Microfluidic high-throughput single-cell mechanotyping: Devices and applications

Cite as: Nanotechnol. Precis. Eng. 4, 045002 (2021); https://doi.org/10.1063/10.0006042
Submitted: 26 April 2021 . Accepted: 24 June 2021 . Published Online: 09 September 2021

Gihoon Choi, Zifan Tang, and Weihua Guan

CALL FOR PAPERS
SPECIAL ISSUE:
Flexible MEMS Sensors and Systems
Microfluidic high-throughput single-cell mechanotyping: Devices and applications

Cite as: Nano. Prec. Eng. 4, 045002 (2021); doi: 10.1063/10.0006042
Submitted: 26 April 2021 • Accepted: 24 June 2021 •
Published Online: 9 September 2021

Gihoon Choi,1,a) Zifan Tang,1 and Weihua Guan1,2,b)

AFFILIATIONS
1 Department of Electrical Engineering, Pennsylvania State University, University Park, Pennsylvania 16802, USA
2 Department of Biomedical Engineering, Pennsylvania State University, University Park, Pennsylvania 16802, USA

a) Present address: Department of Biotechnology and Bioengineering, Sandia National Laboratories, Livermore, California 94550, USA.
b) Author to whom correspondence should be addressed: w.guan@psu.edu

ABSTRACT
The mechanical behavior of individual cells plays an important role in regulating various biological activities at the molecular and cellular levels. It can serve as a promising label-free marker of cells’ physiological states. In the past two decades, several techniques have been developed for understanding correlations between cellular mechanical changes and human diseases. However, numerous technical challenges remain with regard to realizing high-throughput, robust, and easy-to-perform measurements of single-cell mechanical properties. In this paper, we review the emerging tools for single-cell mechanical characterization that are provided by microfluidic technology. Different techniques are benchmarked by considering their advantages and limitations. Finally, the potential applications of microfluidic techniques based on cellular mechanical properties are discussed.
© 2021 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). https://doi.org/10.1063/10.0006042

KEYWORDS
Microfluidic, Single-cell, Cell deformability, Mechanotyping

I. INTRODUCTION
As the basic building blocks for living organisms, cells can effectively adapt to their microenvironment and respond accordingly by altering their biological, chemical, and physical properties. Among these, the mechanical properties of the cell are determined mostly by cellular shells (e.g., plasma membrane), integral structures of the cytoskeleton (e.g., intermediate filaments and microtubules), and the nucleus. To date, various diseases and biological processes have been associated with alterations in cellular mechanical properties. Cellular mechanical properties have been used as potential markers for identifying pathological states. Examples include the decreased red blood cell (RBC) deformability in malaria and in sickle cell anemia, the stiffening of white blood cells (WBCs) in sepsis, trauma, and acute respiratory distress syndrome, the increased cell deformability of invasive cancer cells, and the variation in deformability during stem cell differentiation. To fully exploit the mechanical profiling of cells, detailed measurements with many cells are desirable for statistically significant analysis of cell subpopulations. Current methods for mechanical phenotyping, such as atomic force microscopy (AFM), micropipette aspiration (MA), optical stretching, and acoustic actuation, provide detailed and accurate cell modulus measurements of a small subset of an entire cell population. However, owing to the slow detection speeds, the analyzed sample size is typically limited to less than 100 cells/day. By contrast, fluorescence-activated cell sorting (FACS), a commonly used technique for cell characterization, operates at a throughput of up to 10⁴ cells/s and allows real-time measurement of the cells for sorting purposes.

In this work, recent microfluidic high-throughput techniques for single-cell mechanotyping are reviewed and summarized. Single-cell mechanotyping involves three basic function modules: pressurization (actuation), examination (sensing), and sorting/separation (actuation). We summarize the state-of-the-art microfluidic
techniques that are used in these areas to facilitate high-throughput single-cell mechanical studies. We benchmark these techniques based on their working mechanisms and discuss their advantages as well as ways in which they can be improved. We then summarize various applications based on microfluidic cell mechanotyping, ranging from cell separation to disease diagnosis to drug discovery. Finally, we present perspectives on the opportunities and challenges for further developing and applying microfluidic-based cell mechanotyping.

II. PRESSURIZATION

A. Physical constriction

One of the cell-stretching strategies adopted in microfluidic devices is to flow the cells into a geometric constriction where the channel walls squeeze a single cell. In this method, the level of cell deformation is mainly determined by the channel geometry, such as its width, although the driving force and shape of the constriction edges are also contributing factors. Therefore, the channel geometry needs to be well matched with the cell diameter. Typically, the channel width is designed slightly smaller than the cell diameter to ensure cell deformation. However, the predefined channel geometry often limits the analysis of samples that are polydisperse in size (e.g., whole blood), since the channel geometry is not adjustable.

During the past decade, constriction-based microfluidic technology has enabled high-throughput and precise quantification of individual cell deformability (Fig. 1). Quantification of mechanical properties is achieved by measuring the time-dependent readouts (i.e., transit velocity and transit time), which are often extracted from conductance changes [Figs. 1(a) and 1(b)], frame streams of optical imaging [Fig. 1(c)], and pressure drops across the constriction [Fig. 1(d)]. The best reported measurement throughput so far is ~500 cells/s by electrical measurement. Such a measurement rate is significantly higher than that achievable by conventional deformability characterization methods, such as atomic force microscopy (AFM), micropipette aspiration, and optical tweezers. Owing to their high throughput, constriction-based methods are particularly useful for deformability measurements of biological samples (e.g., RBCs, leukocytes, cancer cells, and stem cells), where a large number of samples need to be characterized to construct reliable statistics. The specific examination techniques (high-speed imaging, electrical measurement, and buoyant mass sensing within a resonant cantilever) to indirectly measure time-dependent readouts will be discussed in Sec. III.

![Figure 1](image-url).

**FIG. 1.** Physical constriction microfluidic device with various geometries and readouts for cell deformability measurement. (a) Conductance changes are monitored as the cell squeezes through the constriction pore. The width of the signal peak indicates the transit time. (b) Two consecutive constrictions with different widths are used for cell size and deformability measurements. The transit time is measured electrically. (c) Size-independent deformability cytometry using real-time adjustable constriction. The system can control the constriction height for samples that are polydisperse in size. The cell transit velocity (or time) information is extracted from the time-dependent cell position in the microfluidic channel. (d) Microfluidic constriction-based pressure manometer. The change in y position directly indicates the pressure drop across the constriction during the cell translocation event.
The time-dependent readouts allow estimation of the elastic modulus or stiffness of cells by the following power-law rheological relation:

\[ t = \left( \frac{\varepsilon_{\text{max}} E}{\Delta P} \right)^{1/\beta} \]

where \( t \), \( \varepsilon \), \( E \), \( P \), and \( \beta \) are time, strain, elastic modulus, pressure drop across the constriction, and power-law exponent, respectively. A higher \( \beta \) value implies more viscous behavior, while a lower \( \beta \) corresponds to more elastic behavior (typically, 0.1–0.5 for living cells). For constriction methods, channel width \( w \) and height \( h \) are important design considerations determining the cell strain \( \varepsilon \). The following equation describes the maximum strain when the cell squeezes through the constriction (\( R_{\text{cell}} \) denotes the cell radius before deformation):

\[ \varepsilon_{\text{max}} = \frac{R_{\text{cell}} - R_{\text{eff}}}{R_{\text{cell}}}, R_{\text{eff}} = \sqrt{\frac{w h}{\pi}}. \]

Typically, \( R_{\text{eff}} \) is designed to be smaller than the \( R_{\text{cell}} \) to ensure cell deformation at constrictions. Although several works have suggested that \( R_{\text{eff}} \) should be half of \( R_{\text{cell}} \), the optimal channel width and height are often determined empirically, since various factors can affect the measurement, such as driving pressure, fluidic channel wettability, and friction.

Constriction-type methods can be categorized as single-channel or multichannel. The single-channel constriction method often faces challenges in distinguishing deformable cells when the cell transit time distributions closely overlap with each other. The transit time difference between cells can be amplified to achieve more sensitive differentiation by using a more extended constriction channel. However, a more prolonged constriction is susceptible to irreversible channel blockage. As a compromise, a multi-constriction design can be considered. The idea is that each constriction channel length is short enough to avoid clogging, while a series of multiple constriction structures amplify the transit time differences [Fig. 2(a)]. A sequential constriction array can also be used in a parallel scheme to increase both transit time difference and measurement throughput [Fig. 2(b)].

Similarly, the use of single constrictions in parallel has been reported to achieve high-throughput measurements at the single-cell level [Fig. 2(c)]. With all parallel constriction methods, bypass channels are commonly used to provide a constant pressure drop across the constriction arrays and prevent clogging. Differential micro-constriction arrays have been used to investigate the cell deformation and relaxation process based on electrical impedance measurements [Fig. 2(d)]. Successive constrictions provide sensitive transit time information \( (T_1 + T_2 + T_3 + T_4) \) as well as the relaxation index \( (T_4/T_1) \) of single cells at a measurement rate higher than 430 cell/min.

Although the physical constriction method is an excellent technique for high-throughput and sensitive measurements of cell deformability, there is a fundamental limitation to be addressed. As the cell walls are in contact with the channel walls, time-dependent transit velocity (or time)-based deformability measurement is convolved with cell size and surface properties of cells and channel walls. To some extent, it is possible to decouple the cell size, using adjustable constriction pores and a physical constriction integrated with a microfluidic Coulter counter (particle size analyzer). However, the approach to precisely decouple quantitative mechanical properties from convolved factors still need room for improvement.

**FIG. 2.** Various multi-constriction methods. (a) Deformability-based flow cytometry using serial constrictions. (b) Microfluidic cell-phoresis device using sequential constrictions in parallel. (c) Quantitative deformability cytometry using a parallel constriction array for rapid and calibrated measurement. (d) Parallelized differential multi-constrictions in series for cell deformation and relaxation measurements.
B. Hydrodynamic stretching

Hydrodynamic approaches measure the mechanical properties of cells by using intrinsic fluid-dynamical stresses that can be tuned by the geometry of the microfluidic channels. While adhesive properties of cells affect deformability measurements in the physical constriction method, contact-free hydrodynamic stretching can decouple this factor and provide direct evidence of cell deformability. Besides, heterogeneous cell sizes can be independently measured and considered in mechanical models to avoid misinterpretation of mechanical properties. Although additional upstream cell focusing (e.g., inertial focusing or viscoelastic focusing) is required for the uniform stress field, the hydrodynamic approach can deform the cell at high rates with sufficient strain (40%–50%).

The hydrodynamic stress exerted on cells in a microfluidic channel can be categorized into shear and compressive forces depending on the dominant flow regime. For example, compressive force is dominant for cell deformation in the inertial flow regime (Reynolds number $Re \gg 1$), while shear force is dominant in the shear regime ($Re \ll 1$). The following equations describe the compressive and shear forces:

$$F_{\text{compress}} \approx \frac{1}{2} \rho U^2 C_D A,$$

$$F_{\text{shear}} \approx 2\pi U \mu r,$$

where the fluid velocity $U$, fluid density $\rho$, viscosity of suspension medium $\mu$, channel cross-sectional area $A$, cell radius $r$, and drag coefficient $C_D$ are experimental or design parameters that can be adjusted to ensure sufficient cell compression. In addition, the work of Armistead et al. showed that the dominant force should be carefully chosen based on each application, since the deformability response showed different sensitivities to forces of the same magnitude but different types. For example, in the shear-dominated regime, there was greater sensitivity to deformations of the cell membrane and the cytoskeleton, while the inertial regime worked better for deformations of the cytosol and nucleus.

In 2012, Di Carlo’s group reported a hydrodynamic-stretching microfluidic device for identifying malignant cells in human pleural fluid samples with a measurement speed of 2000 cell/s. Cells were focused on a narrow streamline near the center of a microfluidic channel and delivered to a cross-junction at a high flow rate, where they underwent mechanical stretching by perpendicular crossflows [Fig. 3(a)]. Cell deformations were captured using a high-speed camera, and images were analyzed offline to extract the cell volume and deformation index (DI). The throughput of the system was limited by accumulation of cells at the stagnation point in the cross-junction. Later work rectified this problem by a unique combination of a self-sheathing flow and a pinched flow, resulting in a significant enhancement of throughput (up to 65,000 cells/s) [Fig. 3(b)]. Further advances were achieved by Otto et al., who developed a real-time deformability cytometry (RT-DC) technique in which cells flowed through a funnel-shaped microfluidic channel and were deformed by hydrodynamic stress and a pressure gradient [Fig. 3(c)]. Since the funnel-shaped channel was wider than the cell diameter, the cells were deformed without physical contact. The deformability was measured by monitoring cell circularity changes in real time, with analysis rates greater than 100 cells/s being achieved. Beyond characterization of deformability, real-time systems have excellent potential for label-free single-cell-level cell sorting based on mechanical properties. Besides, the simple funnel-shaped channel structure is easy to scale up for multiplexing. The parallelism of the flow-induced cell deformation was demonstrated by Ahmmed et al. in multi-sample deformability cytometry. Their array device consisted of 10 funnel-shaped
TABLE I. Summary of various microfluidic techniques to compress cells for deformability characterization. Ellipses (...) indicate undeclared in the cited paper.

<table>
<thead>
<tr>
<th>Channel contact</th>
<th>Critical channel</th>
<th>Channel width × height (μm × μm)</th>
<th>Cell driving</th>
<th>Pressure (kPa)</th>
<th>Cell lines</th>
<th>Deformability measure</th>
<th>Deformation timescale (ms)</th>
<th>Throughput (cells/min)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact</td>
<td>8 parallel constrictions</td>
<td>5 × 9</td>
<td>Pressure</td>
<td>0.1–0.8</td>
<td>Leukemia, breast carcinoma</td>
<td>Transit time</td>
<td>5–10</td>
<td>180</td>
<td>27</td>
</tr>
<tr>
<td>Contact</td>
<td>Single constriction</td>
<td>3 × 5</td>
<td>Pressure</td>
<td>3</td>
<td>RBC</td>
<td>Transit time</td>
<td>1–3</td>
<td>9 × 10³</td>
<td>28</td>
</tr>
<tr>
<td>Contact</td>
<td>2 consecutive constrictions</td>
<td>8 × 8 (size) 5 × 5 (stiffness)</td>
<td>Syringe, Electrophoresis</td>
<td>0.16</td>
<td>HeLa, Jurkat</td>
<td>Transit time</td>
<td>8–23</td>
<td>6 × 10³</td>
<td>30</td>
</tr>
<tr>
<td>Contact</td>
<td>Single constriction</td>
<td>50 × (30 - α) (Adjustable)</td>
<td>Pressure</td>
<td>⋅ ⋅ ⋅</td>
<td>RBC</td>
<td>Transit time</td>
<td>500</td>
<td>⋅ ⋅ ⋅</td>
<td>31</td>
</tr>
<tr>
<td>Contact</td>
<td>Single constriction</td>
<td>5 × 5</td>
<td>Pressure</td>
<td>34.5</td>
<td>RBC and WBC</td>
<td>Pressure change</td>
<td>50</td>
<td>⋅ ⋅ ⋅</td>
<td>32</td>
</tr>
<tr>
<td>Contact</td>
<td>Single constriction</td>
<td>5 × 5</td>
<td>Pressure</td>
<td>6.9</td>
<td>Malaria iRBC</td>
<td>Transit time</td>
<td>70–150</td>
<td>3 × 10⁴</td>
<td>7</td>
</tr>
<tr>
<td>Contact</td>
<td>Constriction pillars</td>
<td>3 × 4</td>
<td>Pressure</td>
<td>⋅ ⋅ ⋅</td>
<td>Malaria iRBC</td>
<td>Transit velocity</td>
<td>⋅ ⋅ ⋅</td>
<td>120</td>
<td>35</td>
</tr>
<tr>
<td>Contact</td>
<td>8 parallel array (100 constrictions/array)</td>
<td>2 × 4</td>
<td>Pressure</td>
<td>0.015</td>
<td>Malaria iRBC</td>
<td>Spatial distribution</td>
<td>⋅ ⋅ ⋅</td>
<td>⋅ ⋅ ⋅</td>
<td>37</td>
</tr>
<tr>
<td>Contact</td>
<td>8 parallel array (100 constrictions/array)</td>
<td>2 × 4</td>
<td>Pressure</td>
<td>0.015</td>
<td>Malaria iRBC</td>
<td>Spatial distribution</td>
<td>⋅ ⋅ ⋅</td>
<td>⋅ ⋅ ⋅</td>
<td>37</td>
</tr>
<tr>
<td>Contact</td>
<td>16 parallel arrays (6 constrictions/array)</td>
<td>5 × 10 (HL60) 9 × 10 (MCF7, MDA-MB-231)</td>
<td>Pressure</td>
<td>28</td>
<td>HL60, MCF7, MDA-MB-231</td>
<td>Transit time</td>
<td>7.5–200</td>
<td>1 × 10³</td>
<td>38</td>
</tr>
<tr>
<td>Contact</td>
<td>64 parallel capillary-like microchannel</td>
<td>6 × 13</td>
<td>Pressure</td>
<td>0.01–1</td>
<td>RBC, HL60</td>
<td>Transit time</td>
<td>200–600</td>
<td>120</td>
<td>39</td>
</tr>
<tr>
<td>Contact</td>
<td>2 parallel arrays (6 constrictions/array)</td>
<td>10 × 20</td>
<td>Pressure</td>
<td>50</td>
<td>MCF7</td>
<td>Transit time</td>
<td>5–10</td>
<td>430</td>
<td>40</td>
</tr>
<tr>
<td>Contact</td>
<td>16 parallel array</td>
<td>5 × 5</td>
<td>Pressure</td>
<td>28</td>
<td>HL60</td>
<td>Transit time</td>
<td>10–100</td>
<td>100</td>
<td>34</td>
</tr>
<tr>
<td>Contact-free</td>
<td>Cross-junction</td>
<td>60 × 30</td>
<td>Syringe pump</td>
<td>⋅ ⋅ ⋅</td>
<td>MCF7</td>
<td>Aspect ratio</td>
<td>0.01</td>
<td>1.2 × 10⁵</td>
<td>41</td>
</tr>
<tr>
<td>Contact-free</td>
<td>Cross-junction</td>
<td>100 × 30</td>
<td>Syringe pump</td>
<td>⋅ ⋅ ⋅</td>
<td>HeLa, Jurkat, MCF7</td>
<td>Aspect ratio</td>
<td>0.01</td>
<td>3.9 × 10⁶</td>
<td>42</td>
</tr>
<tr>
<td>Contact-free</td>
<td>Funnel-shaped channel</td>
<td>20 × 20</td>
<td>Syringe pump</td>
<td>⋅ ⋅ ⋅</td>
<td>HL60, HSC, Whole blood</td>
<td>Circularity</td>
<td>1</td>
<td>6 × 10⁴</td>
<td>43</td>
</tr>
<tr>
<td>Contact-free</td>
<td>Funnel-shaped linear channel array</td>
<td>18 × 20</td>
<td>Pressure</td>
<td>15</td>
<td>MCF10A &amp; 7, PC3, HMS50, MDA-MB-231 &amp; 468, HCC1419</td>
<td>Circularity</td>
<td>2</td>
<td>6 × 10⁴</td>
<td>44</td>
</tr>
<tr>
<td>Optical</td>
<td>Linear optical trap</td>
<td>1000 × 10</td>
<td>⋅ ⋅ ⋅</td>
<td>⋅ ⋅ ⋅</td>
<td>Malaria iRBC</td>
<td>Aspect ratio</td>
<td>20</td>
<td>1.2 × 10³</td>
<td>48</td>
</tr>
<tr>
<td>Optical</td>
<td>Linear optical trap</td>
<td>20 × 200</td>
<td>⋅ ⋅ ⋅</td>
<td>⋅ ⋅ ⋅</td>
<td>RBC and WBC</td>
<td>Aspect ratio</td>
<td>25</td>
<td>6 × 10³</td>
<td>52</td>
</tr>
</tbody>
</table>
fluidic channels that simultaneously deformed cells at a rate of 100 cells/s per channel, allowing analysis of multiple samples with high throughput [Fig. 3(d)].

The abovementioned hydrodynamic approaches have their limitations, however. In the cross-junction method [Figs. 3(a) and 3(b)], uneven cell deformation may occur owing to flow instability and intrinsic asymmetry of the hydraulic resistance displacing the cells from the stagnation point. A key challenge for the funnel-shaped microchannel approach [Fig. 3(c)] is nonuniformity in the shear stress and hydrodynamic pressure within the microchannel, causing irregular cell deformation forces. To address this problem, the cells must be well aligned in the center of the channel using burdensome sheath fluids or various hydrodynamic focusing techniques. Finally, the cell size and shape affect the stress field in the fluid that acts on the cell to deform it. Therefore, novel approaches to decouple these interfering factors are needed for mechanical property measurements.

C. Optical stretching

Another approach to deform cells is through optical forces. In the past, various optically based noncontact cell manipulation methods were reported, including optical tweezers and traps. However, the small magnitude of optically induced forces (less than a couple of hundred piconewtons) is insufficient for stiff cell deformation, which often limits their application to mechanotyping. To address this problem, optical stretchers were developed. The first microfluidic optical stretcher was developed by Guck et al. to study the deformability of circulating cells (e.g., RBCs and human epithelial breast cancer cells). The stretching mechanism was based on the nanonewton-range light-induced surface force exerted on a cell by two nonfocused counterpropagating Gaussian laser beams. While low intensities of the laser beams were used to localize the cells, higher intensities (a few milliwatts) were used to stretch the cells along the axis of the beams. The stretching forces were generated by the momentum transfer that occurred at the interface between the cell and the surrounding medium owing to a refractive index change. As a result, there was sufficient surface force to stretch the localized cell.

The throughput of optical stretchers has gradually been improved. In further work by Guck et al., an optical stretcher was integrated with a microfluidic system that continuously placed cells in the trapping and stretching zone. With automated flow control, a deformability measurement rate of 1 cell/min was achieved. In this system, the rate-limiting factor was determined mainly by the imaging time for observing the small creeping cellular deformation. In 2013, Sawetzki and colleagues characterized the viscoelastic properties of healthy and malaria-infected RBCs (iRBCs) at a measurement rate higher than 20 cells/s using a high-frequency modulated deformation force.

Although the throughput of optical stretchers is higher than that of conventional AFM, micropipette aspiration (0.1 cell/min) is still not comparable to that of the microfluidic-based deformability measurement methods such as physical constriction and hydrodynamic stretching mentioned above. However, the working principle of optical stretchers is independent of flow characteristics, and it thus allows characterization of time-dependent mechanical properties of cells, such as stress relaxation and creep indentation. To avoid radiation damage to cells, the laser intensities and wavelength need to be carefully selected.

Various microfluidic techniques to compress cells for deformability characterization are summarized in Table I.

III. EXAMINATION

A. Imaging (endpoint analysis)

Perhaps the most intuitive way to examine cell deformability is by measuring the changes in cellular shape corresponding to an applied force on cells [Fig. 4(a)]. The cellular deformation is quantified by measuring the DI or stretch ratio (e.g., cell circularity and aspect ratio) using high-resolution imaging. One of the key benefits of the imaging method is the direct observation of cellular motion, providing information about cell deformability and the dynamic behavior of the cell. For example, using a high-speed imaging camera, Forsyth et al. observed three different types of RBC deformation dynamic motion in a microfluidic funnel-shaped capillary at different shear rates: stretching, tumbling, and recoiling. For characterization of the viscoelastic properties of RBC membranes, Tomaiuolo et al. measured the circularity change induced by the converging/diverging flow in multichannel microfluidic devices.

Another imaging-based characterization was achieved by measuring the quantitative parameters (e.g., transit time, transit velocity, and entry time) as indirect indicators of cell deformability as the cell squeezed through a physical constriction [Fig. 4(b)]. In this case, the time-dependent cell position and its corresponding timestamp were monitored using a high-resolution camera. The less-deformable cells spent more time squeezing through the microconstriction than the more deformable ones. For instance, Hou et al. investigated the bioreheological behavior of breast cancer cells in a microscale constriction. In their study, nonmalignant and malignant cells were distinguished by quantitative measurements of entry time, transit velocity, and elongation index from video images.

Several concerns have been reported with regard to imaging methods: (i) high-resolution image-recording setups are expensive, (ii) well-controlled hydrodynamic cell manipulation (e.g., focusing and spacing) is required to locate a cell at the focal spot, and (iii) post-image analysis is a time-consuming process and requires massive image data storage and computational power, limiting the actual throughput (i.e., from sample loading to completion of deformability analysis). For real-world applications, the development of deformability flow cytometry has been changed from endpoint to real-time analysis.

B. Electrical measurements

In constriction-based deformability characterization (Sec. II A), indirect quantitative parameters for cell deformability are often measured by electrical readouts. The transient changes in electrical current are induced by channel blockage during cell translocation, since current disruption occurs owing to the reduced conduction at the physical constriction.

Among various deformability characterization methods, electrical measurements offer the benefits of (i) high throughput,
(ii) simplicity, (iii) automation, and (iv) biocompatibility. However, cell transit time is a complicated function of various parameters: applied pressure, cell size, cell deformability, constriction channel dimensions, and polydimethylsiloxane (PDMS) and cell surface properties. Therefore, precise time-dependent readouts are often required, with well-defined control parameters, to distinguish cell deformability from convolved parameters. In an effort to resolve the cell deformability from the size, Sano et al. used two consecutive constrictions with different channel widths to measure size and deformability during translocation events.

Figures 4(c) and 4(d) depict the simplest two-electrode sensing design. Both schemes measure the impedance changes during a cell translocation event using an impedance analyzer (e.g., a transimpedance amplifier or a lock-in amplifier). An early microfluidics-based impedance sensor for electrical classification of single RBC deformability was devised by Katsumoto et al.. A microfluidic chip with channels integrated with a pair of coplanar microelectrodes was used to measure the normalized resistance changes corresponding to the shape of cell deformation in high-shear microchannel flows. Owing to the positional dependence of the AC measurements, the amplitude obtained could vary based on the z location of the cell from the electrode surface. To enhance measurement accuracy, physical constriction channels with electrical sensing have been proposed. For example, Adamo et al. demonstrated the dependence of transit time, size, and stiffness of HeLa cells by monitoring resistance changes. A similar method was achieved by Zheng et al. to compare the biophysical properties of adult and neonatal RBCs using electrical signatures (namely, transit time, impedance amplitude, and phase). For optimal AC measurements, a frequency range of 10–200 kHz is typically recommended to provide a compromise between temporal resolution, double-layer capacitance, and sensitivity. Electrochemical degradation of electrodes is a significant problem for long-term electrical measurements, since it causes a baseline shift, lower signal-to-noise ratio (SNR), and lower sensitivity. A key benefit of using AC voltage is suppression of electrode polarization, resulting in reduced electrode degradation and fewer air bubbles in microfluidic channels.

C. Mechanical measurements using cantilevers

The first suspended microchannel resonator (SMR) was developed for biomolecular detection by Burg and Manalis. An SMR contains a microfluidic channel embedded in a silicon cantilever. When biomolecules pass through the microfluidic channel, their buoyant mass changes the resonant frequency of the cantilever. Using this principle, SMRs have been used for detection of biomolecules and for measuring the buoyant mass, density, and volume of single cells. The SMR technique has been used to characterize cell deformability and surface friction. Unlike previous applications of SMRs, a physical constriction was added to the embedded microfluidic channel to deform the cells. By monitoring the shift in the resonant frequency, cell entry, transit, and total passage times were extracted to provide an indirect characterization of cell deformability. The physical contact of cells with the constriction channels makes SMR a method of choice when cell friction or retention is of interest. The resonant cantilever is an extremely sensitive microdevice and can measure the center of mass and buoyant mass with precisions of 100 nm and 1 pg, respectively. However, the fabrication of microfluidic-channel embedded cantilevers is complex owing to the multiple steps of wafer thinning and dry etching that are required. In addition, the lack of transparency of the channel limits the ability to use an optically integrated microfluidic system for co-measurement.

D. Real-time measurements

Time-dependent cell deformability measurements are often required to acquire data at a high sampling/frame rate (10 kHz samples/s). While a high sampling rate produces excellent time resolution for monitoring cell deformation, it generates a massive amount...
of data for burdensome off-line analysis, consuming tremendous computational time. Therefore, the true meaning of throughput with considering deformability analysis is much less (∼10 cells/s) than the reported measurement throughput. To address this limitation, the need for real-time deformability measurements has been recognized.

On-the-fly cell deformability measurement was first reported in RT-DC in 2015 by Otto et al. They used an online image analysis algorithm that continuously acquired images from a high-speed CMOS camera at 2000–4000 frames/s and quantified the DI and size (cross-sectional area). The real-time analysis significantly reduced the memory space needed for image recording and computational cost, enabling high-throughput cell deformability characterization (several hundreds of cells per second). A similar imaging-based real-time approach at an even higher frame rate (100 000 frames/s) (several hundreds of cells per second). A similar imaging-based real-time approach at an even higher frame rate (100 000 frames/s) was applied in an inertial microfluidic cell stretcher (iMCS), proving its ability to process an unlimited amount of data. The first real-time electrical measurement was achieved using differential impedance measurement at a sampling rate of 1 MHz. The cell passage time at the constriction was extracted from the electrical signal using a simple peak detection algorithm, which reduced the computational complexity of real-time measurement. This offers great potential for label-free real-time deformability-activated single-cell sorting.

Representative microfluidic deformability characterization devices are summarized in Table II.

### IV. SORTING

#### A. Collective cell separation

Specific cell types and states often exist with other components that are not of interest. Obviously, in a heterogeneous cell population, different subpopulations will have different biophysical and biomechanical characteristics, resulting in a biased analysis. This is the case for mechanotyping research, which investigates cell mechanics to study functional changes in cells during pathological alteration/metastasis and physiological cellular processes (e.g., differentiation, proliferation, and motility). Therefore, a preparative cell separation step based on biophysical properties is required to examine the subpopulations individually.

In recent years, numerous label-free microfluidic approaches have been developed to facilitate qualitative passive separation without reliance on any external forces and based on mechanical properties. These passive techniques contribute to high-throughput separation of subpopulations in continuous flow. However, other performance metrics (e.g., recovery rate, purity, enrichment, and efficiency) vary depending on the different separation mechanisms, microfiltration, deterministic lateral displacement (DLD), compression ridges, crossflow ratchets, and inertial microfluidics (Fig. 5). Although many label-free microfluidic techniques separate subpopulations on the basis of biophysical properties (e.g., size, density, electric polarizability, adhesion, and cell contents), we focus in this review only on deformability-based separation.

#### 1. Microfiltration

Microfiltration is perhaps the most straightforward and intuitive approach to separate micro- and nanoscale cells based on their size and stiffness. Microfabricated porous membranes, micropillars, and weirs are extensively used as filters. Typically, the pore size of the filter is designed close to the cell size to achieve high particle capture efficiency. There are two types of microfiltration methods: dead-end and crossflow filtration. Briefly, the flow direction faces the dead-end filter plane, whereas it is parallel to the filter plane in crossflow. The dead-end approach effectively filters large and stiff cells, but it is prone to clogging, which reduces its selectivity. In crossflow filtration, an additional sheath flow pushes the cells toward the filter, while the crossflow continuously flushes away large and stiff cells captured at the filter interface. This combination of sheath and crossflow enhances purity and throughput by reducing the risk of clogging. To achieve optimal separation performance, the selection of sheath flow and crossflow is critical. For example, an excessive crossflow rate will decrease the chance of filtration, resulting in reduced separation purity. By contrast, de-clogging is less effective with extremely low crossflow, leading to a reduced recovery rate. Although various microfluidic filtration methods have been reported, none of them simultaneously provides high recovery efficiency, purity, and throughput. Furthermore, sample loss and clogging are inherent challenges to be addressed.

#### 2. Deterministic lateral displacement (DLD)

DLD is another well-established passive separation technique that has been extensively explored over the years. The first DLD method was introduced in 2004 by Huang et al. Although early approaches using DLD focused only on size-based separation,

### TABLE II. Summary of representative microfluidic deformability characterization devices.

<table>
<thead>
<tr>
<th>Deformability index</th>
<th>DC</th>
<th>RT-DC</th>
<th>iMCS</th>
<th>SMR</th>
<th>Deformability sensor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell compression</td>
<td>Hydrodynamic stretching</td>
<td>Hydrodynamic stretching</td>
<td>Collision</td>
<td>Constriction</td>
<td>Transit time</td>
</tr>
<tr>
<td>Deformation index</td>
<td>Aspect ratio</td>
<td>Circularity</td>
<td>Aspect ratio</td>
<td>(Passage time)$^{-1}$</td>
<td>Resistance</td>
</tr>
<tr>
<td>Readout</td>
<td>Imaging</td>
<td>Imaging</td>
<td>Imaging</td>
<td>Real-time</td>
<td>Online</td>
</tr>
<tr>
<td>Analysis</td>
<td>Offline</td>
<td>Real-time</td>
<td>Real-time</td>
<td>Off-line</td>
<td></td>
</tr>
<tr>
<td>Throughput (cells/s)</td>
<td>2000–65000</td>
<td>100</td>
<td>450</td>
<td>1</td>
<td>500</td>
</tr>
<tr>
<td>Physical contact</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Refs.</td>
<td>41 and 42</td>
<td>43 and 72</td>
<td>68</td>
<td>10</td>
<td>7</td>
</tr>
</tbody>
</table>
FIG. 5. Various label-free, continuous, and passive bulk cell separation methods based on cell deformability. (a) Porous membrane microfiltration using crossflow. While larger and stiffer white blood cells are captured at the filter entrance, small and softer red blood cells pass through the filter. (b) Deterministic lateral displacement. Repeated laminar flow fields are depicted with a circular-post array. Stiffer cells shift between streamlines as they pass through the post array, while soft cells are deformed and remain in the original streamline. (c) Diagonal compression ridge. Soft cells are displaced in the negative transverse direction from the channel axis, while stiff ones move in the positive transverse direction. Two shear flows align the cells at the channel axis. (d) Resettable cell traps. The constricted diaphragm forms traps to capture the cells of interest, while unwanted cells flow through these traps and are collected in the waste outlet. The relaxed diaphragm enlarges the channel dimensions to clear the microchannel. (e) Microfluidic ratchet sorter. This provides continuous deformability-based cell separation using oscillatory flow and tapered contractions. The more-deformable cells travel farther up than stiffer ones. Crossflow propels the cells in the horizontal direction toward the collection outlets. (f) Inertial microfluidics. The inertial lift force $F_L$ and viscoelasticity-induced force $F_V$ are oppositely directed, and their combined effect thus determines the effective cell position in the channel. Deformable cells tend to stay in the center near the channel axis, while stiff cells move toward the channel walls.

additional sorting targets such as deformability, shape, and internal viscosity have since attracted interest. In 2014, Holmes et al. showed for the first time that there was a direct correlation between cell stiffness and lateral displacement in a DLD device. The deformability-based separation mechanism relies on the structure and geometry of the obstacle array, which determine the laminar flow fields [Fig. 5(b)]. In principle, infinitesimally small nondeformable particles will never switch to another streamline without an external force. However, deformable particles of finite size can move to other laminar flow streams because their hydrodynamic radius (or stretched size) decreases between the micropillars, where the shear stress is greatest. Since the row of micropillars is repeated after a certain distance, the streamline position is related to changes in the array. Therefore, the relative position of
To address clogging have been reported. The idea is to periodically clear the microfluidic channel before blockage by expanding the channel height using active pneumatic pressure control. Tunable height also enables precise control of the degree of cell deformation, and thus the selectivity of separation can be adjusted for different samples. Huang et al. described a proof-of-concept tunable microfilter in 2009. The tunable filter/trap was integrated with conventional dead-end membrane microfiltration methods. Various configurations of valve actuation and flow operation enabled size-dependent selective separation of cells with high separation efficiency (82.6–89.3%) at a reasonable filtration rate (3.3–14.9 μl/min). A similar tunable approach was used in a resettable cell trap (RCT) mechanism introduced by Qin et al. and Beattie et al. The RCT device used cell traps and a flexible diaphragm to achieve size- and deformability-based separation.

The diaphragm periodically closed (constricted) and opened (relaxed) the main flow channel by pneumatic control. When the diaphragm was closed, large and stiff cells were captured in traps, while small and soft cells passed through the constriction. The diaphragm was periodically opened to clear the flow channel to prevent clogging and fouling. This RCT device could center and side fins to support the channel structure when the diaphragm was inflated. Thus, a well-controlled rectangular flow channel to prevent clogging and fouling.

### 3. Compression ridges

Continuous and nondestructive cell separation can be achieved using a periodic diagonal ridge array attached to the top wall of the microfluidic channel. The gap between the diagonal ridge and the channel bottom forms a geometric constriction that compresses cells as they squeeze through periodically. The cell separation trajectory is determined by the interaction between the hydrodynamic drag force and the stiffness-dependent elastic deformation force, in opposing transverse directions. While the hydrodynamic force is dependent on the secondary circulatory flow, the elastic force is a function of cell stiffness. As a result, the cell trajectory gradually diverges at elastic and hydrodynamic equilibrium. The height of the constriction can be adjusted to vary the stiffness-dependent elastic force and thus determine the degree of cell deformation. Therefore, the constriction height is a critical parameter to increase the displacement in the transverse direction, thus enhancing separation resolution. The flow rate is another separation parameter with a direct effect on the hydrodynamic force imposed on cells. It has been experimentally validated that the flow rate does not contribute significantly to separation. The ridge width is usually set close to the cell diameter. While a small width produces insufficient elastic force for successful separation, with a large width there is a high chance of unwanted cell adhesion and irreversible clogging. The ridge angle is typically designed to be 45° to the channel axis, since the maximum hydrodynamic force can then be induced for an optimal separation effect. Ridge spacing is a control parameter for cell relaxation, which can be used to observe cellular viscoelastic behavior. The use of a diagonal compression ridge accommodates a high degree of cell deformation to enhance the separation effect, especially with regard to purity. Another key attribute of this separation technique is that it is less sensitive to variations in cell size.

### 4. Resettable cell traps (RCTs)

Channel clogging is a significant challenge for most passive separation methods. Several tunable microfluidic-based approaches to address clogging have been reported. Galetti et al. first explored a microscale deformability-based ratchet mechanism in 2011. Cell deformability was coupled with a local asymmetry to induce an irreversible ratchet mechanism. The unidirectional cell transport thus obtained suggests the possibility of selective cell separation based on deformability. In later work, a microfluidic ratchet device for high-throughput deformability-based cell separation was demonstrated and used for phenotypic separation of various samples (e.g., leukocytes, cancer cells, and malaria-infected RBCs). The separation mechanism used funnel-shaped constriction arrays, whose width was designed larger than the cell diameter, while the exit side was smaller. This structure only allowed small and deformable cells to ratchet through the tapered constrictions, while large and stiff ones were blocked. The size of the funnel opening gradually decreased from the bottom row (sample inlet side) to the top row (collection outlet side). Thus, a particular diagonal trajectory was formed by oscillatory flow (i.e., clogging and de-clogging flow), which propelled the cell population.

The captured cells were unclotted by a subsequent reverse de-clogging flow and flushed out toward the designated collection outlet by a crossflow. A reverse
flow approach has been used in microfiltration to address clogging. However, the reverse flow often pushed back the initially separated cells, resulting in low filter selectivity. In microfluidic ratchets, compressing cells through a tapered constriction along the direction of the funnel requires less threshold pressure than compressing them in the opposite direction. Such physical asymmetry with oscillatory flows imposes unidirectional and irreversible cell transport, enabling selective cell separation. Advanced microfluidic ratchet-based separation methods stand out in terms of separation rate ($0.5 \times 10^6$ cells/h), purity (98%), and enrichment ($10^4$-fold). In addition, the irreversible cell transport provides excellent selectivity and scalability, with a low risk of clogging.

6. Inertial microfluidics

Various inertial microfluidic techniques for particle and cell manipulation, mostly particle focusing, were first explored by Di Carlo et al. in 2007. In subsequent work, they were able to achieve inertial microfluidic separation by adjusting the particle focusing position based on particle size. The first deformability-based separation using inertial microfluidics was introduced by Hur et al. in 2011. In confined flow, lateral migration of particles can be induced by the effects of the inertial lift force, which is a function of particle Reynolds number. Since the particle Reynolds number depends on intrinsic characteristics, particles with different deformabilities have distinct inertial focusing positions in the microfluidic channel. Hur et al. used this lateral migration phenomenon in a straight microfluidic channel to separate deformable particles without external forces. There are additional lift forces on deformable particles, such as the viscoelasticity-induced force, which is dependent on particle size and rigidity. The balance between the deformability-induced lift force and the inertial lift force determines the equilibrium position in the channel. Therefore, these differences in lateral equilibrium position have been used to separate and collect particles based on their deformability. For example, more-deformable particles move away from the channel walls owing to the extra viscoelasticity-induced force. By contrast, stiffer particles are positioned near the channel wall owing to the dominant inertial lift force [Fig. 5(f)]. More recently, Guzniczak et al. used a spiral microchannel to add curvature, which accelerates the deformable particle displacement and significantly reduces the travel distance compared with a straight microchannel and significantly improves the separation throughput ($\sim 3 \times 10^6$ cells/min). An inertial microfluidic sorter can achieve high throughput for large-scale enrichment without any microfiltration. Thus, clogging is no longer a concern.

B. Active single-cell sorting

In passive sorting, the sorting boundaries cannot be adjusted during an experiment, since the flow geometries are predetermined according to intrinsic cellular mechanical properties. This often limits comparison experiments that require a sorting condition to be varied within the same device. Besides, an individual particle’s quantitative deformability information is inaccessible with passive sorting. Thus, deformability-based active sorting is an attractive option to overcome such limitations. However, surprisingly, there have been only a few works demonstrating streamlined active single-cell/particle sorting capability with deformability characterization.

The first active single-cell sorting method was reported by Faigle et al. in 2015. An optofluidic device was integrated with an optical stretcher to trap, examine, and sort individual cells in...
TABLE III. Summary of the microfluidic deformability-based cell sorting devices.

<table>
<thead>
<tr>
<th>Microfiltration</th>
<th>DLD</th>
<th>Bulk</th>
<th>Compression ridges</th>
<th>Ratchets</th>
<th>Inertial Optofluidics</th>
<th>Acoustic Adhesives Hydrodynamics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separation type</td>
<td>Size exclusion</td>
<td>Streamline around micropost array</td>
<td>Cell compression</td>
<td>Ratcheting</td>
<td>Tripping</td>
<td>Bulk</td>
</tr>
<tr>
<td>Separation mechanism</td>
<td>Active</td>
<td>Optical force (refractive index difference)</td>
<td>Acoustic force</td>
<td>Pressure</td>
<td>Inertial</td>
<td>Bulk</td>
</tr>
<tr>
<td>Throughput (cells/min)</td>
<td>10^4</td>
<td>10^7</td>
<td>10^3</td>
<td>10^5</td>
<td>10^4</td>
<td>10^6</td>
</tr>
<tr>
<td>Purity (%)</td>
<td>80–99</td>
<td>90–98</td>
<td>73–75</td>
<td>71–75</td>
<td>80</td>
<td>73–75</td>
</tr>
<tr>
<td>Enrichment (fold)</td>
<td>140–232</td>
<td>185</td>
<td>100–300</td>
<td>185</td>
<td>183–1800</td>
<td>100–300</td>
</tr>
<tr>
<td>Refs.</td>
<td>76, 83, and 117</td>
<td>91</td>
<td>99, 101, and 102</td>
<td>76 and 79</td>
<td>99, 101, and 102</td>
<td>76 and 114</td>
</tr>
</tbody>
</table>

Choi et al. developed a cytometry-like deformability-activated sorting device that seamlessly integrated single-particle deformability sensing and subsequent active hydrodynamic sorting into a single microfluidic chip. By adopting rapid constriction-based real-time deformability sensing, the system throughput was improved. However, the relaxation time, which is an intrinsic limitation of hydrodynamic sorting, still slows down the sorting process. Besides, it often causes incorrect sorting by missing the sorting timing. Li and Ai reported phenotyping-activated cell sorting, which integrates real-time electrical impedance measurement with acoustic sorting. The impedance measurement can be used to probe the cell transit time at a constriction for characterizing cell deformability. Besides, sensing electrodes can be configured to determine cell viability by measuring cellular impedance. Propagation of traveling surface acoustic waves (TSAWs) generates a radiation force and an acoustic streaming flow-induced drag force on spherical cells, enabling fast active sorting. Like other deformability-activated single-cell/particle sorting techniques, upstream flow cell alignment and well-defined cell spacing will further improve the sorting performance.

Just as fluorescence-activated cell sorting (FACS) allowed identification of the molecular characteristics of a leukocyte subpopulation based on immunophenotypes, mechanotyping-based sorting offers a unique opportunity to understand the molecular underpinnings of cellular mechanics. To this end, there is an urgent need for throughput comparable to that of FACS (30,000–40,000 cells/s) and a reliable device that actively sorts large populations of cells one by one based on their mechanical phenotype.

Microfluidic deformability-based cell sorting devices are summarized in Table III.

V. APPLICATIONS
A. Erythrocytes

Red blood cells (RBCs) or erythrocytes have a unique deformability that allows reversible changes in shape under external forces.
This mechanical property plays a critical role in circulating RBCs by allowing them to carry oxygen and carbon dioxide through the microvessels and fenestrated capillaries of the splenic sinusoids. It has been realized that cell deformability can be altered under various pathophysiological conditions. Thus, measuring RBC deformability can be a valuable indicator to help in understanding hematological diseases and their progression.

In recent years, various microfluidic techniques have been established to measure RBC deformability and thus provides a novel approach to hematological diseases. For example, extensive research has been conducted on malaria by investigating hematological abnormalities. The stiffness of RBCs increases more than 10-fold when they are infected with the Plasmodium falciparum parasite. This mechanical change causes occlusions in the peripheral capillaries and spleen, disrupting oxygen transport to downstream organs and tissues and leading to necrosis. Changes in RBC deformability have also been found in other blood-related diseases, such as sickle cell anemia, sepsis, and diabetes.

The importance of deformability naturally extends to the therapeutic benefits that the microfluidic separation of diseased RBCs or pathologically activated white blood cells (WBCs) can offer. Unadulterated healthy blood cells can be reintroduced to the patient, with abnormal cells being eliminated by microfluidic separation based on cell mechanics. Such dialysis-like therapeutic approaches remove over-activated immune cells such as neutrophils, malignant bone marrow cells after autologous transplantations, stiffer malaria-infected cells, and sickled RBCs during a sickle-cell crisis.

B. Stem cells

Over the past decades, extensive efforts have been made to reveal how the mechanical properties of stem cells affect their pluripotency and differentiation. During the stem cell differentiation process, changes in gene expression and protein abundance modify the cytoskeletal structure, resulting in differences in cell deformability. For example, Pajerowski et al. found that nuclei of human embryonic stem cells become six times stiffer after terminal differentiation. Similarly, Chowdhury et al. found that differentiated mouse embryonic stem cells are 10-fold stiffer than in their undifferentiated stage. Although it has been recognized that biological and mechanical factors are mutually correlated during differentiation, the molecular changes that result in such differences have not yet been fully unveiled. Measuring the mechanical properties of stem cells at each stage of differentiation will provide clues to answer this question.

Separation/enrichment based on stem cell mechanical properties, as a label-free biomarker, has tremendous potential for application in regenerative medicine. Ekpenyong et al. observed that changes in cellular viscoelastic properties determine the fate and function of myeloid precursor cells in the blood and suggested these changes as a cell differentiation marker that could be used for therapeutic purposes. Gonzalez-Cruz et al. also noted that sorting-based enrichment using mechanical biomarkers (i.e., elastic and viscoelastic properties) of adipose-derived stem cells, correlating with the ability to produce tissue-specific metabolites, had implications for cell-based regenerative therapies. Bongiorno et al. reported that cell stiffness as a single-cell osteoblast differentiation biomarker allowed enhanced enrichment of starting cell populations for stem cell therapies. These observations all suggest that the mechanical properties of stem cells are an excellent enrichment target for regenerative therapies.

Pluripotent stem cells have the potential to differentiate into any type of cell in the body. Therefore, implantation of differentiated cells from pluripotent stem cells is a promising approach to cure diseases such as heart failure, retinal and macular degeneration, tendon ruptures, type 1 diabetes, immune-system disorders, and neurological diseases. However, for a wide range of stem cell implementations, there are many risk factors that must be taken into account (e.g., inaccurate stem cell types, variations in differentiation status, proliferative capacity, contamination during in vitro culture and other manipulation steps, irreversibility of treatment, high risk of tumor formation, unwanted immune responses, and the transmission of adventitious agents). According to the U.S. Food and Drug Administration (FDA), quality control is essential in the manufacture of cellular therapy products to reduce such risks in stem cell-based therapies. High-throughput microfluidic devices that separate cells based on their mechanical phenotypes can play an essential role in such quality control by providing the required scalable cell separation tools to eliminate tumor-forming stem cells and to collect mesenchymal stem cells selectively.

C. Cancer cells

It has been realized that cellular mechanical phenotyping is a sensitive biomarker to identify cancer cell malignancy. Thus, high-throughput mechanotyping approaches have long been of great interest in cytopathology research, where sensitive, quantitative, and automated cytological analyses are often required. The nuclear architecture of the cytoplasm (e.g., chromatin condensation, nuclear envelope shape, metaphase nuclei, and the nuclear–cytoplasmic ratio) is an important target in the conventional determination of the potential malignancy of cancer cells. However, the unreliable sensitivity (40%–90%) of techniques based on this approach often means that malignant samples are missed, leading to inappropriate clinical decisions and treatment. Besides, long processing times and expensive assay costs resulting from complex manual sample preparation (e.g., staining for labeling) and prescreening are often burdens for patients in a clinical setting.

Unlike the conventional approach, mechanical phenotyping facilitates label-free assay for rapid identification of malignancy with minimal hands-on work and short processing times. High-throughput quantitative analyses can also provide standardized metrics for risk assessment of malignancy, which is a quite challenging task for qualitative analysis methods such as high-resolution imaging. Currently, mechanotyping-based approaches have been extensively applied to translational cancer research. For example, Yu et al. have recently reported that urothelial cells becomes more deformable during malignant transformation and progression, mainly because of an epithelial–mesenchymal transition pathway. Tse et al. presented a quantitative approach to the diagnosis of malignant pleural effusions with the ability to distinguish leukemias from inflammatory processes using label-free biophysical markers.
Remmerbach et al.\textsuperscript{147} described an approach to the diagnosis of oral cancer based on screening for suspicious lesions in the oral cavity using quantitative biophysical markers. Such examples suggest that mechanotyping-based technologies could have a significant impact on clinical decision-making for various cancers.

D. Drug testing

Many drugs, such as estramustine, discodermolide, and chloroquine, have effects on cytoskeletal or nuclear properties.\textsuperscript{148–150} They influence cellular mechanics by modifying cytoskeletal functions, such as by altering microtubule dynamics or increasing oxidative stress.\textsuperscript{149,150} These changes in cellular mechanical properties provide a potential biomarker for evaluation of drug efficacy and for drug screening. More specifically, drug efficacy can be quickly evaluated by changes in the mechanical properties of cells before and after drug treatment, because mechanical properties are expected to exhibit discernible changes if a drug is effective. On the other hand, as a result of drug resistance, cells may show almost no changes.

It has long been recognized that for each disease, there are differences in clinical response to a drug from patient to patient. Compared with the traditional bulk-lysing approach to drug screening, the measurement of single-cell mechanical properties gives a better representation of drug response and tolerance in a heterogeneous population. Besides, the traditional screening method is based on observations of cell death rates, and it requires hundreds of thousands, or even millions, of tests to find effective drug compounds. Such a time-consuming process is particularly problematic when it is necessary to develop new drugs quickly to deal with the emergence and spread of drug-resistant pathogens (e.g., malaria).\textsuperscript{151} The availability of a microfluidic mechanotyping device that is able to analyze thousands of single cells in a second would provide a new approach for high-throughput and quantitative drug screening and drug candidate selection. In the meantime, mechanotype-based cell sorting can be used to enrich screening libraries for drugs that affect the architecture of the cytoskeleton or the nucleus. Establishing changes in cellular mechanical properties as a standard mode of drug action will provide positive insights and enable advanced personalized medical and drug treatment.\textsuperscript{152}

VI. PERSPECTIVES AND OUTLOOK

Microfluidics has emerged as a promising technology for obtaining quantitative insights into cellular mechanics owing to its ability to carry out manipulation and analysis at the cellular scale. Numerous microfluidic systems have been developed to implement time-dependent characterizations of the size, shape, deformation, and stress/strain relaxation of cells with the aim of understanding the relationships between their mechanical properties and their function. These tools are now laying the foundation for mechanical mechanotyping research to be translated into clinical applications. For a successful translation, the following agenda needs to be addressed in the future.

1. Standardization among various testing approaches. Lack of standardization among various characterization techniques poses analytical challenges and limits cross-study comparison. Standardized cell deformation techniques, detection readouts, experimental protocols and setups, and guidelines for interpreting results are urgently needed for further development of microfluidic single-cell mechanotyping devices.

2. Molecular specificity. Label-free deformability cytometry still lacks molecular specificity, commonly used to monitor cellular physiological states. In standard flow cytometry techniques, molecular specificity is achieved by the use of fluororescent probes.\textsuperscript{153} Similarly, a fluorescent readout can be integrated into real-time deformability cytometry for characterizing both the mechanical and physical properties of cells.\textsuperscript{154} Furthermore, hybrid measurement opens the possibility of investigating correlations between cell mechanics and molecular properties at the single-cell level.

3. High-throughput deformability-activated sorting. One direction of future research would be to increase the throughput of deformability-activated cell sorting. As discussed, real-time deformability analysis enables active sorting based on the mechanical properties of the cell. While automation of continuous-flow individual cell sorting is of great interest to clinicians and cell biologists, the deficiencies in sorting speed compared with traditional FACS (30 000–40 000 cells/s), limits the practical implementation of this technology. However, further improvements in throughput are expected through parallelization of the system using multiplexed techniques.\textsuperscript{155,156}

High-throughput single-cell mechanical phenotyping using microfluidics is in the early stage of translation. Label-free deformability analysis provides additional insight into aspects that are unclear in conventional assays owing to high phenotypic heterogeneity and inconsistent expression of traditional biomarkers.\textsuperscript{156,157} Moreover, label-free sorting offers enrichment of cells of interest and streamlines clinical decision-making. Advances in microfluidic tools will enable further applications, such as molecular delivery\textsuperscript{158,159} and cell-fate decisions,\textsuperscript{160,161} where label-free single-cell mechanotyping is desired.

ACKNOWLEDGMENTS

This work is partially supported by the National Science Foundation under Grant Nos. 1710831 and 2045169. Any opinions, findings, and conclusions or recommendations expressed in this work are those of the authors and do not necessarily reflect the views of the National Science Foundation. W.G. acknowledges the support of the Penn State Center for Biodevices Seed Grant.

REFERENCES


© Author(s) 2021

Nanotechnology and Precision Engineering

4, 045002-17

DOI: 10.1038/s41598-018-06865-x

Diverse and complex microorganisms inhabit diverse environments, including blood. The viscoelastic deformation of red blood cells (RBCs) plays a crucial role in cellular function and pathology. This review highlights the application of viscoelastic deformation in the separation and filtration of blood cells, focusing on recent advances in microfluidic devices for this purpose. The unique mechanical properties of RBCs enable the development of new technologies for cell separation and filtration. The review emphasizes the role of viscoelastic deformation in the selective separation of blood cells with different mechanical properties, such as red blood cells (RBCs), platelets, and white blood cells (WBCs), using microfluidic technology. The review also discusses the clinical implications of these technologies in fields such as hematology, oncology, and infection control.

Key words: Viscoelastic deformation, red blood cell, platelet, white blood cell, microfluidics, cell separation, filtration, blood.

1. Introduction

Blood is a complex biological fluid that circulates throughout the body, delivering oxygen and nutrients to cells and removing waste products. The blood is composed of a liquid phase, plasma, and solid phase, formed from cells that play critical roles in various physiological processes. Red blood cells (RBCs), white blood cells (WBCs), and platelets are the main components of the blood cell fraction. RBCs, which constitute approximately 45% of the blood volume, transport oxygen and carbon dioxide between the lungs and tissues. WBCs, which constitute approximately 5% of the blood volume, play a central role in the immune response. Platelets, which constitute approximately 25% of the blood volume, are involved in the hemostatic process and contribute to blood clotting.

2. Viscoelastic deformation of blood cells

The viscoelastic properties of blood cells are critical for their function and can be exploited for separation and filtration purposes. RBCs are biconcave disc-shaped cells that are highly deformable, allowing them to pass through small capillaries. Their deformability is influenced by their mechanical properties, which can vary based on factors such as age, disease, and genetic background.

3. Microfluidic devices for cell separation and filtration

Microfluidic devices offer a promising platform for the selective separation and filtration of blood cells. These devices are fabricated using microfabrication technologies and can be used for the separation of blood cells based on their mechanical properties or other characteristics. Microfluidic devices can be classified into two main types: those based on viscoelastic deformation and those based on other principles, such as density or electrophoresis.

4. Viscoelastic deformation-based separation and filtration

Viscoelastic deformation-based separation and filtration techniques rely on the different mechanical properties of blood cells, such as elastic modulus or viscosity. These techniques can be used to separate blood cells based on their deformability, which is influenced by factors such as cell age, disease, and genetic background.

5. Applications of microfluidic devices in cell separation and filtration

Microfluidic devices have been applied in a variety of fields, including hematology, oncology, and infection control. They are particularly useful for the selective separation of blood cells, which can be used for diagnostic, therapeutic, or research purposes. Microfluidic devices can be used to separate blood cells based on their mechanical properties or other characteristics, and they offer several advantages over traditional separation methods, such as higher throughput, lower cost, and smaller footprint.

6. Conclusion

The viscoelastic deformation of blood cells provides a promising platform for the development of new technologies for cell separation and filtration. Microfluidic devices offer a unique opportunity to exploit the mechanical properties of blood cells for selective separation and filtration purposes. Future research should focus on the development of novel microfluidic devices for the selective separation and filtration of blood cells, with the goal of improving diagnostic and therapeutic outcomes in various fields.

Acknowledgments

This research was supported by the National Institutes of Health (NIH) grant number T32HL125272.

References

© Author(s) 2021

**Nanotechnology and Precision Engineering**

**REVIEW**


**Gilhoon Choi** received his Ph.D. in electrical engineering from the Pennsylvania State University (2020). He is currently a postdoctoral researcher in the Department of Biotechnology and Bioengineering at the Sandia National Laboratories. His research interests include microfluidics, bioMEMS, and lab-on-a-chip devices as multidisciplinary tools to enable high-throughput analysis of biomolecules and cells. He is also interested in a field-deployable molecular diagnostic system for sensitive, specific, and rapid detection of viral pathogens at the point of need.

**Zifan Tang** is a Ph.D. research assistant in electrical engineering at the Pennsylvania State University, University Park. She obtained her Master’s degree from Tianjin University in 2017. Her current research interests include single-molecule sensing, microfluidics, and developing point-of-care nucleic acid testing devices for diagnostic applications.

**Weihua Guan** received his Ph.D. degree in electrical engineering from Yale University in 2013 and is currently an Associate Professor in the Department of Electrical Engineering at Pennsylvania State University. He has also held a courtesy appointment in the Department of Biomedical Engineering at Penn State. Dr. Guan’s research interests are in the multidisciplinary areas of micro- and nanotechnology, micro/nanofluidics, bioMEMS, lab-on-a-chip devices, and point-of-care devices. His group focuses on developing micro- and nanosensing devices, as well as novel sensing principles toward advanced medical diagnostics and testing. Dr. Guan is a Senior Member of the IEEE and a member of the Engineering in Medicine and Biology Society, the Biophysical Society, and the Biomedical Engineering Society. Among other honors, Dr. Guan is a recipient of the HHMI International Research Fellowship and the NSF CAREER award.