SINGLE CELL LYSIS ON MICROFLUIDIC DEVICES

Maxine A. McClain, Christopher T. Culbertson, Stephen C. Jacobson, and J. Michael Ramsey
Oak Ridge National Laboratory, Oak Ridge, TN 37831-6142 USA

Abstract

Methods to rapidly lyse cells are needed for studying biochemical reaction systems in cells with short half-lives such as protein phosphorylation. Chemical and electrical methods, therefore, were examined to rapidly lyse cells on microfabricated fluidic devices. The most effective cell membrane disruption involved a combination of both chemical and electrical lysing techniques.

Keywords: cell lysis, surfactant, electric field

1. Introduction

Cell lysis is the preliminary step in the analysis of intracellular compounds, such as DNA, RNA or proteins. The lysis process must preserve the integrity of the relevant compounds at their pre-lysis concentrations while releasing them from the cell. Of the various methods to rupture cell membranes in a population of cells, chemical and ultrasonic lysis are the two most common. Electrical lysis is a viable alternative, although it is not commonly used. Of these methods, chemical and electrical lysis are particularly well suited for implementation on a microfluidic platform because their effects can be easily localized.

2. Experimental

The cells used in the experiments were from the Jurkat line of human leukemia cells. The glass chips were fabricated and coated as previously described.[1, 2] A syringe pump was used to draw cells into the lysis chamber. The cells were labeled with membrane permeable probes that become fluorescent after the molecule is internalized and hydrolyzed in the cell. The degree of lysis was determined by 2-D imaging and coincident fluorescence and scattering detection.

3. Discussion

Both chemical and electrical lysis were evaluated separately and together. Sodium dodecyl sulfate and several polyoxyethelyene detergents were tested. With SDS cell lysis was accomplished in less than 1 s. Electrical lysis can be performed even more quickly than chemical lysis, which is significant for analyzing transient species. Direct current and alternating current electric fields over a range of voltages were effective in compromising the cell membrane. At low frequencies, DC biased AC fields were effective for lysis and did not produce the same level of Joule heating and bubble formation that the equivalent DC field did. Cell lysis generated with a DC biased AC

field and with solubilizing detergent in the side arms is shown in figure 1A. In this figure the cells are brought to a cross intersection hydrodynamically. At the intersection they are exposed to an electric field and lysed within two video frames (<66 ms). In figure 1B, the same region is shown without the electric field applied; no cell lysis is observed. The cell images look elongated in frames 3-7 because the fluid below the cross intersection is traveling at a faster velocity than that above the cross. The speed of membrane disruption correlated with the magnitude of the applied field. The effective range of field strengths was between 150 and 500 V/cm peak-to-peak for square wave 50 Hz AC fields with a 300 to 400 V/cm DC offset. A combination of chemical and electrical lysis provided the best results. The electrical lysis was fast and the chemical lysing agents kept cell membrane debris solubilized.

Acknowledgements
Oak Ridge National Laboratory is managed and operated by UT-Battelle, LLC under contract DE-AC05-00OR22725 with the U.S. Department of Energy

1. Hjerten, S. *J. Chromatogr.* **1985,** *347*, 191-198.
2. Jacobson, S. C.; Hergenröder, R.; Koutny, L. B.; Ramsey, J. M. *Anal. Chem.* **1994,** *66*, 2369-2373.

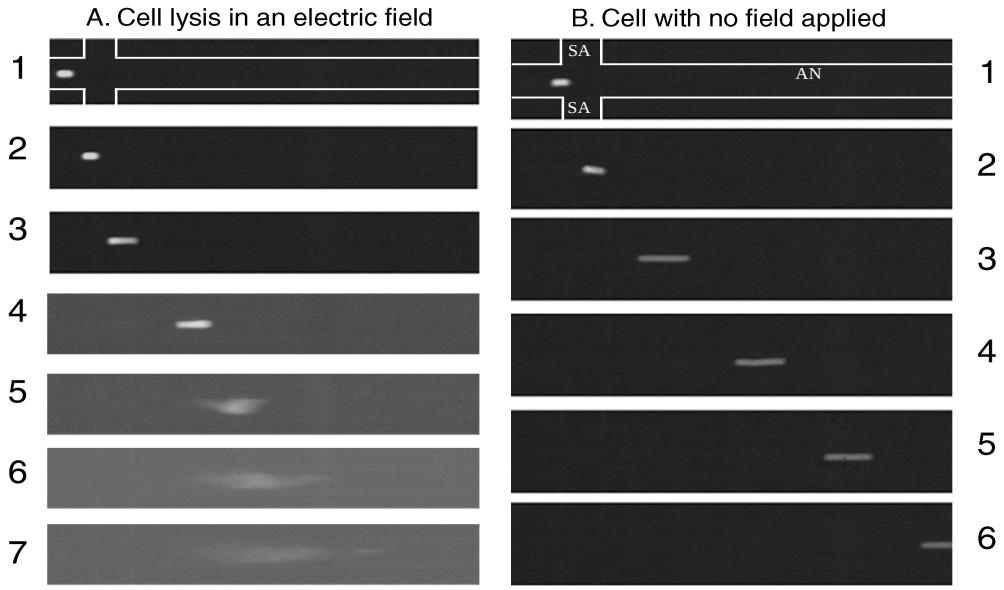

Figure 1. Jurkat cells loaded with calcein are hydrodynamically moved through a cross intersection with (A) and without (B) an electric field applied between the side arms (SA) and the analysis channel (AN). The images of the cells look elongated in the AN channel because of the increase in velocity in this channel.