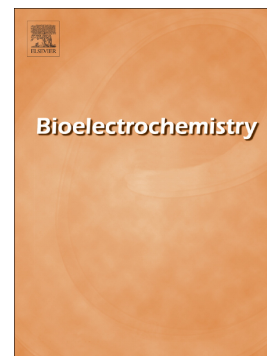


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Single Cell Dielectrophoresis Study of Apoptosis Progression Induced by Controlled Starvation

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Abstract

Nutrient depletion in fed-batch cultures and at the end of batch cultures is among the main causes of stress on cells and a trigger of apoptosis. In this study, we investigated changes in the cytoplasm conductivity of Chinese hamster ovary (CHO) cells under controlled starvation. Employing a single-cell dielectrophoresis (DEP) cytometer, we measured the DEP response of CHO cells incubated in a medium without glucose and glutamine over a 48-hour period. Using the measured data in conjunction with numerical simulations, we determined the cytoplasm conductivity of viable and apoptotic cell subpopulations. The results show that a small subpopulation of apoptotic cells emerges after 24 to 36 hours of starvation and increases rapidly over a short period of time, less than 12 hours. The apoptotic cells have a dramatically lower cytoplasm conductivity, ~ 0.05 S/m, than viable cells, ~ 0.45 S/m. Viability of starvation cultures was measured by fluorescent cytometry, DEP cytometry, and trypan blue exclusion assays. DEP, Annexin V, caspase-8, and 7-AAD assays show a similar decline in viability after 36 hours of starvation and indicate a very low viability after 48 hours. Trypan blue exclusion assay fails to detect early-stage viability decline and estimates a much higher viability after 48 hours.

Keywords: Apoptosis, CHO, cytoplasm conductivity, dielectrophoresis, flow cytometry, starvation

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