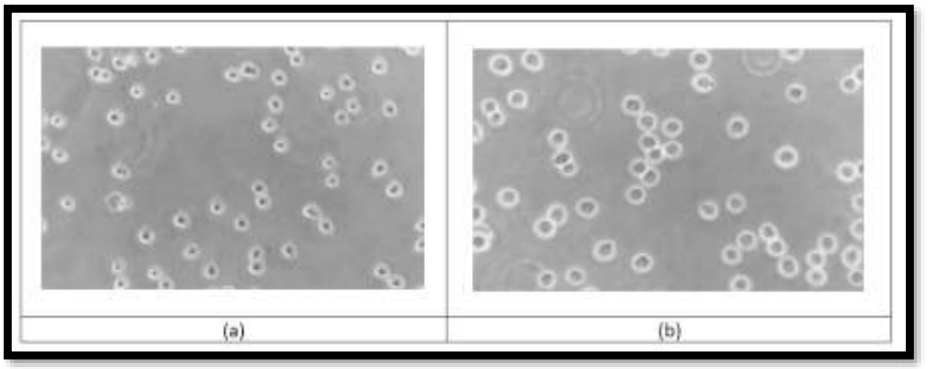

Fig. 5. MCF-7 morphological changes; (a) Non electroporated cells (b) Electroporated cell

5. Conclusion

Electroporation has proven to be a highly effective technique in various fields of biotechnology and biomedical engineering. Its diverse applications encompass gene therapy, electrochemotherapy (ECT) electro-fusion, electro-sterilization, and tumour tissue ablation. Notably, electrochemotherapy has exhibited significant advancements, progressing to both pre-clinical and clinical trial stages. Thus, comparatively it has proven that electrochemotherapy is the effective treatment with other existing cancer treatments. In this study, the fundamental ideas of electroporation were addressed. In an anti-cancer investigation, both approaches were found to be associated depending on the amount of their threshold and application. Tualang and Kelulut honey will be further investigated on the anti-cancer properties incorporated with electroporation process. This technique will be tested on method on breast cancer cell MCF-7 and cervical cancer cell HeLa to study on the cell proliferation, cell growth and morphology. The outcome of this research may lead to electrochemical pathways for cervical cancer treatment.

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