EFFECT OF INTRACELLULAR MOLECULES ON CELLS VIABILITY AFTER ELECTROPORATION

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During electroporation (EP), short electrical pulses are used to enhance the permeability of cell membranes. This method is commonly utilized to facilitate the transfer of substances into cells or extract intracellular molecules from cells. EP has a wide range of applications, in different fields, like biotechnology, food industry and medicine. Nevertheless, the full potential of EP remains undiscovered. Among the various subjects under assay, scientists are exploring methods that helps to improve cell viability after EP. Researchers demonstrated that during irreversible EP (when all cells die after treatment) intracellular molecules are released from cells, then the medium is obtained and known as supernatant (SN). When new cells were electroporated in SN, it was determined that cells viability increase, compare to cells that was treated in standard EP medium. One of the theories, that through EP cells with SN medium have similar amount of intracellular molecules inside and outside, therefore cells releases less of intracellular molecules through EP and are less affect by stress and more viable. During our investigation, we intended to evaluate whether the use of intracellular molecules could change cell viability after EP with different parameters.

Electroporation was carried out on Chinese hamster ovarian (CHO) cells positioned between stainless-steel electrodes, using a low-conductivity electroporation medium (0.1 S/m, 270 mOsmol, pH 7.2). CHO cells were EP with 1 or 8 high voltage (HV) pulses of 100 µs duration, pulse intensity was changed from 0.8 kV/cm to 3.6 kV/cm and repeating at 1 Hz frequency. Cell viability were determined by using colorimetric MTS (metabolic activity) assay and flow cytometry (by counting amount of cells) 24 hours after treatments.

Results revealed that CHO cells viability were increased after EP with SN, MTS assay results shown that cells were more viable with 8 HV and 1 kV/cm, 1,2 kV/cm ir 1,4 kV/cm electric pulses. The maximum change in cell viability recorded using 1 kV/cm and 8 HV electric pulses was 48% ± 12% in EP medium and 70% ± 12% in EP SN medium. With flow cytometry cells viability were statistically significantly different with SN medium using these parameters: 8 HV and 1 kV/cm ir 1,2 kV/cm. From the obtained results, we can notice that the cells exposed to 8 HV electric pulsed supernatant has a positive effect on cell viability after EP, although this effect of EP SN medium is not seen when cells are exposed to 1 HV. Investigation of SN medium with addition MTS and flow cytometry assays demonstrated that cells viability with SN increases due intracellular molecules, not due to cells that were mistakenly transferred with SN.

In conclusion, SN can increase cell viability, but electric pulse parameters should carefully chosen.

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