INVESTIGATION OF ANXA4 FUNCTION IN CELL PLASMA MEMBRANE PERMEABILIZATION, RESEALING AND CELL VIABILITY POST-ELECTROPORATION

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Electroporation (EP) is a technique where the application of brief, high-voltage electric pulses leads to the permeabilization of the cellular plasma membrane, potentially through the formation of electro pores. However, the precise mechanism of electropermeabilization remains unexplained.[1] Critical parameters influencing the efficacy of EP include the intensity, duration, and number of electric pulses, with excessive levels of any parameter potentially compromising cell viability.[2] Post-EP, the recovery of plasma membrane integrity is essential for cell survival, highlighting the role of annexin family proteins, particularly annexin A4 that is known to be involved in membrane repair processes after activation by Ca^{2+} ions. Despite ongoing research spanning over four decades, information about the impact of proteins on the restoration of cell plasma membrane post-EP is missing, despite numerous publications on calcium influence on cell plasma membrane recovery after EP. This study aimed to investigate the influence of Ca^{2+} ions on the recovery of the cells membrane following EP. Response of wild-type MCF7 cells, with an intact annexin A4 gene, in contrast to MCF7-ANXA4^{-} knockout (KO) cells, where annexin A4 gene's expression was disrupted were investigated.

Cell viability was assessed using the MTS assay, while the dynamics of plasma membrane repair and electropermeabilization were evaluated via flow cytometry, specifically through the quantification of propidium iodide permeable cells. Electroporation was conducted using a single 100 μs electric pulse at various intensities, with a $CaCl_2$ concentration set at 2 mM. Interestingly, we determined that MCF7-WT cells are less sensitive to the adverse effects of electroporation compared to MCF7-ANXA4^{-} (KO) cells. Furthermore, results showed that calcium had a negative impact on the viability of both cell lines, despite having a positive impact on reducing electropermeabilization level. In contrast, cell plasma membrane recovery after pulsing in calcium medium had a significant negative impact on cell plasma membrane recovery by increasing amounts of permeable cells in both cell lines 35 mins after EP. These observations suggest that annexin A4 may play a critical role in the plasma membrane repair and cell viability after electropermeabilization when calcium ions are involved, challenging the presumed importance of this protein in the cellular recovery process.

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[2] B. Jakštys, et al. "Correlation between the loss of intracellular molecules and cell viability after cell electroporation," Bioelectrochemistry, 135: 107550, 2020.