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Sensitive cell identification in impedance flow cytometry using optimized dielectric buffer properties and low cost microchannels

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Summary: Impedance flow cytometry is a promising technique to identify cells label-free. In this study, dielectric properties of buffer solutions are examined together with 3D focusing to achieve sensitive cell identification using low-cost microfluidic chips based on co-planar electrodes and wide channels. The efficiency for identification of polystyrene beads and HEK, C28, and C2C12 cells under various buffers and sheath flow conditions is analyzed.

Keywords: Impedance flow cytometry, cell identification, label-free, co-planar electrodes, 3D flow focusing

Background

Isolating and sorting single cells is of critical importance in biology, biotechnology, precision medicine [1] and point-of-care diagnostics [2]. Fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) are well-established, commercially available methods. Although these conventional techniques offer high-throughput and are widely used [3], they have significant drawbacks, including expensive markers, time-consuming labeling [4], and the absence of specific markers for certain cells [4], limiting their use for cell therapy [5]. These limitations have motivated research toward non-invasive, label-free alternatives such as impedance flow cytometry, which identifies cells based on their impedance at different frequencies. However, impedance measurements depend not only on the cells but also on the buffer, electrode configuration, channel geometry and cell-to-electrode distance. Developing a system that provides reproducible signals for identical cells while sensitively differentiating distinct cell types remains challenging. A key approach is to match the electrode volume as closely as possible to the cell volume [6] maximizing electric field perturbation and impedance change. In addition, with a channel size comparable to the cell size, cell positioning becomes more consistent, removing variation in the signal due to position changes. On the other hand, a smaller channel increases the risk of clogging, as well as the fabrication complexity and costs. Innovative ideas were implemented to address this issue, like modifying the electrode configuration [7], using a channel constriction [8] or using an insulating sheath fluid [6], [9].

Theory

When a cell or particle passes through the electric field between two electrodes, the electrical current changes, corresponding to an impedance change. An effective way to reduce the noise of the signal and eliminate the impedance of the buffer impedance is to use a differential electrode configuration, with the current change due to the cell in the first electrode pair compared to a second empty electrode pair. As a cell passes over the first electrode pair, the difference of the measured currents of both electrode pairs is proportional to the difference of the inverse of the both impedances:

$$\frac{1}{Z_{\text{CiB}}(f)} - \frac{1}{Z_{\text{B}}(f)} = \frac{i_1(f)}{V} - \frac{i_2(f)}{V} = \frac{\Delta i(f)}{V} \quad (1)$$

where i_1 and i_2 are the two measured currents of each electrode pair at frequency f for a chosen voltage V, and $Z_{\text{CiB}}, Z_{\text{B}}$ are the impedances of the cell in buffer and of the buffer. The cell identification is maximized when two different cells produce the largest difference in differential current Δi. This is ideally achieved when the buffer has a larger impedance compared to the cell $Z_{\text{B}}(f) \gg Z_{\text{CiB}}(f)$. However, this is challenging to achieve: since the cell is itself surrounded by buffer, increasing the buffer's impedance also increases the one of the cell in buffer, and especially if the channel's cross-section is large compared to the cell (which is economical and robust against clogging). Δi should also be significantly larger than the minimal resolvable current of the instrument to ensure trustworthy results. One approach to solve this problem is to use two buffers: a conductive buffer close to the cell and an isolating buffer used as a sheath flow. Both buffers should have similar osmotic pressure as the cells. With its high conductivity (1.6 S/m), phosphate-buffered saline (PBS) is ideal for a conductive buffer. In theory, a solution of 10g sucrose into 100mL of deionized water achieves

the same osmotic pressure as PBS, with a very low conductivity (1e-3 S/m).

Methods

Here, the validity of the presented theory was tested using a custom-built trans-impedance amplifier FGPA-based, with a wide (180um x 80um) and inexpensive microfluidic chip mounted on co-planar electrodes. A 3D printed mold was used to cast the PDMS microfluidic chip. The cell is focused in the center of the channel using hydrodynamic 3D focusing, with a sheath fluid, allowing a reproducible position of the particle. Polystyrene beads and several cells lines (HEK, C28, C2C12) were measured for several sheath and core buffers of specific conductivities, using PBS, a 10% sucrose solution and mixes of both. The differential current Δi, the noise and the signal-to-noise ratio (SNR) were compared. The impact of the buffer configuration on cell discrimination was studied using support vector machine (SVM), which finds the hyperplan that separates best all cell populations.

Results

When the core and sheath buffers are similar, Δi increased with conductivity, and the noise remained constant. In such configuration, as the channel is much larger than the particle, using PBS as the core and sheath fluids seems to provide the largest signals. Cells and beads lead to different conclusions when the conductivity of the sheath fluid was reduced while keeping a core of PBS. For beads, the signal intensity linearly decreased, whereas for the cells, different behavior were observed for different cells. Figure 1 illustrates changes of current produced by HEK cells in a PBS core, with different sheath fluids. The imaginary part remained constant, but the real part was reduced, leading to a lower amplitude. For C28, the amplitude remained constant but the similar phase shift was observed. The size difference between both cell types could explain this behavior. In all cases, the lowest noise was observed with a PBS core and sucrose as sheath fluid, leading in fine to a higher SNR. Interestingly and surprisingly, SVM accurately identified all cell populations in nearly all configurations, and no general ideal configuration could be identified. Only the identification of beads size was proved more successful when both the core and the sheath fluids were the most conductive. Our results suggest that 3D focusing in combination with co-planar electrodes can be used to identify cells sensitively in a wide microfluidic chip.

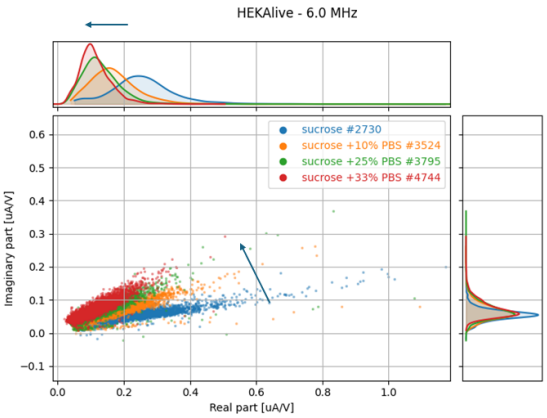

Fig. 1: HEK cells in a core of PBS surrounded by various sheath fluids, at 6 MHz, (#nb of cells)

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