ISO-ACOUSTIC FOCUSING FOR SIZE-INSENSITIVE CELL SEPARATION BASED ON ACOUSTIC PROPERTIES

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ABSTRACT

We report a novel size-insensitive method, iso-acoustic focusing (IAF), that can, for the first time, analyze and separate cells based on their intrinsic acoustic properties. We characterize the IAF system using two different cell lines and apply it to measuring the acoustic impedance of two subgroups of white blood cells (WBC): neutrophils and lymphocytes. We find cell type specific differences in acoustic properties that indicates that this method has potential for cell phenotyping and cell separations.

KEYWORDS: Cell phenotyping, Blood cells, Acoustophoresis

INTRODUCTION

Separation methodologies based on the intrinsic properties of cells (e.g., size, deformability, electrical properties) have enabled new applications within microfluidics. Among these, acoustophoresis is a gentle and robust method that has been demonstrated for concentrating, washing and sorting cells [1]. However, the strong size dependency in acoustophoresis has hampered the development of separators based on the underlying properties of cell density and compressibility. Here we investigate cell migration in an acoustic field where the acoustic impedance of the suspending medium increases monotonically until cells reach their point of zero acoustic contrast, the iso-acoustic point (IAP), such that the cells' final positions reflect their individual intrinsic properties.

EXPERIMENTAL

Cells in suspension were drawn from a pressurized test tube and were flow laminated along either side of a rectangular cross section (375 µm by 150 µm) silicon microchannel sealed by a glass lid. Liquid of higher acoustic impedance than the initial cell medium was introduced through a central inlet. A piezoelectric transducer, glued to the back of the chip, generated a 2.0 +/-0.1 MHz ultrasonic standing wave across the width of the channel such that a pressure node was located at the channel center and along the whole length, Figure 1(a).

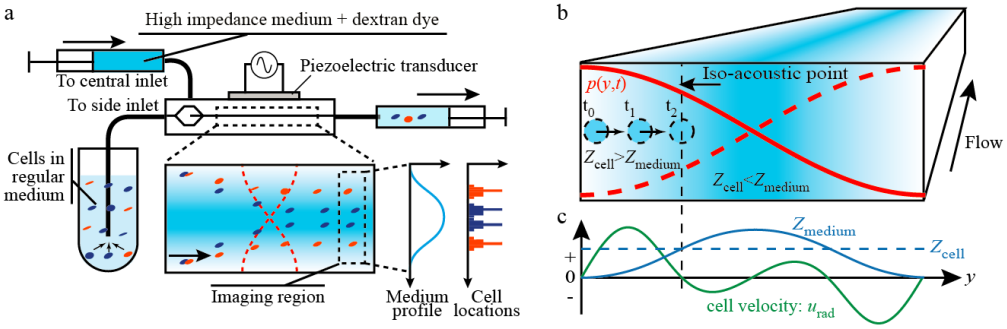

Figure 1: IAF system. (a) Medium of *high acoustic impedance was infused through a central inlet and cells were introduced through the sides' inlet. By the end of the channel, cells are spatially separated according to acoustic impedance. (b) Cells flowing in a microchannel are deflected sideways towards the node of an acoustic resonant pressure field. (c) The acoustic impedance (Z) of the medium can be tailored so that as the cell approaches the nodal position the acoustic impedance increases gradually. When the acoustic impedance of the cells match that of the surrounding liquid their sideways transverse velocities become zero so that their sideways position (y) reflects their individual acoustic impedances.*

RESULTS AND DISCUSSION

In IAF, cells flowing in a microchannel are exposed to an acoustic field that deflects them sideways into a gradient of increasing acoustic impedance of the surrounding liquid, until they reach a point where their acoustic impedance ($Z = \sqrt{(\rho/\kappa)}$) matches that of the liquid, Figure 1(b). Formation of such a gradient is difficult since liquid acoustic impedance and density are linked; an acoustic impedance gradient has an associated density gradient that is unstable and will equilibrate quickly due to gravity. Here we use the acoustic field to stabilize and shape a smooth gradient, through an intricate interplay between diffusion, gravity, acoustic streaming [2] and acoustic radiation acting on the medium itself [3]. In IAF, cells and media rearrange according to their acoustic impedance so that high-impedance matter is pushed to the acoustic pressure node in the channel center.

A requirement for IAF is to create an acoustic impedance gradient with minimal increase in viscosity. Screening across a range of gradient media, we chose iodixanol for its fast diffusivity, low toxicity and low viscosity for high acoustic impedance. To form a gradient of acoustic properties, iodixanol-containing media is injected in the central inlet of a trifurcation while cells in normal cell media are injected in the side branches, Figure 1(c).

To extract cells' acoustic impedances, after reaching their iso-acoustic point, we image both a proxy of the iodixanol gradient and the cells' position within that gradient, Figure 2 (a-c). Off chip bulk measurements of the acoustic impedance of iodixanol allows inference of the acoustic impedance of the media, and indirectly, of the cells, Figure 2 (d and e).

Characterization using murine BAF3 pro-B cells show that IAF is insensitive to variations in acoustic field strength, Figure 2(f), and microchannel flow rate, Figure 2(g). When we assay the acoustic impedance of a variety of cell types, we find that acoustic impedance is dependent on cell type, Figure 3(a), suggesting the potential of IAF for both cell separation and analysis. Finally, we analyzed WBCs from healthy human red blood cell (RBC) lysed blood, determining that the acoustic impedance of neutrophils and lymphocytes can be distinguished, Figure 3(b). Notably, the BAF3 cells have higher impedance (i.e. migrate closer to channel center) than MCF7 cells despite being smaller, emphasizing the size-insensitivity of the method; larger cells experience larger acoustic forces and would be found closer to the center in a size-based separation scheme.

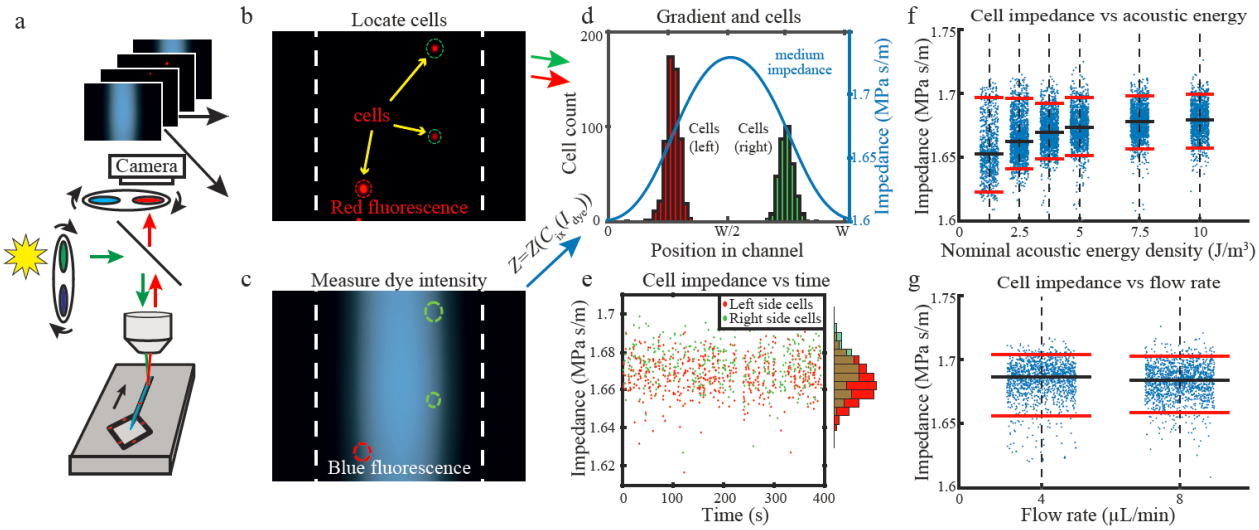

Figure 2: The acoustic impedance gradient is inferred via (a) sequential imaging of (b) cells and (c) fluorescent dextran tracer gradient. (d) Standard solutions of iodixanol and dextran dye were analyzed to convert from fluorescence intensity to acoustic impedance. (e) Scatter plot of inferred BAF3 cell acoustic impedances from both sides of the device over time. (f-g) Scatter plot of the inferred BAF3 cell impedance versus (f) acoustic energy density and (g) flow rate. Black horizontal lines show median and red lines show the 5th and 95th percentiles.

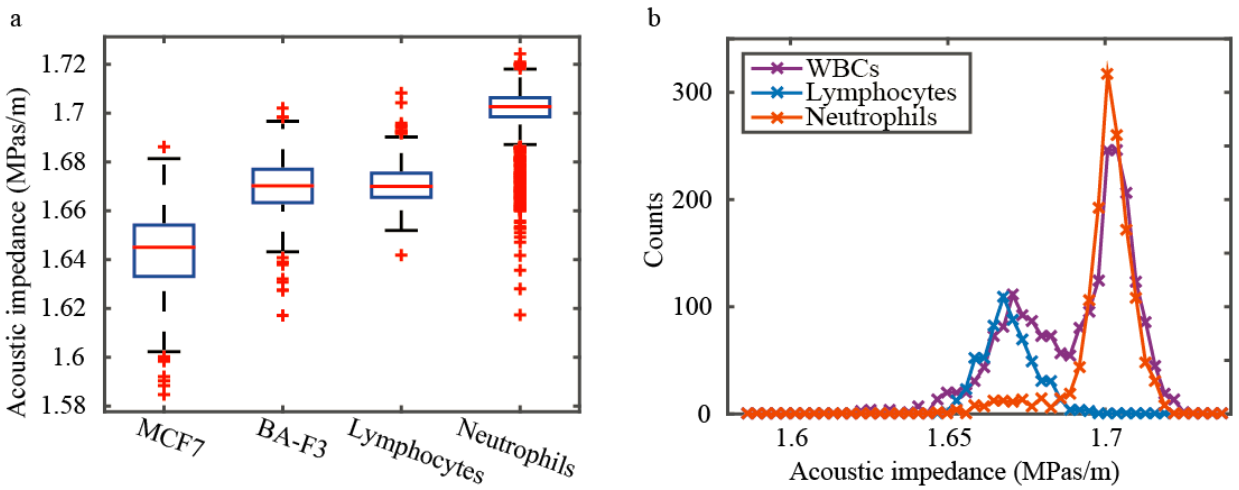

Figure 3: Acoustic impedance measurements reveal that some cell types have similar acoustic impedances (mouse BAF3, human lymphocytes), whereas others (human MCF7, lymphocytes and neutrophils) differ. Red vertical lines show median, blue box show 25 and 75 percentile, whiskers extend to 1.5 times the box height and red markers indicate outliers. (b) Measurement of fresh human leukocytes from RBC-lysed blood reveals two peaks that overlap well with measurement of density-gradient purified fresh human lymphocytes and neutrophils from the same donor.

CONCLUSION

These experiments show for the first time that we can tune the acoustic contrast between cell and liquid to be positive, negative or zero [4-6] and that the acoustic contrast of cells can be tuned to direct them to their iso-acoustic point in a smooth gradient. Together, these results introduce IAF as a new addition to the suite of microfluidic intrinsic separation techniques.

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