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The Application of Micropipette Aspiration in Molecular Mechanics of Single Cells

Micropipette aspiration is arguably the most classical technique in mechanical measurements and manipulations of single cells. Despite its simplicity, micropipette aspiration has been applied to a variety of experimental systems that span different length scales to study cell mechanics, nanoscale molecular mechanisms in single cells, bleb growth, and nucleus dynamics, to name a few. Enabled by micro/nanotechnology, several novel microfluidic devices have been developed recently with better accuracy, sensitivity, and throughput. Further technical advancements of microfluidics-based micropipette aspiration would have broad applications in both fundamental cell mechanics studies and for disease diagnostics. [DOI: 10.1115/1.4029936]

Introduction

The study of cell mechanics can be traced back to the late 17th century when Robert Hooke and Antony van Leeuwenhoek observed Brownian motions of tiny creatures in water under simple optical microscopes [1]. To date, it is well recognized that cells and tissues' response to mechanical stimuli and their mechanical properties do appear to change with the development of human diseases [2]. To give a few examples, shear stress exerted by blood flow on endothelial cells can be mechanotransduced into biochemical signals to trigger atherosclerosis [3,4]. Environmental factors like allergens, air pollutants, and viruses can induce airway epithelial cells to initiate biochemical responses from several relevant cell types through cell–cell communications to cause contraction in airway smooth muscle cells [5]. Under the influence of migratory and chemotactic stimuli, several regulatory proteins of the actin cytoskeleton network respond by assembling membrane protrusions, resulting in cancer cell migration to metastasize to distant organs through blood circulation [6,7].

The study of cell mechanics requires tools which can apply or measure small forces at a small scale, matching the cell size. A number of techniques exist to mechanically probe living cells, including atomic force microscopy, magnetic twisting cytometry, optical trapping, micropipette aspiration, shear flow, and stretchable substrates [8]. Scientists and biomedical Engineers began developing micropipette aspiration for cell mechanics studies since the 1970s. Due to its simplicity, micropipette aspiration is still a widely adopted technique to study cell mechanics. An extensive review article by Hochmuth published in 2000 on micropipette aspiration on living cells provided a general overview of using continuum liquid and solid models to measure material properties such as cortical tension, Young's modulus, and viscoelasticity of living cells [9]. In this current review, we will first briefly revisit the working principle and the infrastructure of micropipette aspiration systems. Then, we will focus mainly on primary research works highlighting some applications of micropipette aspiration in studying cellular mechanics at both nano- and microscales and the recent technology advancement of the integration of micropipette aspiration with microfluidics.

Working Principle of a Micropipette Aspiration System

The schematic of a traditional micropipette aspiration system is shown in Fig. 1. Micropipette aspiration measures the mechanical properties of single cells by the observation of cell deformation upon pressure suction. For a detailed description of the instrumentation of micropipette aspiration and procedures, refer to Refs. [10,11]. A conventional micropipette aspiration system generally consists of: (1) a set of micromanipulators which controls the positioning of a glass capillary micropipette, (2) a pressure generator by the hydrostatic pressure generated by the differential water level between two columns of reservoirs. The water level is regulated by a syringe pump and a valve, (3) a pressure gauge which measures the suction pressure generated on the micropipette, and (4) an optical microscope which observes cell deformation upon micropipette aspiration and the images are captured by a camera.

Manual operation is usually required in the operation of a traditional micropipette aspiration system, in which the micromanipulator positions a pulled glass micropipette in close proximity to a cell in the cell suspension chamber and a negative pressure is applied to aspirate the cell into the micropipette. Cell deformation is recorded through imaging on an optical microscope at varying known suction pressures. Based on this simple principle that the cell must be deformed in which a known force or stress and its deformation are measured, micropipette aspiration has been applied in numerous ways from examining passive material properties and mechanical responses in lipid vesicles and cells, to measuring molecular adhesion and active cell contractility against mechanical load.

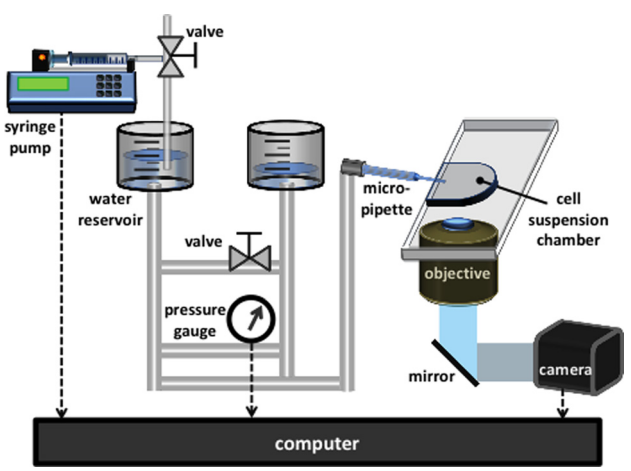

Fig. 1 Experimental setup of a traditional micropipette aspiration system

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Study of the Membrane Elasticity Using Lipid Vesicles

Numerous pioneering studies using micropipette aspiration were conducted using lipid bilayer vesicles. These cell-sized lipid vesicles can be prepared by rehydrating dried synthetic or natural lipids in aqueous buffer. At the low tension regime, lipid bilayer is assumed to be incompressible [12], thus the total membrane area is constant. However, there is a loss of configurational entropy for the lipid molecules by suppressing membrane undulation leading to an increase in an optically resolvable increase in the relative change of apparent membrane area α [13]. It has been shown that α relates to the $\ln(\tau_m)$ by the following expression:

$$\alpha \cong \frac{1}{8\pi\kappa_B\beta}\ln(\tau_m) \tag{1}$$

where κ_B is the bending rigidity of the lipid bilayer, τ_m is the lipid bilayer tension (more about this in the Study of the Material Properties of Single Cells or Nucleus section) and β is $1/k_BT$. Thus, the bending rigidity can be obtained by a linear fit of α versus $\ln(\tau_m)$ at the low pressure regime. For lipid bilayer vesicles, this usually takes on the value between 10 and 40 k_BT, depending on membrane composition.

At the high tension regime, the membrane is tense so the response is governed by membrane area dilation [14]

$$\alpha \cong \frac{(\tau_m)}{K_A} \tag{2}$$

where κ_A is the apparent area modulus.

Micropipette aspiration has been a principle tool for membrane biophysicists interested in measuring elastic properties of lipid bilayers [15]. To highlight a few such studies, application of micropipette aspiration to large unilamellar vesicles has provided detailed understanding of how membrane composition affects bilayer elasticity and rupture tension [16,17], as well as the phase behavior over different sterol concentrations [18]. It has also enabled the study of the effect of tension on channel-forming peptide such as gramicidin [19].

Study of the Material Properties of Single Cells or Nucleus

Perhaps the simplest model for analyzing micropipette aspiration result is based on the Young–Laplace equation. This model basically assumes a homogeneous elastic membrane holding a drop of homogeneous Newtonian liquid inside. Under micropipette aspiration and weak osmotic pressure difference, the cell deforms with a constant volume. When the cell is aspirated into the micropipette forming a perfect semi-hemisphere, in which the protrusion length is equal to the radius of the micropipette opening, the membrane tension of the cell can be calculated under the force balance with the liquid's internal pressure. This model is known to fit very well for soft cells which behave like a liquid such as a neutrophil [9,20]

$$\Delta p = 2T_m\left(\frac{1}{R_P}-\frac{1}{R_C}\right), \quad \left(\Delta p = \Delta p_c \quad \text{at} \quad \frac{L_P}{R_P}=1\right) \tag{3}$$

where T_m is the membrane tension (analogous to τ_m for the analysis of lipid bilayer vesicles), L_p is the protrusion length of the cell into the micropipette, R_c is the radius of the cell outside the pipette, and R_P is the radius of the mouth of the micropipette. When there is an increase in suction pressure such that the protrusion length is larger than the radius of the mouth of the micropipette ($L_P/R_P > 1$), a liquid droplet will flow into the pipette.

While the above treatment works well for a continuum model for a liquidlike neutrophil, the aspiration length increases linearly with the suction pressure for a viscoelastic solidlike chondrocyte so that the slope of the plot of aspiration pressure versus L_p/R_p is proportional to the elasticity of the cell [21]. An aspirated elastic cell can be modeled as an infinite, homogeneous half-space, and the Young's modulus E has been shown to have the following expression [22]:

$$E = \frac{\frac{3\Delta p\Phi}{2\pi}}{\left(\frac{L_p}{R_p}\right)} \tag{4}$$

where Φ is a constant which is determined by the geometry of the micropipette, which typically takes a value of 2.1, so the coefficient $3\Phi/2\pi$ is about one. An equivalent cortical tension can also be computed for a solidlike cell as follows:

$$(T_c)_{\text{equivalent}} \approx 2.2ER_P \tag{5}$$

Micropipette aspiration is regarded as a pioneering technique for cell mechanical measurement, and it also can be applied to the measurement of mechanical properties of cell nucleus [23]. Studies have been conducted ranging from determining the rheological properties and the influence of specific nuclear membrane proteins on mechanical properties in isolated nuclei to studying nuclear deformation during different stages of stem cell differentiation [24–26].

The use of micropipette aspiration under different mechanical models has enabled new knowledge in understanding the bulk mechanical behaviors during different biological processes. In addition, mechanical stimuli exerted on the cell can trigger a cascade of biochemical signaling with a time scale of seconds [27]. Contractile or stiffening response of cells occurs with a longer time scale of minutes [28], which can possibly affect the mechanical measurement of cell mechanical properties. It has been reported that breast cancer cells become more deformable upon successive extension and relaxation events due to the reorganization and alignment of cytoskeletal network [29]. Thus, researchers need to be cautious when interpreting micropipette aspiration results from single cells as it is difficult to deconvolve passive mechanical properties from active cellular responses.

Molecular Adhesion Measurement

Micropipette aspiration can be also be used for measuring interbilayer interactions. The most prevalent application is for measuring receptor–ligand interaction or receptor–receptor interaction. Adhesion between individual cells and cells with their environment is critical for their proper functioning. An elastic cell, such as a red blood cell (RBC), can act as a sensitive biomembrane force probe (BFP) that serves as a transducer for reporting the force history applied to a bond. The measurement would require the use of two micropipettes, the first aspirate on a cell expressing a receptor of interest and is allowed to bind to another cell held on an opposing micropipette expressing another receptor of interest. This has been applied for measuring the kinetic rate constants in cell adhesion between Fc receptors and IgG by probing single receptor–ligand bond (Fig. 2(*a*)) [30]. Using the same concept, ligands and soluble portion of its cognate receptor can be immobilized onto glass microspheres and computer-driven linear-piezo translator holding the ligand-attached target glass microsphere can move with respect to the fixed BFP attached to the second receptor-attached target microsphere. This approach has allowed the discovery of a mechanism for the "catch bond" response of the leukocyte adhesion bonds (Fig. 2(*b*)) [31]. Using a similar experimental design involving two different cells, cell binding kinetics for the adhesion molecule cadherin has been quantified by measuring intercellular binding probability versus contact time between a Chinese hamster ovary (CHO) cell expressing cadherin aspirated in one micropipette and a RBC functionalized with cadherin aspirated in another micropipette (Fig. 2(*c*)) [32]. These studies demonstrated more advanced and versatile uses of micropipette aspiration for nanomechanics of biological molecules.

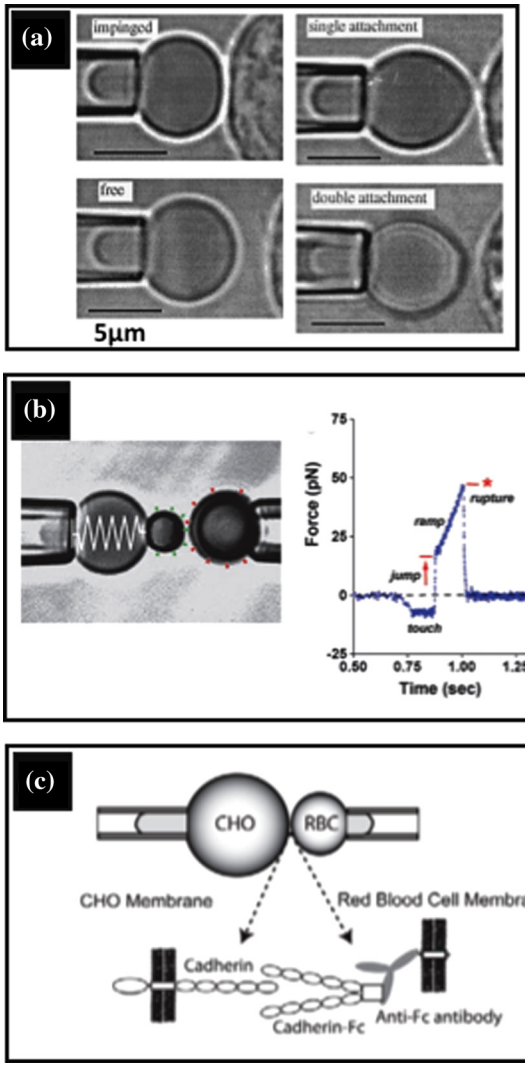

Fig. 2 Study of cell–cell interaction using micropipette aspiration for cell manipulations. (*a*) Measure of cadherin binding force between CHO cells and RBCs (Reproduced with permission from Elsevier–Cell Press [30]), (*b*) measurement of the bond strength of a single receptor–ligand bond pair (Reproduced with permission from PNAS [31]), and (*c*) trace of the adhesion force of P-selectin glycoprotein ligand 1 in leukocyte (Reproduced with permission from *Journal of Cell Science*–The Company of Biologists [32]).

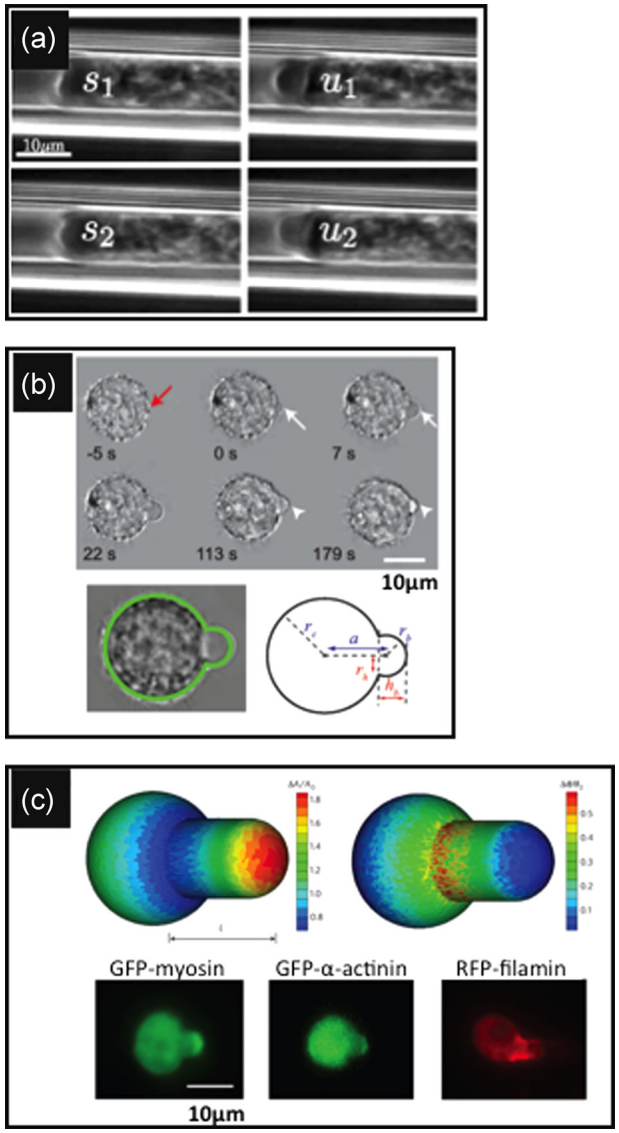

Fig. 3 Molecular mechanics of single cell by micropipette aspiration. (*a*) Bleb-based cell motility (Reproduced with permission from PNAS [34]), (*b*) organization of cytoskeletal proteins (Reproduced with permission from PNAS [33]), and (*c*) measurement of cortical tension on cell blebbing induced by laser ablations (Reproduced with permission from *Nature Materials*–Nature Publishing Group [38]).

Study of the Molecular Mechanism in Single Cells

Our discussion in an earlier section presented the use of micropipette aspiration for quantifying mechanical behaviors of cells that could be treated as a more liquidlike or a more solidlike material. The complex mechanical properties of the cells can be attributed to the biophysical properties of cytoskeletal assemblies. One of the dominant features of cytoskeletal assemblies related to micropipette aspiration experiments is the actin cortex. Actin cortex, also known as actomyosin cortex, is a thin meshwork of actin filaments, myosin motor proteins and associated protein beneath the plasma membrane and provides mechanical strength and support to the plasma membrane. The tension measured by micropipette aspiration encompasses the cortical tension from the actin cortex, due to its direct and indirect connection to the plasma membrane [33]. Over the past few years, micropipette aspiration has enabled mechanistic studies into the role of cortical actin networks in regulating mechanical behaviors of cells.

Contraction of actomyosin cortex could lead to cortex unbinding from the plasma membrane resulting in spherical membrane protrusions known as blebs. Using micropipette aspiration, Brugués et al. studied the dynamics between the cytoskeletal cortex and the plasma membrane under the interaction of myosin-generated contractile stress on bleb growth and showed that cortex dynamics can generate spontaneous oscillations of cell forward movement and cell retraction (Fig. 3(*a*)) [34]. Micropipette aspiration has also revealed the existence of a critical tension below which bleb cannot expand (Fig. 3(*b*)) [33]. Given the renewed interest of a cell migration mechanism without actin–myosin based cell motility, these studies have general implication for basic cell biology.

By studying the time-dependent response of the cortical tension of neutrophils during phagocytosis, it has been shown that neutrophils have the ability to modulate their cortical tension by enabling or resisting further extension of their surface areas [35]. Another example is from a recent finding that cortical tension decreases during meiosis that depends on the assembly of cortical actin network [36].

In addition, micropipette aspiration experiments have also enabled some molecular understanding of cortex mechanics.

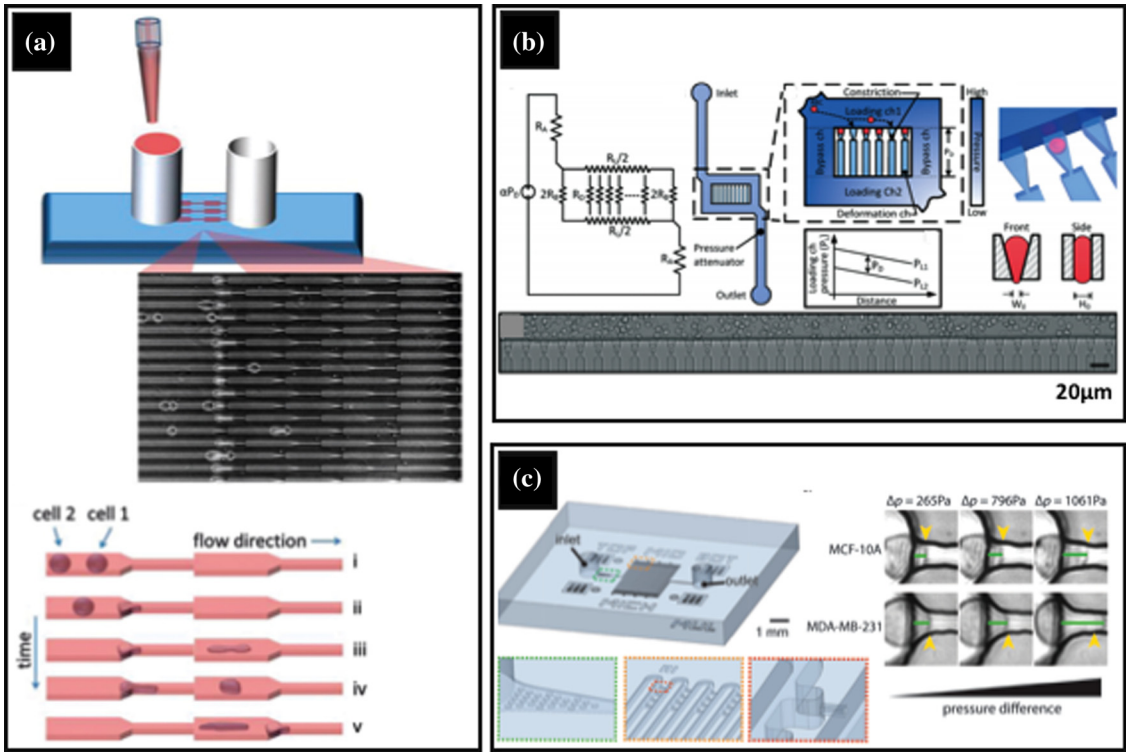

Fig. 4 Micropipette aspiration enabled by microfluidics (*a*) a serial micropipette array to study breast cancer viscoelastic deformation (Reproduced with permission from *Integrative Biology*–RSC Society [43]), (*b*) a microfluidic plunger device to study the deformability of RBCs (Reproduced with permission from *Lab on a Chip*–RSC Society [45]), and (*c*) a μFPA to measure mechanical properties of cancer cells (Reproduced with permission from *Lab on a Chip*–RSC Society [46])

Using the amoeba *Dictyostelium discoideum* as a model system, it has been shown that mutants lacking myosin II could not retract during cell aspiration, providing some early evidence of the role myosin II plays in cortex mechanics [37]. More recently, Luo et al. examined the molecular mechanism for cellular mechanosensing in *D. discoideum* and discovered that forces exerted by micropipette aspiration are shared between myosin II and other actin crosslinker proteins (e.g., α-actinin and filamin) that have distinct responses to dilation and shear (Fig. 3(*c*)) [38]. These studies demonstrate the utility of micropipette aspiration for more detailed biophysical and molecular understanding of the origin of cortical mechanosensing.

Integration of Micropipette Aspiration With Microfluidics Technology

First emerged in the 1970s, the working principle of micropipette aspiration has not changed for almost 40 years where pressure difference across a small orifice is used to apply suction pressure to deform single cells. Up to now, all the pioneering work and associated applications have been conducted on standard micropipette aspiration setups. Recently, there has been more effort on working toward the automation of micropipette aspiration systems. Vision-based robotic system was built to control the micromanipulator and pressure difference exerted by the water tank [39]. Despite the possibility of automation, there are drawbacks for a wide adaptation of micropipette aspiration systems for cell biological studies. First, one of the key components in a traditional micropipette aspiration system is the glass capillary micropipette. The size and the parallel geometry of the mouth of glass capillary micropipette are critical for both the accuracy and sensitivity of the cell mechanical measurement. However, the manufacture of glass micropipette is not trivial and requires expertise. Although commercially available micropipettes for aspiration studies exist, several custom made parts are required in the setup of a micropipette aspiration system. A second limitation of a micropipette aspiration experiment is that only a single cell can be aspirated at a time. Typically, it takes about 10 mins to complete one measurement. This greatly limits the throughput of micropipette aspiration experiments. Physiological deterioration of cells and environmental fluctuations can also affect measurement accuracy.

Motivated by the idea that cell mechanical properties represent a label free biophysical marker, which can potentially be used in clinical scenarios as a rapid diagnostics, several groups have combined microfluidics with the principal concepts of micropipette aspiration. The impetus for utilizing microfluidics is that it can parallelize the measurements thereby increasing throughput. Over the past decade, microfluidics has shown considerable promise in biology research that can potentially augment or replace traditional experimental approaches [40]. Replacing glass "micropipettes" with microfluidic channels made from the elastomer polydimethylsiloxane (PDMS) has an inherent advantage in that there is a significantly greater experimental flexibility in the design of devices based on the evolution of the concept behind micropipette aspiration.

A simple principle that has been used early in the implementation of microfluidics with cell mechanics measurement draws on the fact that stiff sickle cells would clog and lyse in constricted microfluidic channels by mechanical stress. Shelby et al. constructed microfluidic channels with different constricted channel widths and evaluated the deformability of *Plasmodium falciparum*-infected RBCs at different stages [41]. Similarly, using a network of bifurcating microfluidic channels, the transit time across the microfluidic channels was used as a quantitative metric to measure cell stiffness of neutrophils with hemotalogic diseases [42]. Taking this idea further, a microfluidic device with multiple channels each with a serial micropipette with constrictions was used to subject cells to repeated deformation (Fig. 4(*a*)) [43,44]. These studies would be practically impossible with a standard

micropipette aspiration setup, in which a single glass capillary pipette is used.

The parallel nature of microfluidic devices for aspirationlike setup was also recently demonstrated by Myrand-Lapierre et al. [45]. In this work, there is a parallel array of deformation microchannels with a funnel constriction such that when a cell is trapped, the applied pressure is focused across the cell (Fig. 4(*b*)). The multiplex fluidic plunger (MFP) used a sawtooth pressure waveform to squeeze each cell through constriction channels. By analyzing these cell deformation events, the threshold deformation pressure can be quantified. Truly inspired by conventional micropipette aspiration and to take advantage of the unique features afforded by microfluidics, we have developed microfluidic micropipette aspiration for single cells [46]. The novel microfluidic pipette array (μFPA) device consists of parallel micropipette walls that enable high throughput aspiration of single cells (Fig. 4(*c*)) [46]. μFPA is a multilayer PDMS device that comprises of an array of trapping structures, in which each trapping array contains a micropipette that aspirates on a single cell. The device is capable to autonomously trap cells and the pressure exerted on the trapped cells depends only on the volume flow rate, which is controlled by a single syringe pump. Coupled with semi-automated image analysis and using similar mechanical analysis described in an earlier section, we can determine cortical tension and Young's modulus of single cells at a much higher throughput than traditional micropipette aspiration. Such development would enable mechanical measurement of a heterogeneous population of cells and could potentially simplify the use of micropipette aspiration to needing only a syringe pump.

The implementation of micropipette is not restricted to just applying pressure to cells against solid wall constrictions. The force to aspirate on single cells can come from hydrodynamic forces. Dudani et al. implemented a pinched-flow mechanism to hydrodynamically stretch single cells with exceptional high throughput [47]. In this work, hydrodynamic compression force generated by cross-flows from branched side channels impinges on cells flowing along the main microfluidic channel and deforms the cell. Another work from the same group measured cell deformability of malignant cells in pleural effusions using a similar concept of deformability cytometry [48]. Hydrodynamic micropipette currently has the highest throughput for quantifying cell deformability and foreseeably can be applied in clinical diagnostics through large scale cell mechanophenotyping.

Concluding Remarks and Future Perspectives

Despite its simplicity, micropipette aspiration has been applied to numerous applications in nano and cell mechanics. An intrinsic drawback of traditional micropipette aspiration system is the low throughput of experimental measurement that requires skilled operation of custom instrumentation. In recent years, several emerging techniques based on microfluidics with their fundamental concepts rooted in micropipette aspiration provide a versatile and simple platform to measure mechanical information of single cells. Given the growing interest of mechanobiology and the use of mechanical information as a label-free biomarker, such development will likely find applications in clinical diagnostics and medicine. While the current implementations of microfluidic micropipette aspiration have focused on single cell measurements, it would be potentially interesting to implement the idea of biomolecular force probe in microfluidics devices. Such a development would enable the parallelization of single molecule nanomechanics studies. The ability to perform micropipette aspiration in parallel in microfluidic devices can greatly facilitate faster data collection to provide statistically meaningful data and push the envelope of micropipette aspiration utility.

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