DIELECTROPHORETIC (DEP) CYTOMETER: LABEL-FREE ELECTRONIC SENSING OF PHYSIOLOGICAL CHANGES IN CELLS

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ABSTRACT

We designed and developed a sensor that allows us to electronically monitor changes in physiological properties of biological cells. Based on the ability to simultaneously detect and dielectrophoretically (DEP) actuate cells, we can observe unique changes in the electronic detection signature that are directly related to polarizability of the cell. The important novel feature of this work is a differential electrode array that allows us to relate changes in the cell polarizability -– attributable to changes in physiological properties -- to a simple measurable physical variable such as the amount of vertical cell translation during DEP actuation.

KEYWORDS

DEP cytometry, label-free electronic biosensor, impedance biosensor, electronic detection of early apoptosis, single-cell analysis

INTRODUCTION

Continual monitoring of cell physiology and its response to different environmental stimuli or drugs is essential in biomedical research and industry, and generates a need for instruments and techniques that can provide quantitative understanding of cellular processes.   Some of the most important physiological changes in the cell are associated with programmed cell death, or apoptosis [1], crucial in organism development and in maintaining the normal balance of the living and dying cells (homeostasis).   The ability to predict apoptosis during its early stages, when it is still reversible, would be of great benefit because it would allow interventions resulting in, for example, a more efficient production of biopharmaceuticals, or more successful treatments of diseases in which homeostasis is disrupted (e.g., cancer, autoimmune diseases, AIDS, neurodegenerative diseases).

Changes in cell volume, observed early in apoptotic process, have been associated with ionic flux over the cell membrane [2].   This exchange of ions leads to a slight change in ionic composition of the cytoplasm and manifests macroscopically as a change in cell polarizability, which, in turn, influences cell response to DEP actuation.   We designed a novel differential electrode array that enables us to modulate electronic signatures produced by the cells during their flow through the microfluidic channel in a way that captures the amount of vertical cell translation effected by DEP actuation.   Subsequent analysis of signature modulations allows us to correctly assign cells to different subpopulations based on their differing dielectric properties that result from subtle changes in physiology [3].

DESIGN PRINCIPLE

Differential electrode array and its position in a microfluidic channel are illustrated in Fig. 1 (a) and (b).   The electrode array extends into wider pads, partly shown in Fig. 1(a), that provide contacts for the electrode wires extending from the microwave resonator.   In this way, the microfluidic channel is coupled to the microwave resonator to create a sensor.   Using a microwave interferometric approach described previously in [4,5], the sensor can identify vertical translations smaller than 0.5 µm.   Cells are detected at gigahertz frequencies as they flow in a liquid suspension above a coplanar differential electrode array (Fig. 1(a)), fabricated at the bottom of the microfluidic channel.
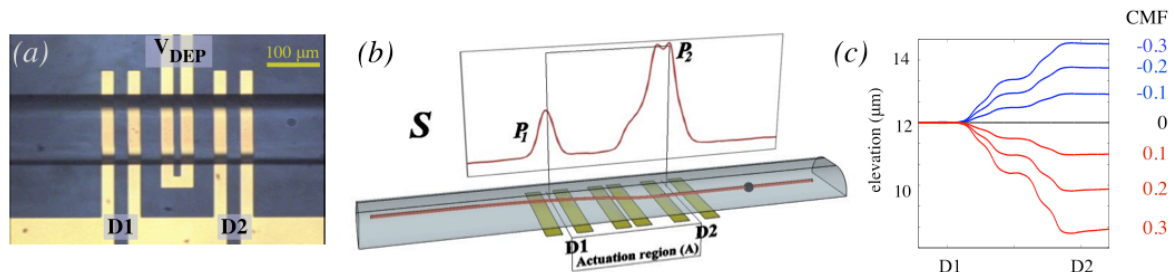

Figure 1: (a) Micrograph of the electrode array; (b) Schematic representation of a pDEP actuated cell trajectory with a corresponding electronic signature, S; (c) Simulations of trajectories for a CHO cell, initially at 12 µm elevation, for different polarizabilities (CMF). DEP force on a cell and extent of the resulting elevation change are controlled by the amplitude of applied DEP signal -- set to $V_{DEP} = 8\ V_{pp}$ in this case.

Two detection sites, D1 and D2, flank the actuation region, A (Fig. 1(b)), throughout which the cells are independently dielectrophoretically (DEP) actuated using frequencies in the range of 0.1 to 10 MHz. A cell flowing above the electrode region produces a distinctive electronic signature, S. Amplitude of S is proportional to the squared amplitude of the detection electric field at the location of the cell center of mass when the cell is directly above the detection site D1 or D2, as shown in Fig. 1(b). Simulated trajectories of actuated cells that start at the same elevation but experience different amount of deflection because of their different polarizabilities are depicted in Fig. 1(c).

Without actuation (no DEP), a cell remains at the same elevation during its flow between D1 and D2, and the signal amplitude P_2 at D2 matches the amplitude P_1 at D1 (Fig. 2 (a)). Actuation is effected by an attractive (pDEP) or repulsive (nDEP) force resulting from the applied DEP signal; it leads to an elevation change whose extent is evident in the trajectories of cells – *cf.* Fig. 1(c) -- simulated for different polarizabilities, expressed in uniformly spaced steps of Clausius-Mossotti factor (CMF). The amount and direction of vertical displacement within the actuation region depend on the specific dielectric properties of the cell, and uniquely modify profiles of electronic signatures collected during the experiment. These modifications can be quantified by defining the "force index," ϕ:

$$\phi = \frac{P_2 - P_1}{P_2 + P_1} \begin{cases} > 0, P_2 > P_1 \text{ (pDEP)}, \\ < 0, P_2 < P_1 \text{ (nDEP)}. \end{cases} \qquad (1)$$

Related examples of electronic signatures are given in Fig. 2(b).

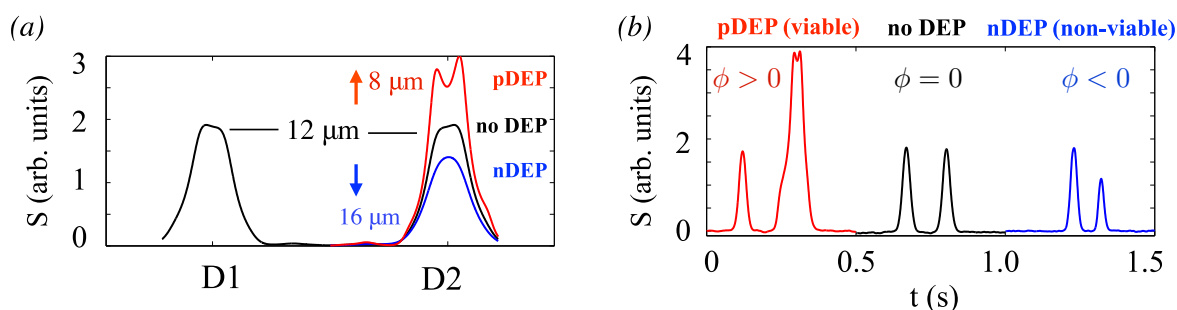Figure 2. Electronic signatures, S: (a) Simulations show how amplitude of S diminishes with elevation. Symmetric profile of S (black) is expected when no DEP is applied. Changes in profile for 4 µm elevation changes caused by pDEP (red) and nDEP (blue) are illustrated for a cell initially at 12 µm. (b) Experimental signatures for CHO cells, collected at f_{DEP} = 6 MHz. Force index, ϕ, is used to quantify cell dielectric properties.

EXPERIMENT

The concept of our device is illustrated on a quantitative study performed on a population of Chinese hamster ovary (CHO) cells involved in a bioprocess. CHO cells expressing a human-llama chimeric antibody (EG2) were cultured in a 3 L glass bench-top bioreactor (Applikon, Foster City, CA) at 37°C, 40% dO$_2$, 7.2 pH and 200 rpm. Batch culture was maintained through growth, stationary and declining stages. During this time (~120 h), the physiological state of cells was monitored with Trypan blue staining, as well as with two Guava 8HT (EMD Milipore, Danvers, MA) fluorescent flow cytometer assays: Nexin and ViaCount. Samples were taken every 24 hrs for the first four days and then every 6 hrs for the next 24 hrs.

For each measurement, 5-10 mL samples were taken from the bioreactor, cells were spun down, and reconstituted in a low conductivity (0.17 S/m) medium (22.9 mM sucrose, 16 mM glucose, 1 mM CaCl$_2$, 16 mM Na$_2$HPO$_4$), at a dilution of ~10^6 cells/mL. Cells in a low conductivity suspension were pumped through the microfluidic channel at a rate of 40 to 100 cells a minute. Experimental signatures were collected for about 5 minutes as the cells passed over the electrodes, first with 6 MHz DEP signal on, and then again with no DEP signal, to obtain a control group.

RESULTS AND DISCUSSION

During the first four days of cell monitoring, cell viability remained high (~90% and above) according to all four techniques. However, to estimate viability, each assay monitors different events in cell physiology. Consequently, they disagree on the rates of viability decline. Trypan blue selectively stains cells whose cell membranes are already compromised; ViaCount assay relies on a proprietary mixture of two DNA-binding proteins (one that can permeate the membrane, the other that cannot) to distinguish between healthy viable, apoptotic, and necrotic cells; Nexin assay detects the fluorescence resulting from the selective binding of protein annexin V to phosphatidyl serine (PS) heads on the non-cytoplasmic side of the membrane. The latter event occurs only after the PS molecules flip from the inner leaflet of the cell membrane to the outer one, and is generally regarded as the onset of apoptosis. Finally, DEP cytometer determines viability as percent of pDEP signatures in total number of cell signatures.

In Fig. 3, we present the results obtained in the final 24 h of the experiment. Comparison of viability estimates among the four different techniques (Fig. 3(a)) shows that the DEP cytometer estimates agree with those of the annexin V assay, which detects the early apoptotic changes.   Fig. 3(b) documents a shift in the state of cell health over the last 24 h of the experiment. As confirmed by the standard fluorescent flow cytometry assays, force index distribution shows a rapid switch from a healthy viable to an early apoptotic population.

CONCLUSIONS

A novel differential electrode array enables us to make non-invasive sensitive measurements of the DEP cell actuation using differential detection of cell altitudes.   This makes it possible to relate the variations in cell polarizability to the changes in physiological properties of the cell.   Simultaneous comparison with three standard techniques for monitoring cell viability confirmed the ability to observe a rapid transition from a healthy viable population of cells to an early apoptotic cell population using inherent properties of the cells as a marker of their physiological state.   All-electronic approach makes this sensor easy to miniaturize and integrate into a lab-on-chip type of application.

(a)

Assay	% viability at approx. hours				
	96	102	108	114	120
Trypan blue	96.9	93.8	85.7	80.0	78.7
ViaCount*	89.5	88.8	74.5	56.5	35.8
Nexin (annexinV)	82.6	72.0	44.1	21.9	13.9
DEP cytometer	93.0	76.1	46.6	25.8	9.5

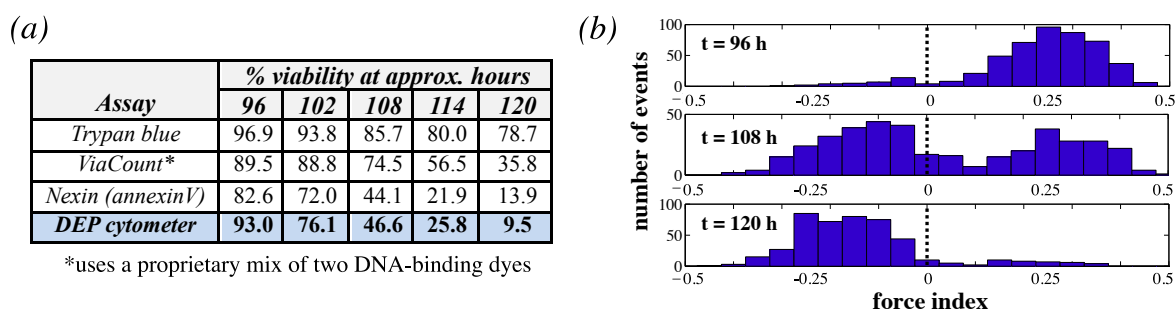*uses a proprietary mix of two DNA-binding dyes

(b)

Figure 3. (a) DEP cytometer viability assessment was simultaneously compared with three standard cell monitoring techniques; it agrees with the annexin V assay, which can detect early apoptotic changes.   Trypan blue staining depends on a cell membrane being compromised and is therefore not a reliable predictor of early changes in cell subpopulations [6].   (b) Force index histograms for CHO cells over a 24 h period show a clear separation of cells into two populations based on their dielectric properties, directly related to viability. As confirmed by standard assays, the incipient population on the left of the top plot consists of early apoptotic cells. Within 24 h, we observe that 90% of the cells undergo early apoptotic changes (bottom plot).

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