A COMPARATIVE STUDY OF ELECTROPORATION-INDUCED CELL DEATH IN SUSPENSION AND MONOLAYER CULTURES

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Electroporation (EP) is a technique that uses short electric pulses to create pores in cell plasma membrane and has been utilized for malignant tissue ablation since 2005 [1]. If the amplitude of applied pulsation is sufficiently high, the cells eventually die due to homeostasis disruption and membrane damage. This process is called irreversible electroporation (IRE) and is used to target cancerous tumors and, as of recently, cardiac tissue, in order to treat certain types of arrhythmias [2, 3]. In the past years, many studies have evaluated the efficiency of IRE both *in vitro* and *in vivo*, however, these studies are not necessarily directly comparable, as different electrode configurations, medium compositions and electrical parameters are often used, and not all data is available publicly [3]. Thus, in this study, it was aimed to elucidate optimum conditions for IRE that would utilize lowest energy and achieve highest cell mortality rates.

Chinese hamster ovary cells were subjected to EP using stainless steel electrodes. During the evaluation of viability of electroporated suspended cells, the suspension was placed between parallelly-arranged electrode plates. The viability of the treated cells in suspension was evaluated by measuring their fluorescence after staining with propidium iodide, an otherwise membrane-impermeant dye, 20 min post-EP via flow cytometry. Clonogenic assay was also employed to assess cell survival. The same EP conditions were applied to cell monolayers, and the extent of electroporation was evaluated. The cells had been plated in the wells of 24-well plates 48 h prior to the experiment. Reversible and irreversible electroporation zones were identified using fluorescent microscopy. For imaging, the dead and permeable cells were stained with propidium iodide, and the viable cells were stained using calcein-AM.

The scope of irreversible electroporation was determined under varying electroporation parameters i.e., the number of pulses, pulse duration and amplitude while maintaining the same total single pulse energy. In cell suspensions, the decrease in cell viability is proportional to electric field strength, and ultimately plateaus after using a high number of pulses. The areas of reversible and irreversible cell monolayer electroporation were compared, and these results were contrasted with the results of viability of cells that were electroporated in suspension. Preliminary experiments show that irreversibly electroporated area grows more slowly than the area of reversible electroporation.

[1] Miller et al. (2005). Cancer cells ablation with irreversible electroporation. Technology in cancer research and treatment, 4(6), 699–705.
[2] Lavee et al. (2007). A novel nonthermal energy source for surgical epicardial atrial ablation: irreversible electroporation. The heart surgery forum, 10(2), E162–E167.
[3] Casciola et al. (2022). Human cardiomyocytes are more susceptible to irreversible electroporation by pulsed electric field than human esophageal cells. Physiological reports, 10(20), e15493.