SMGroup

SM Journal of Biomedical Engineering

Review Article

Microfluidics for Single Cell Mechanical Characterization: A Review

Ravetto A[1], Anderson PDA[2,3], Bouten CVC[1,3] and den Toonder JMJ[2,3]*

[1]*Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands*
[2]*Department of Mechanical Engineering, Eindhoven University of Technology, The Netherlands*
[3]*Institute for Complex Molecular Systems, Eindhoven University of Technology, The Netherlands*

Article Information

Received date: Jan 30, 2017
Accepted date: Mar 02, 2017
Published date: Mar 08, 2017

***Corresponding author**

den Toonder JMJ, Eindhoven University of Technology, Eindhoven, The Netherlands, Tel: +31 40-247 5706; Email: J.M.J.d.Toonder@tue.nl

Keywords Cell mechanics; Microfluidics; Diagnostics; Cell analysis; Disease screening

Abbreviations AFM: Atomic Force Microscopy; PDMS: Polydimethylsiloxane; RBC: Red Blood Cell; WBC: White Blood Cell; CTC: Circulating Tumor Cell; DI: Deformation Index

Abstract

Mechanical characterization of cells may be used to distinguish and to select certain types of cells and, more importantly, to discriminate between healthy and diseased cells. This means that mechanical properties of cells could be used as markers to screen and diagnose for diseases like cancer, malaria, and cardiovascular diseases, without the need for biochemical assaying. Conventional cell mechanical characterization methods are not suitable for practical clinical application since they are tedious and have a low throughput. Microfluidic technology holds great promise to realize single cell mechanical characterization devices that are suitable for clinical use. In recent years, many devices based on different microfluidic principles for characterizing cell mechanical properties have been introduced in the literature and science has developed up to a point at which the next steps must be taken to enable the actual translation into the clinic. In this paper, we review the different methods discussing advantages and disadvantages, and we conclude on the major challenges that need to be tackled next to enable the translation towards clinical application.

Introduction

The mechanical properties of cells are determined by the properties and organization of the cells' nucleus, membrane and cytoskeletal components [1-7]. Actin, in particular, is a cytoskeletal component that provides most of the mechanical integrity to the cell and is involved in important biomechanical functions, such as cell motility [3,5,6,8-11]. Understanding the link between cell structure and cell mechanics provides insight into the mechanisms of cell function in normal biological processes as well as in a diseased state, since for a number of diseases significant changes in the organization of cytoskeletal and membrane structures of human cells take place during disease development, which affect the mechanical and adhesive properties of the cell [2,12-17]. For instance, it was shown that cancer cells undergo a significant increase of deformability and adhesive properties [18-20] probably due to a strong reduction and dynamic changes of actin and keratin structures [13,19,21-23]. Also, the etiology of some diseases is the result of changes in the structural and mechanical properties of cells [14]. In the case of malaria, for example, the invasion of the parasite Plasmodium in red blood cells results in an up to ten-fold increase of effective cell stiffness within the first 48 hours [15], which is the direct cause of obstruction of flow through the microvasculature. This cell sequestration causes an impaired clearance by the spleen and results in complications and changes of the disease, which leads to the advanced stage of malaria [15,19]. Moreover, it was shown that the clogging of capillaries in the microcirculation is mainly due to cytoskeletal and mechanical alterations of blood cells and that this can result in ischemia and circulatory problems [8,24,25].

Mechanical properties therefore may be used to distinguish and to select certain types of cell and to discriminate affected cells from healthy cells or cells in particular stages of a disease. As such, cell mechanical properties may be viewed as a selective marker and a diagnostic indicator for the incidence and progression of disease, and therefore may be used as a basis to screen and diagnose for diseases. The investigation of changes in cell structural and mechanical properties might also give insight into the underlying mechanisms of disease processes. Lastly, the link between cell mechanics and disease might be used to identify targets for therapeutic intervention and might provide information about the efficacy of drugs used to treat a disease. For instance, Ruef et al. showed a reduced stiffness of activated neutrophilic granulocytes by treatment with anti-inflammatory drugs called phosphodiesterase inhibitors [26]. This leads to decreased obstruction of microcirculation in patients with leukostasis.

Many studies have been performed to deform single cells and investigate the relation between cellular response and cellular components such as the cytoskeletal structure [27]. Single cell studies allow to separate the behavior of each cell from the response of the cell population and to observe the heterogeneity among cells of the same type. This is fundamental to the study of disease and disease progression, since only a few cells within a total cell population may determine the pathophysiological state and its development. Moreover, the progress in microtechnology allowed the development

of cell probing techniques that are able to generate and sense forces and displacements, to a precision of picoNewtons and nanometers respectively, for analysis at the cellular and subcellular level.

Several experimental techniques have been developed to deform local regions of the cell, such as Atomic Force Microscopy (AFM) [28,29], while other techniques rather manipulate the whole cell, such as micropipette aspiration [30-34], microplate manipulation [35-37] and optical/magnetic tweezers [21,38-41]. However, these techniques have several limitations, such as laborious sample preparation and low throughput. Thus, 'microfluidics' has gained interest as an attractive alternative for the study of single cell mechanics, due to several advantages compared to conventional biomechanical techniques [42-44]. Microfluidics is the science and technology of manipulating and analyzing fluidics at small scale, typically between micrometers and millimeters. Microfluidic devices typically contain (networks of) microchannels in which fluids, as well as their constituents such as cells, can be precisely manipulated and observed [45]. Important applications of microfluidic devices are in medical diagnostics such as miniaturized immunoassays or nucleic acid-based diagnostics, as well as cell and tissue analysis, including the relatively new field of organ-on-a-chip in which human organ functions are mimicked in a physiologically relevant manner [46-49]. Microfluidic technologies are also appealing from a cell mechanics point of view, due to their small scale and the quantitative analysis at the cellular and subcellular level. In fact, microfluidic devices are able to perform fast and reliable biomechanical analysis using reduced quantities of samples and reagents. Since in-vivo microenvironments can be closely mimicked the devices enable real-time analysis of cellular response to mechanical and chemical stimuli in physiological and pathological conditions. Other advantages of microfluidic devices are the well-developed fabrication process and materials. Most devices are fabricated with a precise and reproducible photolithography method using transparent material, such as Polydimethylsiloxane (PDMS) or glass, allowing easy microscopic visualization for cell analysis. Finally, microfluidic devices offer the possibility of high-throughput mechanical measurements with a variety of cell probing principles.

Since microfluidic devices permit to manipulate physical and chemical cues in a capillary-like microenvironment, they have been extensively used as in-vitro model systems for studying the role of blood cell mechanics in hematological diseases [8,15,24,50]. In addition, microfluidics has emerged as a platform technology for the study of cell biomechanics at the cellular and the molecular levels, and has been used to gain an insight into several human diseases such as cancer [13,18,20,51-54], malaria [11,13,42,55] and cardiovascular diseases [50,56-58].

In the following, a concise review of the state-of-the-art of microfluidic technologies for studying mechanical properties of cells is presented. We first discuss the advantages and limitations of microfluidic technology. Following, we summarize the existing microfluidic devices designed for characterizing mechanical properties of cells, based on their mechanism of cell deformation. Finally, we present a perspective on the future directions and challenges of microfluidic technology for cell mechanical characterization.

Characteristics of Microfluidic Devices for Cellular Biomechanics

Microfluidic technology provides an attractive alternative for the study of cell mechanics due to its unique features beyond traditional macroscopic techniques as indicated above [42,59]. In fact, microfluidics enables mechanical analyses in a controlled cellular microenvironment and using significantly smaller samples. In the next section, we will elaborate on the advantages and disadvantages of microfluidics for cellular biomechanics.

Advantages

To increase the throughput of testing, mechanical cell characterization methods have been developed that are based on studies of populations of cells, such as micropore filtration. It is well known that individual cells can differ from each other even within a genetically identical population [60,61]. Cell population assays, relying on ensemble averaging, are not able to characterize these differences between individual cells. Thus, the development of a single cell technique for isolation and analysis of individual cells will improve our understanding of the heterogeneity of cell populations, which is fundamental for disease research, for regenerative medicine, for diagnostics and for drug discoveries. Microfluidics enables the manipulation of single cells since the typical dimensions of microfluidic features (such as channel width) are very similar to the cellular scale, typically in the order of tens of micrometers, and the cellular microenvironment can be controlled with high spatial and temporal resolution. At the same time, speed and frequency of analysis can be high, allowing for high throughput testing.

One of the main challenges of cell mechanical studies is the difficulty of handling specimens with an extremely small size scale that is not easily manageable for human manipulation [62]. The spatial positioning of the specimen is fundamental for evaluating cell properties using standard operations, such as fluorescent microscopy analysis, and for investigating cell interactions with their microenvironment [63]. Human manipulation of these small samples is laborious, time consuming and often requires significant technical skills [32,33]. Microfluidics allows integrating most of the common laboratory functions, such as cell lysis or cell separation, by miniaturizing the macroscopic systems. In fact, by integrating and designing microchannel structures, or even actuators, microfluidic experimental platforms can allow for precise manipulation and positioning of single cells [62,64].

The Reynolds number describes the ratio of inertial and viscous terms in the momentum equation for fluid flow. It is defined by $Re=\rho UD/\mu$, where ρ is the fluid density, U the fluid velocity, D a characteristic length scale such as the channel's hydraulic diameter and μ the characteristic fluid viscosity. The small channel dimensions in microfluidic devices cause the Reynolds number (Re) to be extremely small. As a result, the flow in microfluidic channels is almost always laminar. The fluid behavior at small scale is then highly controllable and predictable. This characteristic enables a precise control of the experimental conditions and the straightforward integration of several laboratory functions in a microfluidic device, such as the formation of stable gradients [65-67]. For example networks of branching microchannels present in microfluidic devices were designed and used to produce chemicals gradients within the channels in order to carry out drug assays [68]. Another advantage of microfluidic systems is the temporal stability of processes, since the small channel sizes decrease the time required for chemical and drug molecules to reach the cells of interest.

Microfluidic systems can mimic the in vivo micro-environment of the cells such as pH, biochemical gradients, temperature and mechanical stimuli, allowing real-time studies of cellular response to mechanical stimulus under almost physiological conditions. For instance, in the study of blood cell deformation, microfluidic devices can mimic the capillaries in which cells are flowing, whose dimensions (from 1 to 7,5 μm, with mean 3,7 μm) are in the same order as of microfluidic channels [69]. The flow conditions used in the microfluidic analyses can also be comparable to physiological situations.

Recent progress in microfabrication techniques enables the integration in microfluidic systems of laboratory procedures with increased sensitivity. Since microfluidics devices are microscopy compatible, cell mechanical measurements can then be designed to obtain real-time data at multiple time points and over a large range of parameters, which results in much more data being available than the one-point measurements in traditional approaches. Furthermore, microfluidic devices allow the implementation of a large range of input conditions for the screening of a high number of cells.

Disadvantages

The characteristic laminar flow of microfluidic channels can also be viewed as a limitation for certain applications, especially when fast mixing of fluid is required. In this case, the flow must be manipulated such that chaotic advection accelerates diffusion and therefore induces mixing. This can be achieved in various ways, for example by integrating passive flow structures in microchannels that create flow patterns resulting in chaotic advection, or by the actuation of magnetic microparticles in the fluid, acting as microstirrers mixing the fluid [70-74].

The small reagent volumes used in microfluidic devices can pose challenges for handling these low amounts of fluids. Furthermore, some difficulties can arise from adaptation of biological protocols to small microfluidic scales, such as media supplements for cell culture.

Since most biological procedures require oxygen/carbon dioxide exchange, the materials used for microfluidic devices have usually high gas permeability, for example Polydimethylsiloxane (PDMS). This can lead to fluid evaporation, drying of the system and consequent change in media osmolarity.

Microfluidics for Mechanical Characterization of Cells

Most microfluidics-based devices for mechanical characterization of cells have been applied to circulating cells. Cells circulating in the blood are a combination of subgroups of cells, including Red Blood Cells (RBCs), platelets, and different classes of White Blood Cells (WBCs) and, in the adverse case of cancerous disease, Circulating Tumor Cells (CTCs) [18]. The wide diversity of these cells and their non-adhesive properties pose significant challenges for the isolation and characterization of cell mechanical properties. Due to its characteristic advantages, microfluidics has been employed for blood cell analyses in healthy and diseased conditions [75-77], such as plasma separation and mechanical studies of RBCs [15,77-84], platelets [41,85], WBCs [8,24,86] and CTCs [20,87,88].

In order to mechanically characterize blood circulating cells, the microfluidic device must exert a certain deformation or force to the cell. In the next sections, we illustrate the state-of-the-art of experimental microfluidic tools, based on the mechanism used to deform or load the cell. Table 1 lists, in a concise manner, the published microfluidic devices for mechanical characterization of cells, indicating the technique used, the cell types studied, the parameters varied, and a summary of the main results.

Flow induced

Cells can be exposed to fluid stresses in a microfluidic channel by generating either shear flow in narrow channels [80,83,89] or extensional flow in two orthogonal microchannels [87,90]. The Deformation Index (DI) is often used as a quantification parameter for cell deformability. The DI is defined as (X-Y)/(X+Y), assuming an ellipsoidal shape with X being the long axis and Y the short axis of the ellipsoid.

Forsyth et al. [83] deformed RBCs in a straight microfluidic channel by pressure-driven flow to study cell dynamics upon treatment with chemical agents known to affect cell mechanics, namely diamide and glutaraldehyde (Figure 1A). They revealed three different types of RBC motion due to the increased shear rate in the constriction, namely stretching, tumbling and recoiling. Katsumoto et al. [91] characterized RBCs deformability by detecting the resistance change when an RBC passed along the surface of embedded electrodes in the microchannel. In fact, the resistance profile turned out to be correlated with the shape of the RBC under shear deformation, giving information about cell deformability.

Gossett et al. [87] reported a hydrodynamic-stretching microfluidic device for identifying cancerous malignant cells with a high throughput of 2000 cells/s flowing through microchannels (Figure 1B). By using inertial forces, cells were focused to the center of a junction of two channels where the cells underwent mechanical stretching. Cell deformation was captured using a high-speed camera. The DI was subsequently extracted by analyzing cell images. Gosset et al. demonstrated that cancerous cells are more deformable than benign cells.

The advantage of these flow induced methods is that the throughput can in principle be very high; however the timescale of the probing is determined completely by the flow, which makes it difficult to probe time-dependent behavior of the cells.

Optical stretcher

The principle of the optical stretcher is based on the surface force exerted on a cell by two slightly divergent Gaussian beams [92-96]. The setup consists of a microchannel and two laser fibers located at both sides of the passage way of the cell. At low laser intensities, the flowing cell is trapped and, at higher intensities, the cell is deformed in a controlled way by the laser beams [6]. The deformability of different circulating cells was characterized using the optical stretcher, such as RBCs [38,97], cancer cells [6,21] and myeloid cells [96].

Guck and colleagues [38,98] developed a microfluidic optical stretcher to study the deformability of suspended cells, such as RBCs and human epithelial breast cancer cells (Figure 1C). They showed that cancer cells with higher metastatic potential deformed more than control cells. Mauritz et al. [99] also characterized the viscoelastic properties of healthy and malaria-infected RBCs. They showed that infected RBCs have increased rigidity due to the internalized parasite Plasmodium falciparum.

Table 1: Microfluidic devices for mechanical characterization of single cells on the basis of cell deformation.

Method	Technique	Cell type	Parameters	Main results	References
Flow induced	Straight micro channel High shear flow	RBCs	Deformation Index (DI)	Measured shear modulus (3.7 mN/m) is in good agreement with previous results	[89]
	Hyperbolic converging microchannel Extensional flow	RBCs	Deformation Index (DI)	Extensional flow is more efficient than shear flow in inducing cell deformation	[90]
	Straight channel Pressure-driven stretching	RBCs	Extent of deformation and cell dynamic	Three types of cell motion. Cellular viscosity governs cell behavior in pressure-driven flow	[83]
	Channel with micro membrane electrodes Shear stress	Normal and rigidified RBCs	Deformation rate Electrical resistance	Normal RBCs have an ellipsoidal shape while rigidified RBCs maintain a biconcave shape. The electrical resistance signal is correlated to RBCs deformation	[91]
	Two orthogonal micro channels Hydrodynamic stretching	Cancer cells; stem cells	Deformation Index (DI)	Carcinoma cells are highly more deformable; stem cell pluripotency is associated with increased deformability	[87]
Optical stretcher	Two laser-beams	RBCs and fibroblasts	Extent of deformation	Optical deformability can be used to distinguish between different types of cells	[38]
	Two laser-beams	Normal, cancerous and metastatic epithelial cells	Extent of deformation	Cancerous cells deform more than normal cells. Metastatic cancerous cells deform even more than non-metastatic	[21]
	Two laser-beams	Differentiating myeloid cells	Creep compliance	Compliance measurements reveal softening during differentiation of myeloid cells	[96]
Compression	Actuated PDMS membrane	Epithelial cells	Cell viability	Epithelial cells were deformed and lysed under the compression of the membrane	[102]
	Actuated PDMS membrane	Monocytic and fibrobastic cells	Recovery time constant	Cell type can be distinguish on the basis of the characteristic recovery time constant	[104]
Aspiration	Glass micropipette	Neutrophils	Cortical tension	The measured cortical tension was 0.024 dyn/cm. The change in cortical tension can represent an indicator of cell activation state.	[31]
	Micro-aspirator chip –40 cells traps	Suspended Hela cells	Young modulus	High-throughput measurement of cell Young modulus	[54]
	Funnel-shaped constrictions	Malaria-infected and normal RBCs	Minimum cylindrical diameter MCD (related to area and volume)	Results showed a high MCD for infected RBCS	[81]
	Funnel-shaped constrictions	Neutrophils, lymphocytes and cancer cells	Cortical tension	The measured cortical tensions were consistent with previous studies performed with traditional techniques	[108]
	Funnel-shaped constrictions	Malaria-infected and normal RBCs	Cortical tension and pressure required to squeeze out cells	Diseased RBCs from different malaria stages were shown to be from 1,5 to 200 times stiffer than normal RBCs	[109]
	Glass micropipette	Monocytic cells (non-treated, activated and actin-disrupted)	Compressive and shear moduli	Activated cells become less compressibile but more deformable. Actin-disrupted cells have extremely low compressive and shear moduli	[111]
Constriction deformation	Single constriction channel	Malaria-infected and normal RBCs	Cell blockage vs Passage	Malaria-infected cells easily block the channel	[15]
	Two identical constrictions (manometer)	RBCs and fixed RBCs	Excess pressure drop	The pressure drop of RBC passage is enhanced after fissative treatment	[112]
	Bifurcating channels network	Neutrophils and monocytic cells (healthy, sepsis and leukostasis infected)	Transit time distribution	Diseased cells were stiffer than the normal ones. This observation was based on the increased transit time and occlusion of diseased cells	[50]
	Single constriction channel	Malaria-infected and normal RBCs	Transit and recovery time	Malaria-infected cells were stiffer than non-infected RBCs. RBCs in a later stage of the disease easily got destroyed by the passage in the constriction	[99]
	Single constriction channel	Leukocytic cells (non-treated and actin/myosin treated)	Cell entry time, transit velocity and relaxation time	Actin has an important role in cell entry time and transit velocity but not in cell relaxation time. Myosin is not responsible for these trafficking stages.	[8]
	Single constriction channel	Epithelial cells and cancer epithelial cells	Entry time, elongation index and transit velocity	Cancer cells were shown to be less stiff than normal epithelial cells since they exhibit shorter entry time	[20]
	Single constriction channel	Leukocytes and ARDS (inflamed) leukocytes	Entry time	ARDS leukocytes exhibit significantly longer entry time (increased stiffness)	[113]
Constriction deformation [cont.]	Single constriction + electrodes	Cancer cells	Electrical impedance	Combination of biomechanical and bioelectrical parameters, could provide a higher cell classification success rate	[116]

	Microbarriers-based filter	Cancer cells	Cell separation	Cancer cells with high metastatic potential are less stiff than normal cells. They change shape and pass through the filter exiting the separation device	[114]
	Single constriction channel	Suspended Hela cells (normal and actin-disrupted)	Transit time	Control Hela cells have a longer transit time compared to actin disrupted cells	[115]
	Single constriction + electrodes	Adult and neonatal RBCs	transit time, impedance amplitude ratio, and impedance phase increase	Multiple parameters in combination can provide a higher classification success rate	[78]
	Comb-like filter	Leukocytes and inflamed leukocytes	Cell blockage stage	Inflamed leukocytes stopped at an early stage of the filter due to their increased stiffness	[24]
	Two successive constrictions separated by a chemical stimulation component	Monocytic cells	Entry time, transit time	Differences in entry and transit times were detected after chemical cues are delivered to the cells through an integrated porous membrane	[118]

The optical stretcher method has the advantage that the mechanical stretching mechanism is in principle independent from the flow characteristics. This makes it possible to do both high-throughput and time-dependent mechanical characterization of cells. At higher laser intensities, there is the risk of photo-toxicity affecting the cell behavior.

Compression

Compressive forces can be applied to cells by a deformable membrane integrated in microfluidic devices [100]. These multi-layer devices consist of a thin elastomeric membrane sandwiched between two orthogonal fluid channels, namely the flow and the control channels. The cells in the flow channel are compressed when pressure in the control channel is applied to deflect the membrane. Hohne et al. [101] revealed that this method is capable of characterizing soft objects with a Young's modulus in the range of 102-105 Pa, which is a relevant range for cell mechanical studies. Kim et al. [102,103] used a device with an actuated membrane to measure cancer cell deformation and to characterize "bulge" formation on the cellular membrane (Figure 1D). They also showed that this device enables to distinguish between healthy and breast tumor cells. We designed a microfluidic device based on the principle (Figure 1E), and used it to characterize the viscoelastic properties of leukocytic and fibroblastic cells [104] by measuring cell relaxation time upon releasing the compression. We showed that it is possible to distinguish cell type on the basis of their viscoelastic response.

The compression method can result in quantitative characterization of cell mechanical properties, and in principle multiple cells could be probed simultaneously. A disadvantage is that the cells directly contact the (solid) walls of the device and the deformable membrane so that the cell's response is not just caused by its mechanics but also by (biochemical) adhesive interactions between the cell and the solids.

Aspiration

Micropipette aspiration is a conventional technique for measuring the mechanical properties of single cells. The method is based on the partial aspiration of a cell into a glass micropipette that has a diameter of 1 to 5 μm. A mathematical model permits the calculation of the Young's modulus and of the cell viscosity, on the basis of the observed cell elongation [105]. The concept of conventional micropipette aspiration has been adopted in microfluidic applications [106,107]. Kim et al. [107] used microfluidic devices with a series of 40 funnel-shaped constrictions to deform single cells in parallel. Cells were trapped by different flow resistances, and they were simultaneously deformed by application of a negative hydrostatic pressure. Guo et al. [108,109] developed a system based on cell deformation through a series of funnel-shaped constrictions (Figure 1F). They used this platform to measure the deformability of several cell types, such as neutrophils, lymphocytes and cancer cells [108] and to detect mechanical stiffening in malaria-infected RBCs [109]. Gifford et al. [110] also used a system based on wedge-shaped channels to measure surface area and volume of RBCs. Herricks et al. [81] adapted this technique to study the deformability of a large population of RBCs at different malaria infection stages. They showed that the filterability of RBCs is well predicted by the minimum cylindrical diameter, which is calculated from cell surface area and volume.

Needham et al. [31] used the micropipette system to fully aspirate a neutrophil and measure the surface stress as a function of the surface area dilation of the highly ruffled cellular membrane. In [111], we describe a similar method, adapted from micropipette technology. The device we used is named the Capillary Micromechanics device. We used this technique for studying the changes of deformability of monocytic cells upon cell activation. The device [111] measures the pressure-induced deformation of cells as they are deformed in a tapered glass microcapillary (Figure 1G). This approach allows for the calculation of both compressive and shears moduli from a single experiment, over a large range of physiologically relevant deformations.

Aspiration enables the quantitative characterization of cell mechanical properties, however at rather low throughput and over limited time scales. Also, the (biochemical) interaction between the cells and the capillary walls may influence the observed behavior next to the plain mechanical properties of the cells.

Constriction deformation

Constriction-based microfluidic devices have been widely used to investigate the mechanical properties of circulating cells, such as red blood cells [15,55,78,112], white blood cells [8,24,50,113] and Circulating Tumor Cells (CTCs) [20,114].

Such a device contains one or more channel sections with a width that is marginally smaller than the diameter of the tested cells. Thus, single cells driven through such a constriction channel are squeezed and deformed by the channel walls. Due to the capacity of resembling

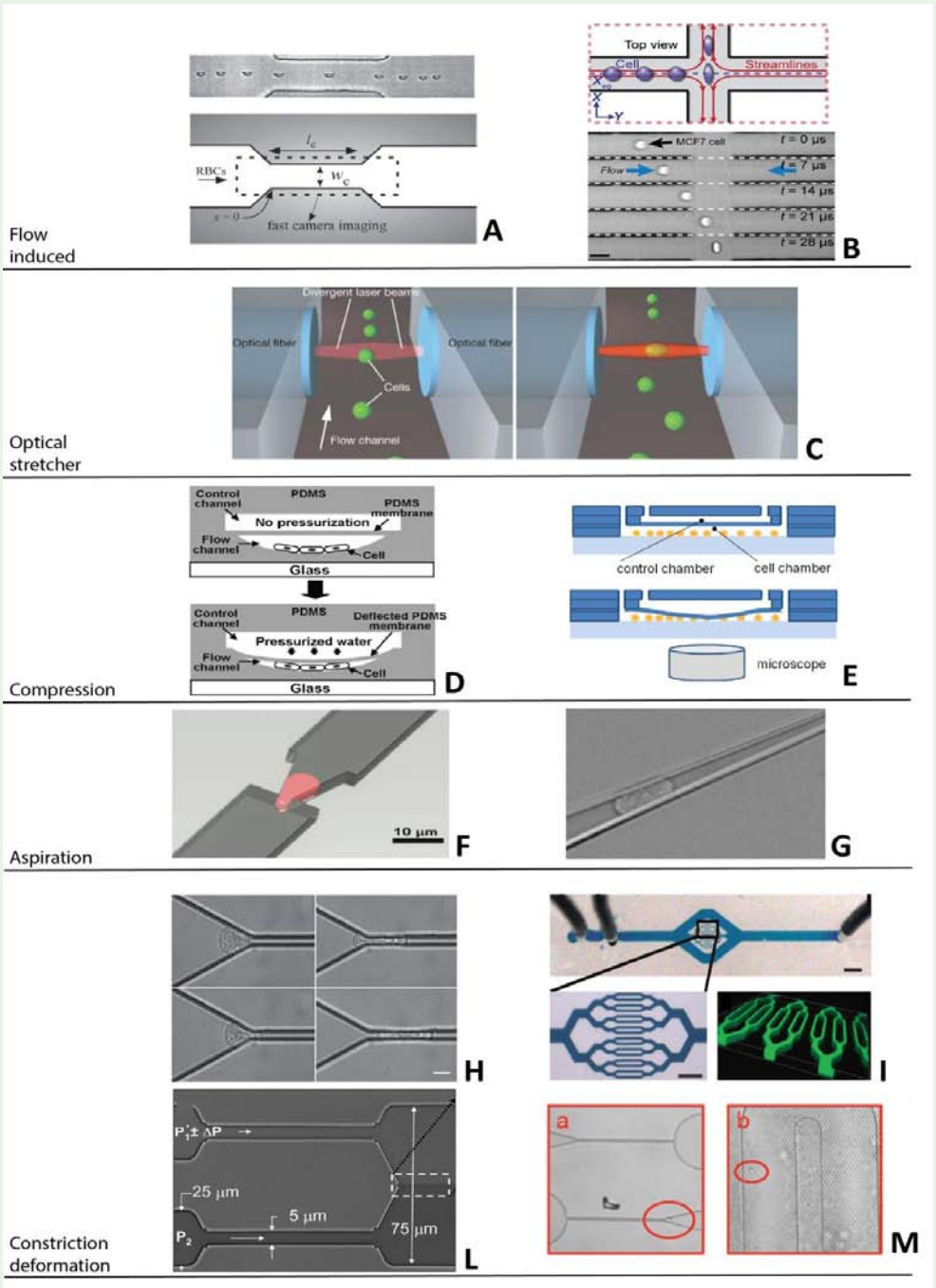

Figure 1: Examples of microfluidic tools for mechanical characterization of circulating cells.
Flow induced: cells can be deformed by flow in a narrow channel (A) or in two orthogonal channels (B). Optical stretcher: cells are deformed in a microchannel by two laser beams located at both sides of the channel (C). Compression: cells are compressed by an integrated deformable membrane (D-E). Aspiration: the method of conventional micropipette aspiration can be adapted in microfluidic devices as funnel-shaped constrictions (F) or pressure deformation in a tapered glass pipette (G). Constriction deformation: cells can be probed in a single narrow constriction (H) or in a branching network (I). The concept of cell deformation in the channel can be adapted by investigating different parameters, such as hydrodynamic resistance (L), or by coupling this system with other procedures, such as chemical manipulation (M). (A) Reprinted from [83] with permission from Elsevier (http://dx.doi.org/10.1016/j.mvr.2010.03.008). (B) Adapted from [87] with PNAS permission (http://dx.doi.org/10.1073/pnas.1200107109). (C) Reprinted from [21] with permission from Elsevier (http://dx.doi.org/10.1529/biophysj.104.045476). (D) Reprinted from [102] with permission from Elsevier (http://dx.doi.org/10.1016/j.snb.2007.05.050). (E) Adapted from [104] with permission. (F) Adapted from [108] with permission of The Royal Society of Chemistry (http://dx.doi.org/ 10.1039/C2LC40205J). (G) Adapted from [111] with permission. (H) Reprinted from [8] with permission from Elsevier (http://dx.doi.org/10.1016/j.bpj.2009.02.037). (I) Adapted from [50] with permission of The Royal Society of Chemistry (http://dx.doi.org/10.1039/b802931h). (L) Adapted from [112] with PNAS permission (http://dx.doi.org/1073/pnas.0507171102) (Copyright (2006) National Academy of Sciences, U.S.A.). (M) Adapted from [110] with permission.

the constrictive situation of an in-vivo capillary, this approach bears physiological relevance since it enables to give insight into blood cell behavior flowing through in vivo capillaries. The system can be easily adapted to different cell types by changing the flow rate, which determines the driving force exerted to the cell, and the width of the constriction, which determines the amount of deformation on the cell.

Cell deformability can be related to multiple trafficking parameters in the constriction, such as entry time, transit time, cell elongation, cell velocity and shape recovery time. These parameters can be determined from recordings made with a high-speed camera connected to a microscope.

Shelby et al. [15] used this approach to characterize RBC deformability and to detect changes between healthy and malaria-infected cells in different stages of the disease. They showed that healthy RBCs were able to flow through the constrictions while stiffer late disease stage RBCs readily blocked the narrow channels (that had a width of 2-4 μm). By observation of the 2 μm constriction channel, Shelby et al. were also able to describe the pitting phenomenon occurring in the spleen where parasites are removed without destroying the RBCs. In a similar way, Handayani et al. [99] confirmed the increased stiffening of malaria-infected RBCs by a constriction-based microfluidic device. Bow et al. [55] developed the so-called "deformability cytometer" by combining the constriction method with fluorescent measurement to study the deformability of malaria-diseased RBCs. Bow et al. performed a simulation of RBCs using a Dissipative Particle Dynamics (DPD) simulation to infer quantitative biomechanical characteristics of individual RBCs and to interpret the effect of the parasite on cell deformability. Bow et al. showed a correlation between mechanical properties, such as entry time, and biochemical properties, such as surface markers related to malaria.

Gabriele et al. [8] used constriction based microfluidic devices for studies of leukocytes. They investigated the role of actin and myosin II on cellular deformation in a constriction channel, mimicking cell sequestration in the lung micro-vessels during acute lung injury (Figure 1H). Gabriele et al. showed that actin organization has a distinct role in entry time and in the extent of cell deformation in the transit stage within the constriction. They also found that cell shape and orientation at the channel entrance can influence entry and transit times. Preira et al. [24] used a comb-like filter in series to separate leukocytes on the basis of cell deformability. They showed that leukocytes of patients affected by acute respiratory distress syndrome got separated in the devices due to increased stiffness compared to cells of healthy subjects.

Rosenbluth et al. [50] developed a device consisting of a network of channels successively bifurcating into increasingly narrower constriction channels to study sepsis and leukostasis state in relation to blood cell transit time in the channels (Figure 1I). The dependence of transit times on cell size and cell deformability was measured to determine a transit time distribution. Rosenbluth et al. demonstrated a clear difference in distribution of transit times between cells affected by leukostatis and healthy cells.

Hou et al. [20] used a constriction-based microfluidic device to distinguish between benign breast epithelial cells and non-metastatic tumor cells by measuring the entry time, the deformation index and the transit velocity. Cancerous cells were described as more deformable than the benign cells in relation to the shorter entry time in the constriction. Zhang et al. [114] used a similar approach based on micro-barriers to detect more deformable cancer cells.

Abkarian et al. [112] developed a novel technique called a microfluidic manometer, similar to a fluidic pressure compactor, to measure the excess pressure drop due to the passage of a cell in a constriction channel (Figure 1L). The setup consists of two identical channels, the reference and the test channels, that are connected downstream. Fluid flow was generated in both the channels but cells were flown only in the test channel. The increase in hydrodynamic resistance due to the presence of the cell in the constriction channel leads to a displacement of the downstream fluid-fluid interface, which correlates to the cell stiffness. They showed pressure drop variations due to changes in the RBCs mechanical properties upon treatment with the fixative drug glutaraldehyde.

Adamo et al. [115] developed a high throughput (up to 14 cells/s) constriction-based device to measure the deformability of suspended Hela cells. They demonstrated that control cells have longer transit time compared to cells treated with drugs depolymerizing actin.

Besides measurement of transit parameters, microfluidic constrictions can be coupled with other measurement techniques to achieve multiple analyses for cell characterization. For instance, Chen et al. [116,117] developed a microfluidic device for electrical and mechanical characterization of single cells. The method is based on impedance spectroscopy combined with cellular deformation. A single cell was deformed in a constriction channel by negative pressure and the cellular impedance was measured via two Ag/AgCl electrodes. They distinguished with high precision breast cancer cells from their multi-drug resistant counterparts, which were treated with anti-cancer drug. Zheng et al. [78] used a similar system for high-throughput (100-150 cells/s) biophysical characterization of RBCs, which may be used eventually for measurement of multiple biophysical disorders in RBCs of patients with sepsis, malaria, or sickle cell anemia.

We developed and used a constriction device that enables to combine cell mechanical characterization with chemical manipulation [118]. The device consists of two crossing channels separated by a porous membrane. This multi-layer device was used to study drug-induced changes in leukocytic cell mechanics by diffusion of chemicals through the porous membrane (Figure 1M). The results showed that the system can detect differences in entry time and cell velocity after the actin disruption agent Cytochalasin-D was delivered to the cells through the porous membrane.

The devices using constrictions to probe cell mechanics give quantitative information of cell mechanical properties. When multiplexing the constriction channels, relatively high throughput can be achieved. A disadvantage is that the range of timescales at which the cell can be characterized is limited because this is set by the flow. Additionally, the cell by definition interacts with the walls through adhesive effects or other (biochemical) interactions which adds an unknown to the analysis.

Table 2: Major challenges for microfluidic tools for single cell mechanical characterization and the possible direction of future research.

Major challenges	Research direction
Sample throughput	Adjust device design or flow parameters to reach clinically relevant cell numbers
Ease-of-use and automation	Integration of automated systems to reduce user intervention and development of software for data analysis
Standardization	Correlation with gold standard biophysical analyses and establishment of standard protocols
Biological validity	Accurate evaluation of the specificity of microfluidic analysis and of the influence of the *in-vitro* microfluidic environment
Limited readouts	Combination with biochemical analyses and detection/isolation within microfluidic chip for further investigations
Clinical relevance	Validation using real patient samples and integration of multiple laboratory function in a compact chip

Conclusions and Outlook

The development of novel platforms for the mechanical manipulation and analysis of single cells is necessary to gain more and quantitative insight into the relation between cell properties and cell function, in particular in a disease state. Microfluidic devices have emerged as a promising approach for mechanical characterization of single cells due to the several small-scale advantages. Clearly, considering the numerous microfluidic approaches to characterize cell mechanics published to date, we conclude that this development has great potential to lead to clinical applications of microfluidic systems as diagnostic or drug screening tools.

Despite the considerable growth in microfluidic development, several challenges remain to be solved to enable the translation from scientific research to clinical application, in particular reliably applying the methods to real patient samples, increasing the throughput, and automation of the analysis. Table 2 indicates the major challenges, in our view, and the research directions needed to tackle them.

Most of the microfluidic tools for mechanical characterization of cells have been tested with cell lines and with a model of the disease. Measurement of actual patient cells usually introduces significant challenges compared to the analysis of the cell lines. Among other factors, clustering upon cell separation and the high adhesive characteristics of patient cells can make single cell analysis in a microfluidic device particularly difficult. Thus, the translation of the microfluidic device to clinical application introduces additional factors to be evaluated. For instance, when testing circulating cells, after leukocytic cells are collected from the blood, cell deformability starts to change. Since many experimental parameters, such as temperature or CO_2 concentration, might affect cell state and deformability, it is essential to establish a standard testing protocol and standard circumstances in which experimental conditions are maintained as constant as possible and cells are tested within a limited period of time.

Another main challenge for microfluidic approaches for cell mechanical characterization is the sample throughput. In order to obtain clinically relevant data, the developed device should be able to sensitively test a large number of cells (at least 50-100 cells/min) within a reasonable time frame (maximum an hour for analyses performed at room temperature). In most published studies, the focus is on analysis of pure populations of cells with a known condition. For a clinical application, a heterogeneous population with cells of an unknown state is tested. Since the device needs to perform the detection and the selection of the diseased cells, the amount of analyzed cells will be significantly higher compared to the current throughput. An accurate and high throughput analysis is also clinically relevant due to the possible rarity of diseased cells within the cell population.

Ease of use and analysis automation is often undervalued in the design of microfluidic chips for research purposes. In order to reach clinical applications, the device should work with minimal user intervention in a fully automated way and the analysis should follow a simple protocol that will result in clear and easy readable outputs. Proper cell selection and analysis of the data need further research and technical development to improve the automation and standardization of microfluidic research systems, protocols, and analysis methods. The development of image analysis software is advised for evaluating cell mechanical properties from videos of the cells flowing in the microfluidic channel, albeit this may not result in real-time readouts. Another desirable solution for the improved automation of a mechanical biosensor might be the integration of a sorting approach to collect the cells with specific mechanical properties for further biochemical analyses.

A final challenge lies in the interpretation of the measured data: when is a property an indicator of diseased or healthy state? This requires careful analysis and validation with clinical data. In fact, the results from microfluidic devices can be used to learn this and improve our understanding.

In conclusion, despite the challenges, microfluidic-based biophysical measurements can potentially be used for single cell analysis and, in combination with biochemical analyses, hold great potential as future tools for clinical point-of-care diagnosis and drug screening platforms.

References

1. Discher D, Dong C, Fredberg JJ, Guilak F, Ingber D, Janmey P, et al. Biomechanics: cell research and applications for the next decade. Ann Biomed Eng. 2009; 37: 847-859.
2. Janmey PA, McCulloch CA. Cell mechanics: integrating cell responses to mechanical stimuli. Annu Rev Biomed Eng. 2007; 9: 1-34.
3. Fletcher DA, Mullins RD. Cell mechanics and the cytoskeleton. Nature. 2010; 463: 485-492.
4. Di Carlo D. A mechanical biomarker of cell state in medicine. J Lab Autom. 2012; 17: 32-42.
5. Ananthakrishnan R, Guck J and Käs J. Cell mechanics: recent advances with a theoretical perspective. Recent Res Devel Biophys. 2006; 5: 39-69.
6. Lincoln B, Erickson HM, Schinkinger S, Wottawah F, Mitchell D, Ulvick S, et al. Deformability-based flow cytometry. Cytometry A. 2004; 59: 203-209.

7. Maniotis A, Chen CS and Ingber DE. Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. Proc Natl Acad Sci. 1997; 94: 849-854.

8. Gabriele S, Benoliel A, Bongrand P and Théodoly O. Microfluidic investigation reveals distinct roles for actin cytoskeleton and myosin II activity in capillary leukocyte trafficking. Biophys J. 2009; 96: 4308-4318.

9. Howard TH, Oresajo CO. The kinetics of chemotactic peptide-induced change in F-actin content, F-actin distribution, and the shape of neutrophils. J Cell Biol. 1985; 101: 1078-1085.

10. Richelme F, Benoliel AM and Bongrand P. The leukocyte actin cytoskeleton. Bull Inst Pasteur. 1996; 94: 257-284.

11. Downey GP, Elson EL, Schwab B, Erzurum SC, Young SK and Worthen GS. Biophysical properties and microfilament assembly in neutrophils: modulation by cyclic AMP. J Cell Biol. 1991; 114: 1179-1190.

12. Ingber DE. Mechanobiology and diseases of mechanotransduction. Ann Med. 2003; 35: 564-577.

13. Suresh S, Spatz J, Mills JP, Micoulet A, Dao M, Lim CT, et al. Connections between single-cell biomechanics and human disease states: gastrointestinal cancer and malaria. Acta Biomater. 2005; 1: 15-30.

14. Lee GY, Lim CT. Biomechanics approaches to studying human diseases. Trends Biotechnol. 2007; 25: 111-118.

15. Shelby JP, White J, Ganesan K, Rathod PK, Chiu DT. A microfluidic model for single-cell capillary obstruction by Plasmodium falciparum-infected erythrocytes. Proc Natl Acad Sci U S A. 2003; 100: 14618-14622.

16. Miller LH, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. Nature. 2002; 415: 673-679.

17. Suresh S. Mechanical response of human red blood cells in health and disease: Some structure-property-function relationships. J Mater Res. 2006; 21: 1871-1877.

18. Suresh S. Biomechanics and biophysics of cancer cells. Acta Mater. 2007; 55: 3989-4014.

19. Lam WA, Rosenbluth MJ, Fletcher DA. Chemotherapy exposure increases leukemia cell stiffness. Blood. 2007; 109: 3505-3508.

20. Hou HW, Li QS, Lee GY, Kumar AP, Ong CN and Lim CT. Deformability study of breast cancer cells using microfluidics. Biomed Microdevices. 2009; 11: 557-564.

21. Guck J, Schinkinger S, Lincoln B, Wottawah F, Ebert S, Romeyke M, et al. Optical deformability as an inherent cell marker for testing malignant transformation and metastatic competence. Biophys J. 2005; 88: 3689-3698.

22. Korb T, Schlüter K, Enns A, Spiegel HU, Senninger N, Nicolson GL, et al. Integrity of actin fibers and microtubules influences metastatic tumor cell adhesion. Exp Cell Res. 2004; 299: 236-247.

23. Katsantonis J, Tosca A, Koukouritaki SB, Theodoropoulos PA, Gravanis A and Stournaras C. Differences in the G/total actin ratio and microfilament stability between normal and malignant human keratinocytes. Cell Biochem Funct. 1994; 12: 267-274.

24. Preira P, Grandné V, Forel JM, Gabriele S, Camara M and Theodoly O. Passive circulating cell sorting by deformability using a microfluidic gradual filter. Lab Chip. 2013; 13: 161-170.

25. Downey GP, Doherty DE, Schwab B, Elson EL, Henson PM, Worthen GS. Retention of leukocytes in capillaries: role of cell size and deformability. J Appl Physiol. 1990; 69: 1767-1778.

26. Ruef P, Craciun E, Altfelder F, Simon C, Frommhold D, Koch L, et al. Effects of phosphodiesterase (III/IV)-inhibitors and cytokines on mechanical properties of neutrophilic granulocytes in neonates and adults. Clin Hemorheol Microcirc. 2010; 45: 301-310.

27. Kim DH, Wong PK, Park J, Levchenko A, Sun Y. Microengineered platforms for cell mechanobiology. Annu Rev Biomed Eng. 2009; 11: 203-233.

28. Rosenbluth MJ, Lam WA, Fletcher DA. Force microscopy of nonadherent cells: a comparison of leukemia cell deformability. Biophys J. 2006; 90: 2994-3003.

29. Mahaffy RE, Shih CK, MacKintosh FC and Käs J. Scanning probe-based frequency-dependent microrheology of polymer gels and biological cells. Phys Rev Lett. 2000; 85: 880-883.

30. Buttrum SM, Drost EM, MacNee W, Goffin E, Lockwood CM, Hatton R, et al. Rheological response of neutrophils to different types of stimulation. J Appl Physiol. 1994; 77: 1801-1810.

31. Needham D, Hochmuth RM. A sensitive measure of surface stress in the resting neutrophil. Biophys J. 1992; 61: 1664-1670.

32. Evans E and Yeung A. Apparent viscosity and cortical tension of blood granulocytes determined by micropipet aspiration. Biophys J. 1989; 56: 151-160.

33. Tsai MA, Frank RS, Waugh RE. Passive mechanical behavior of human neutrophils: power-law fluid. Biophys J. 1993; 65: 2078-2088.

34. Rinker KD, Kirkpatrick AP, Ting-Beall HP, Shepherd RD, Levin JD, Irick J, et al. Linoleic acid increases monocyte deformation and adhesion to endothelium. Atherosclerosis. 2004; 177: 275-285.

35. Thoumine O, Ott A. Time scale dependent viscoelastic and contractile regimes in fibroblasts probed by microplate manipulation. J Cell Sci. 1997; 110 : 2109-2116.

36. Desprat N, Richert A, Simeon J, Asnacios A. Creep function of a single living cell. Biophys J. 2005; 88: 2224-2233.

37. Fernández P, Pullarkat PA, Ott A. A master relation defines the nonlinear viscoelasticity of single fibroblasts. Biophys J. 2006; 90: 3796-3805.

38. Guck J, Ananthakrishnan R, Mahmood H, Moon TJ, Cunningham CC and Käs J. The optical stretcher: a novel laser tool to micromanipulate cells. Biophys J. 2001; 81: 767-784.

39. VM Laurent, S Hénon, E Planus, R Fodil, M Balland, D Isabey, et al. Assessment of mechanical properties of adherent living cells by bead micromanipulation: comparison of magnetic twisting cytometry vs optical tweezers. J Biomech Eng. 2002; 124: 408-421.

40. Irmscher M, de Jong AM, Kress H, Prins MW. Probing the cell membrane by magnetic particle actuation and Euler angle tracking. Biophys J. 2012; 102: 698-708.

41. van Zijp HM, Schot CCMM, De Jong AM, Jongmans N, Van Holten TC, Roest M, et al. Measurement of platelet responsiveness using antibody-coated magnetic beads for lab-on-a-chip applications. Platelets. 2012; 23: 626-632.

42. Hou HW, Lee WC, Leong MC, Sonam S, Vedula SRK and Lim CT. Microfluidics for applications in cell mechanics and mechanobiology. Cell Mol Bioeng. 2011; 4: 591-602.

43. Vanapalli SA, Duits MH, Mugele F. Microfluidics as a functional tool for cell mechanics. Biomicrofluidics. 2009; 3: 12006.

44. Mu X, Zheng W, Sun J, Zhang W, Jiang X. Microfluidics for manipulating cells. Small. 2013; 9: 9-21.

45. Whitesides GM. The origins and the future of microfluidics. Nature. 2006; 442: 368-373.

46. Beebe DJ, Ingber DE, den Toonder J. Organs on Chips 2013. Lab Chip. 2013; 13: 3447-3448.

47. Huh D, Torisawa YS, Hamilton GA, Kim HJ, Ingber DE. Microengineered physiological biomimicry: Organs-on-Chips. Lab Chip. 2012; 12: 2156-2164.

48. Ahrberg CD, Manz A and Chung BG. Polymerase chain reaction in microfluidic devices. Lab Chip. 2016; 16: 3866-3884.

49. Han KN, Li CA, Seong GH. Microfluidic chips for immunoassays. Annu Rev Anal Chem (Palo Alto Calif). 2013; 6: 119-141.

50. Rosenbluth MJ, Lam WA, Fletcher DA. Analyzing cell mechanics in hematologic diseases with microfluidic biophysical flow cytometry. Lab Chip. 2008; 8: 1062-1070.

51. Lin HK, Zheng S, Williams AJ, Balic M, Groshen S, Scher HI, et al. Portable filter-based microdevice for detection and characterization of circulating tumor cells. Clin cancer Res. 2010; 16: 5011-5018.

52. Zheng S, Lin HK, Lu B, Williams A, Datar R, Cote RJ, et al. 3D microfilter device for viable Circulating Tumor Cell (CTC) enrichment from blood. Biomed Microdevices. 2011; 13: 203-213.

53. Cross SE, Jin YS, Rao J and Gimzewski JK. Nanomechanical analysis of cells from cancer patients. Nat Nanotechnol. 2007; 2: 780-783.

54. Wirtz D, Konstantopoulos K, Searson PC. The physics of cancer: the role of physical interactions and mechanical forces in metastasis. Nat Rev Cancer. 2011; 11: 512-522.

55. Bow H, Pivkin IV, Diez-Silva M, Goldfless SJ, Dao M, Niles JC, et al. A microfabricated deformability-based flow cytometer with application to malaria. Lab Chip. 2011; 11: 1065-1073.

56. Saito H. Mechanical properties of rat bone marrow and circulating neutrophils and their responses to inflammatory mediators. Blood. 2002; 99: 2207-2213.

57. Worthen GS, Schwab B, Elson EL and Downeyt GP. Mechanics of stimulated neutrophils: cell stiffening induces retention in capillaries. Science. 1989; 245: 183-186.

58. Du L, Zhou J, Zhang J, Yan M, Gong L, Liu X, et al. Actin filament reorganization is a key step in lung inflammation induced by systemic inflammatory response syndrome. Am J Respir Cell Mol Biol. 2012; 47: 597-603.

59. Chen J, Li J, Sun Y. Microfluidic approaches for cancer cell detection, characterization, and separation. Lab Chip. 2012; 12: 1753-1767.

60. Zheng Y and Sun Y. Microfluidic devices for mechanical characterisation of single cells in suspension. Micro Nano Lett. 2011; 6: 327-331.

61. Lecault V, White AK, Singhal A, Hansen CL. Microfluidic single cell analysis: from promise to practice. Curr Opin Chem Biol. 2012; 16: 381-390.

62. Franke TA, Wixforth A. Microfluidics for miniaturized laboratories on a chip. Chemphyschem. 2008; 9: 2140-2156.

63. Lim CT, Zhou EH, Li A, Vedula SRK and Fu HX. Experimental techniques for single cell and single molecule biomechanics. Mater Sci Eng C. 2006; 26: 1278-1288.

64. Sinton D. Microscale flow visualization. Microfluid Nanofluidics. 2004; 1: 2-21.

65. Saadi W, Rhee SW, Lin F, Vahidi B, Chung BG and Jeon NL. Generation of stable concentration gradients in 2D and 3D environments using a microfluidic ladder chamber. Biomed Microdevices. 2007; 9: 627-635.

66. Jeon NL, Dertinger SKW, Chiu DT, Choi IS, Stroock AD and Whitesides GM. Generation of solution and surface gradients using microfluidic systems. Langmuir. 2000; 16: 8311-8316.

67. Dertinger SKW, Chiu DT, Jeon NL and Whitesides GM. Generation of gradients having complex shapes using microfluidic networks. Anal Chem. 2001; 73: 1240-1246.

68. Nguyen NT, Shaegh SA, Kashaninejad N, Phan DT. Design, fabrication and characterization of drug delivery systems based on lab-on-a-chip technology. Adv Drug Deliv Rev. 2013; 65: 1403-1419.

69. Doerschuk CM, Beyers N, Coxson HO, Wiggs B and Hogg JC. Comparison of neutrophil and capillary diameters and their relation to neutrophil sequestration in the lung. J Appl Physiol. 1993; 74: 3040-3045.

70. Stone HA, Stroock AD and Ajdari A. Engineering flows in small devices: microfluidics toward a lab-on-a-chip. Annu Rev Fluid Mech. 2004; 36: 381-411.

71. Campbell CJ and Grzybowski BA. Microfluidic mixers: from microfabricated to self-assembling devices. Philos Trans R Soc London Ser A. 2004; 362: 1069-1086.

72. Stroock AD, Dertinger SK, Whitesides GM, Ajdari A. Patterning flows using grooved surfaces. Anal Chem. 2002; 74: 5306-5312.

73. Gao Y, van Reenen A, Hulsen MA, de Jong AM, Prins MWJ and den Toonder JMJ. Chaotic fluid mixing by alternating microparticle topologies to enhance biochemical reactions. Microfluid Nanofluidics. 2014; 16: 265-274.

74. Kang TG, Hulsen MA, Anderson PD, den Toonder JM, Meijer HE. Chaotic mixing induced by a magnetic chain in a rotating magnetic field. Phys Rev E Stat Nonlin Soft Matter Phys. 2007; 76: 066303.

75. Toner M, Irimia D. Blood-on-a-chip. Annu Rev Biomed Eng. 2005; 7: 77-103.

76. Dimov IK, Basabe-Desmonts L, Garcia-Cordero JL, Ross BM, Park Y, Ricco AJ, et al . Stand-alone self-powered integrated microfluidic blood analysis system (SIMBAS). Lab Chip. 2011; 11: 845-850.

77. Beech JP, Holm SH, Adolfsson K, Tegenfeldt JO. Sorting cells by size, shape and deformability. Lab Chip. 2012; 12: 1048-1051.

78. Zheng Y, Shojaei-Baghini E, Azad A, Wang C and Sun Y. High-throughput biophysical measurement of human red blood cells. Lab Chip. 2012; 12: 2560-2567.

79. Handayani S, Chiu DT, Tjitra E, Kuo JS, Lampah D, Kenangalem E, et al. High deformability of Plasmodium vivax-infected red blood cells under microfluidic conditions. J Infect Dis. 2009; 199: 445-450.

80. Yaginuma T, Oliveira MSN, Lima R, Ishikawa T and Yamaguchi T. Human Red Blood Cell Behavior under Homogeneous Extensional Flow in a Hyperbolic-Shaped Microchannel. Biomicrofluidics. 2013; 7: 054110.

81. Herricks T, Antia M and Rathod PK. Deformability limits of Plasmodium falciparum-infected red blood cells. Cell Microbiol. 2009; 11: 1340-1353.

82. Mills JP, Qie L, Dao M, Lim CT and Suresh S. Nonlinear elastic and viscoelastic deformation of the human red blood cell with optical tweezers. Mech Chem Biosyst. 2004; 1: 169-180.

83. Forsyth AM, Wan J, Ristenpart WD and Stone HA. The dynamic behavior of chemically 'stiffened' red blood cells in microchannel flows. Microvasc Res. 2010; 80: 37-43.

84. Higgins JM, Eddington DT, Bhatia SN, Mahadevan L. Sickle cell vasoocclusion and rescue in a microfluidic device. Proc Natl Acad Sci U S A. 2007; 104: 20496-20500.

85. Nam J, Lim H, Kim D, Shin S. Separation of platelets from whole blood using standing surface acoustic waves in a microchannel. Lab Chip. 2011; 11: 3361-3364.

86. Shevkoplyas SS, Yoshida T, Munn LL, Bitensky MW. Biomimetic autoseparation of leukocytes from whole blood in a microfluidic device. Anal Chem. 2005; 77: 933-937.

87. Gossett DR, Tse HTK, Lee SA, Ying Y, Lindgren AG, Yang OO, et al. Hydrodynamic stretching of single cells for large population mechanical phenotyping. Proc Natl Acad Sci. 2012; 109: 7630-7635.

88. Wuang SC, Ladoux B and Lim CT. Probing the chemo-mechanical effects of an anti-cancer drug emodin on breast cancer cells. Cell Mol Bioeng. 2011; 4: 466-475.

89. Korin N, Bransky A, Dinnar U. Theoretical model and experimental study of red blood cell (RBC) deformation in microchannels. J Biomech. 2007; 40: 2088-2095.

90. Lee SS, Yim Y, Ahn KH and Lee SJ. Extensional flow-based assessment of red blood cell deformability using hyperbolic converging microchannel. Biomed. Microdevices. 2009; 11: 1021-1027.

91. Katsumoto Y, Tatsumi K, Doi T and Nakabe K. Electrical classification of single red blood cell deformability in high-shear microchannel flows. Int J Heat Fluid Flow. 2010; 31: 985-995.

92. Sraj I, Eggleton CD, Jimenez R, Hoover E, Squier J, Chichester J, et al. Cell deformation cytometry using diode-bar optical stretchers. J Biomed Opt. 2010; 15: 047010.

93. Ashok PC. Optical trap as a micro-manipulation tool in biology. 1-15.

94. Teo SK, Goryachev AB, Parker KH and Chiam KH. Cellular deformation and intracellular stress propagation during optical stretching. Phys Rev E Stat Nonlin Soft Matter Phys. 2010; 81: 051924.

95. Bellini N, Vishnubhatla KC, Bragheri F, Ferrara L, Minzioni P, Ramponi R, et al. Femtosecond laser fabricated monolithic chip for optical trapping and stretching of single cells. Opt. Express. 2010; 18: 4679-4688.

96. Lautenschläger F, Paschke S, Schinkinger S, Bruel A, Beil M, Guck J. The regulatory role of cell mechanics for migration of differentiating myeloid cells. Proc Natl Acad Sci USA. 2009; 106: 15696-15701.

97. Lai CW, Hsiung SK, Yeh CL, Chiou A and Lee GB. A cell delivery and pre-positioning system utilizing microfluidic devices for dual-beam optical trap-and-stretch. Sensors Actuators B Chem. 2008; 135: 388-397.

98. Guck J, Ananthakrishnan R, Moon TJ, Cunningham CC, Käs J. Optical deformability of soft biological dielectrics. Phys Rev Lett. 2000; 84: 5451-5454.

99. Mauritz JMA, Tiffert T, Seear R, Lautenschläger F, Esposito A, Lew VL, et al. Detection of Plasmodium falciparum-infected red blood cells by optical stretching. J Biomed Opt. 2010; 15: 1-3.

100. Unger MA, Chou HP, Thorsen T, Scherer A and Quake SR. Monolithic microfabricated valves and pumps by multilayer soft lithography. Science. 2000; 288: 113-116.

101. Hohne DN, Younger JG and Solomon MJ. Flexible microfluidic device for mechanical property characterization of soft viscoelastic solids such as bacterial biofilms. Langmuir. 2009; 25: 7743-7751.

102. Kim YC, Kang JH, Park SJ, Yoon ES and Park JK. Microfluidic biomechanical device for compressive cell stimulation and lysis. Sensors Actuators B Chem. 2007; 128: 108-116.

103. Kim YC, Park SJ, Park JK. Biomechanical analysis of cancerous and normal cells based on bulge generation in a microfluidic device. Analyst. 2008; 133: 1432-1439.

104. Du G, Ravetto A, Fang Q and den Toonder JMJ. Cell types can be distinguished by measuring their viscoelastic recovery times using a microfluidic device. Biomed Microdevices. 2011; 13: 29-40.

105. Liu X, Wang Y and Sun Y. Cell Contour Tracking and Data Synchronization for Real-Time, High-Accuracy Micropipette Aspiration. IEEE Transactions on Automation Science and Engineering. 2009; 6: 536-543.

106. Tan WH, Takeuchi S. A trap-and-release integrated microfluidic system for dynamic microarray applications. Proc Natl Acad Sci U S A. 2007; 104: 1146-1151.

107. Kim W and Han A. A micro-aspirator chip using vacuum expanded microchannels for high-throughput mechanical characterization of biological cells. MicroTAS. 2010; 253-255.

108. Guo Q, Park S, Ma H. Microfluidic micropipette aspiration for measuring the deformability of single cells. Lab Chip. 2012; 12: 2687-2695.

109. Guo Q, Reiling SJ, Rohrbach P, Ma H. Microfluidic biomechanical assay for red blood cells parasitized by Plasmodium falciparum. Lab Chip. 2012; 12: 1143-1150.

110. Gifford SC, Derganc J, Shevkoplyas SS, Yoshida T, Bitensky MW. A detailed study of time-dependent changes in human red blood cells: from reticulocyte maturation to erythrocyte senescence. Br J Haematol. 2006; 135: 395-404.

111. Ravetto A, Wyss HM, Anderson PD, den Toonder JM, Bouten CV. Monocytic cells become less compressible but more deformable upon activation. PLoS One. 2014; 27: 9.

112. Abkarian M, Faivre M and Stone HA. High-speed microfluidic differential manometer for cellular-scale hydrodynamics. Proc Natl Acad Sci. 2006; 103: 538-542.

113. Preira P, Leoni T, Valignat M, Lellouch A, Robert P, Forel J, et al. Microfluidic tools to investigate pathologies in the blood microcirculation. Int J Nanotechnol. 2012; 9: 529-547.

114. Zhang W, Kai K, Choi DS, Iwamoto T, Nguyen YH, Wong H, et al. Microfluidics separation reveals the stem-cell-like deformability of tumor-initiating cells. Proc Natl Acad Sci. 2012; 109: 18707-18712.

115. Adamo A, Sharei A, Adamo L, Lee B, Mao S, Jensen KF. Microfluidics-based assessment of cell deformability. Anal Chem. 2012; 84: 6438-6443.

116. Chen J, Zheng Y, Tan Q, Shojaei-Baghini E, Zhang YL, Li J, et al. Classification of cell types using a microfluidic device for mechanical and electrical measurement on single cells. Lab Chip. 2011; 11: 3174-3181.

117. Chen J, Zheng Y, Tan Q, Zhang YL, Li J, Geddie WR, et al. A microfluidic device for simultaneous electrical and mechanical measurements on single cells. Biomicrofluidics. 2011; 5: 14113.

118. Ravetto A, Hoefer IE, den Toonder JM, Bouten CV. A membrane-based microfluidic device for mechano-chemical cell manipulation. Biomed Microdevices. 2016; 18: 31.