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Research review paper

Mechanical properties of single cells: Measurement methods and applications

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ABSTRACT

Cell mechanical properties, e.g. elastic and shear modulus, play vital roles in cell activities and functions, such as cell growth, cell division, cell motion, and cell adhesion. Measurement of single-cell mechanical properties has attracted great interest from both academia and industry, due to its importance in a variety of applications, such as cell separation, disease diagnostics, immune status analysis and drug screening. Therefore, accurate, robust and sensitive methods for measuring the mechanical properties of single cells are highly desired. In this review, we classify ten most commonly used methods for measuring single-cell mechanical properties into three main categories based on measurement locations, (1) cell surface (2) cell interior and (3) whole cell, and discuss their utilizations with examples. In addition, we discuss directions for future research, such as improving throughput, automating the probing of cell mechanical properties and integrating different methods to achieve simultaneous measurements of mechanical properties of both cell surface and interior. The above are all necessary to overcome the limitations of current technologies in the mechanical characterization of single cells.

1. Introduction

Mechanobiology focuses on the interactions between mechanical stimuli and cellular biology, which includes (1) the mechanisms of cells to sense, transduce and respond to mechanical stimuli and (2) the characterization of cellular mechanical properties (Kim et al., 2009). The intrinsic and extrinsic mechanical forces have significant impacts on cell behaviours and tissue homeostasis (Panciera et al., 2017), and alterations of mechanical forces can lead to the remodeling of tissues, such as bone (Galea et al., 2017; Sundh et al., 2018) and blood vessels (Liu et al., 2018b; Min and Schwartz, 2019; van Haaften et al., 2018). In addition, mechanical forces are related to the cell fate switching, pattern formation, tissue development in embryo, stem cell differentiation and function in adult tissue (Mammoto et al., 2013). A variety of tools have been developed to study the mechanobiology of cells, such as microfluidic platforms to characterize the effects of shear stress, interstitial flow and stiffness gradient on cells (Polacheck et al., 2013), and cellular force measurement techniques with the assistance of image processing algorithm (Ghanbari et al., 2012; Liu et al., 2007). More detailed information about mechanobiology and techniques used to measure the

Abbreviations: AMs, Acoustic methods; ABZ, Albendazole; AFM, Atomic force microscope; CS, Chitosan; CLL, Chronic lymphocytic leukemia; CTCs, Circulating tumour cells; CMC, Complement-mediated cytotoxicity; DNN, Deep neural network; DC, Deformability cytometry; DLD, Deterministic lateral displacement; DEP, Dielectrophoresis; ECM, Extracellular matrix; *E. histolytica*, *Entamoeba histolytica*; *E. coli*, *Escherichia coli*; fs, Femtosecond; FACS, Fluorescence-activated cell sorting; FIB, Focused ion beam; HS-AFM, High-speed AFM; HUVECs, Human umbilical vein endothelial cells; IRM, Interference reflection microscopy; IAF, Iso-acoustic focusing; LTM, Laser tracking microrheology; LINC, Linker of the nucleoskeleton and cytoskeleton; MDCK, Madin-Darby canine kidney; MTs, Magnetic tweezers; MTC, Magnetic twisting cytometry; MMs, Microfluidic methods; MA, Micropipette aspiration; mAFM, Modified atomic force microscopy; MORE, Morpho-rheological; NETs, Neutrophil extracellular traps; NLRC4, NLR family CARD domain-containing protein 4; OS, Optical stretcher; OTs, Optical tweezers; PPT, Parallel-plate technique; PTM, Particle-tracking microrheology; RT-FDC, Real-time fluorescence and deformability cytometry; RBCs, Red blood cells; SNACS, Size-normalized acoustic scattering; SSAW, Standing surface acoustic waves; SMR, Suspended microchannel resonator; VICs, Valve interstitial cells.

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response of cells to mechanical stimuli, such as stretching, surface morphology and surface stiffness can be referred to recent review articles by other research groups (Darnell and Mooney, 2017; Kamble et al., 2016; Matellan and del Río Hernández, 2018; Mehlenbacher et al., 2017; Roca-Cusachs et al., 2017).

Here, we mainly concentrate on the mechanical properties of cells. Cell mechanical properties refer to the deformability or the resistance to deformation of a cell when subjected to mechanical forces. Assessment of mechanical properties of cells implies the characterization of cell deformation in response to the mechanical force over time, which can be described by the theory of stress and strain. With different deformation modes (e.g. linear extension, shear deformation), different parameters (e.g. Young's modulus, shear modulus) can be obtained to depict the mechanical properties of cells (Mohammed et al., 2019). Due to the viscoelastic properties, cells can exhibit elastic and viscous characteristics simultaneously, resulting in the stress relaxation (decrease of the required stress to keep a constant strain) or creep (increase of strain under a constant stress). The mechanical properties of cellular components (e.g. cell membrane, cytoskeleton, nucleus and other organelles) are integral of whole-cell mechanical properties (Starodubtseva, 2011). Investigating the mechanical properties of cellular components and the linkage between these components can contribute to the insight of the comprehensive mechanical properties of cells. Cell membrane, cytoskeleton, nucleus and other organelles establish a thoroughly connected network and play important roles in the mechanobiology of single cells. The membrane can distribute the external loads applied on cells to the compressive and tensile components inside cells, and transduce the stresses and strains from the cell membrane through the cytoskeleton to the nucleus. In addition, the cytoskeleton is mainly composed of microfilaments, intermediate filaments and microtubules. The microfilaments can not only maintain the positions of intracellular organelles but also resist the overall deformation induced by external stimuli, while the intermediate filaments can resist tension and bear larger deformation (300% strain) without breaking (Qin et al., 2009) and establish a thoroughly connected network by connecting with other cytoskeletal elements, organelles and cell membrane. The microtubules are conducive to the single-cell mechanical behaviours by resisting compression. As the mechanosensory destination, nucleus is tightly integrated into the cell structural network through the linker of the nucleoskeleton and cytoskeleton (LINC) complexes, and plays a central role in the mechanosensing and mechanotransduction of single cells by regulating mechanical behaviours under external stimuli (Isermann and Lammerding, 2013). Single-cell analysis, the study of cell-to-cell variations within a cell population, is an effective method to characterize diseases,

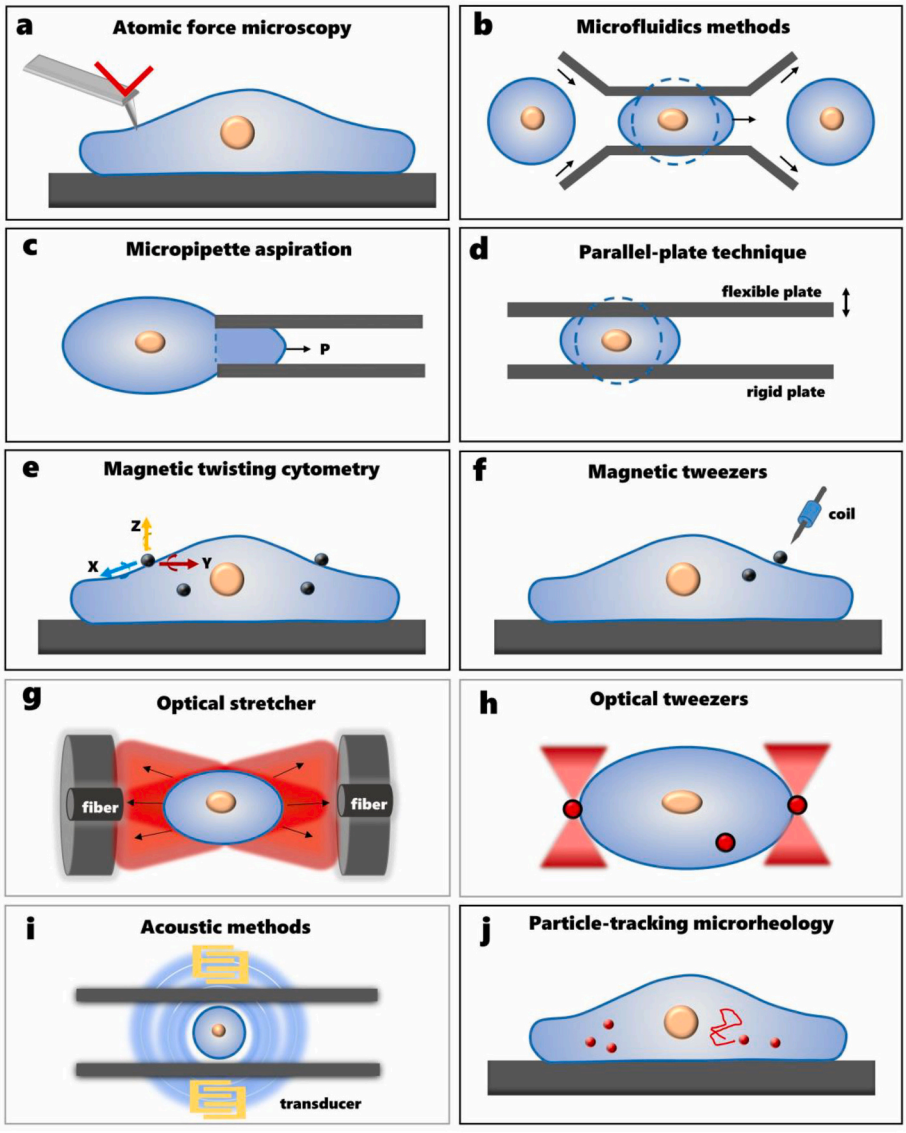

Fig. 1. Schematic illustration of different methods used for measuring single-cell mechanical properties. (a) Atomic force microscope (AFM). (b) Microfluidics methods (MMs). (c) Micropipette aspiration (MA). (d) Parallel-plate technique (PPT). (e) Magnetic twisting cytometry (MTC). (f) Magnetic tweezers (MTs). (g) Optical stretcher (OS). (h) Optical tweezers (OTs). (i) Acoustic methods (AMs). (j) Particle-tracking microrheology (PTM). Please note that measurement modes (contact or non-contact) are indicated by colors of boxes: contact (black) and non-contact (gray). MMs can work with three main approaches: micro-constriction, extensional flow and shear flow, the micro-constriction approach (b) is a contact measurement while the extensional flow and shear flow approaches are non-contact.

accelerate drug development, identify stem cell differentiation and uncover cancer and physiological functions in embryos and adults (Heath et al., 2015; Hodzic, 2016; Lawson et al., 2015; Mizrak et al., 2019). Previous studies have demonstrated that the mechanical properties of single cells have significant impacts on cell activities, such as cell growth, cell division, cell motion, and cell adhesion (Borowska-wykre et al., 2012; Gudipaty et al., 2017). Moreover, the capability to measure the changes in mechanical properties (e.g. Young's modulus and shear modulus) at the single-cell level has been demonstrated to be useful for a wide range of applications, such as cell separation (Ding et al., 2014; Otto et al., 2015), disease diagnostics (Darling and Di Carlo, 2015; Remmerbach et al., 2009; Suresh, 2007; Swaminathan et al., 2011; Yadav et al., 2019), immune status analysis (De Vlaminck et al., 2014) and drug screening (Kavallaris, 2010; Zhang et al., 2015). Therefore, accurate, robust and sensitive methods for measuring the mechanical properties of single cells are highly demanded.

This manuscript is an overview of the measurement methods (Fig. 1) used to assess cell mechanical properties. Atomic force microscope (AFM, Fig. 1a) was developed in 1986 and was initially used to acquire images of hard surface samples with atomic resolution (Binnig et al., 1986). Radmacher et al. (1992) pioneered the use of AFM to measure living cells in 1992, and AFM is now one of the most commonly used approaches to measuring single-cell mechanical properties due to its high accuracy. Microfluidic methods (MMs, Fig. 1b) have been used to characterize single-cell mechanical properties with a high-throughput in the range of 10^3–10^4 cells/s (Calistri et al., 2018; Chen et al., 2011; Whitesides, 2006; Zheng et al., 2013) for a range of biomedical and clinical applications (Liu et al., 2018a; Serra et al., 2017; Yamada et al., 2017a). Micropipette aspiration (MA, Fig. 1c) was invented in 1954 to measure the surface tension of a sea urchin egg on the cell membrane (Mitchison and Swann, 1954). MA remains as one of the primary methods to obtain the viscoelastic properties of both cell local regions and whole cell and is known to have measurement accuracy comparable to that of AFM. Other widely used active methods for single-cell mechanical properties measurements are based on the involvement of external fields (e.g. mechanical torque, magnetic, optical, and acoustic fields) and they include parallel-plate technique (PPT, Fig. 1d), magnetic twisting cytometry (MTC, Fig. 1e) (Chen et al., 2016b; Zhang et al., 2017), magnetic tweezers (MTs, Fig. 1f) (De Vlaminck and Dekker, 2012), optical stretcher (OS, Fig. 1g) (Yang et al., 2016a), optical tweezers (OTs, Fig. 1h) (Khakshour et al., 2017), and acoustic methods (AMs, Fig. 1i) (Hartono et al., 2011). Particle-tracking microrheology (PTM, Fig. 1j) (Lozoya et al., 2016) is a passive method that can measure single-cell mechanical properties by recording the random motion of fluorescent beads in cells using a high-magnification fluorescence microscope. Besides the methods introduced above, there are other possible methods for measuring single-cell mechanical properties, such as dielectrophoresis (DEP). Researches have used DEP for investigating the deformation of cells (Guido et al., 2012; Teng et al., 2017; Urbano and Clyne, 2016). Despite the capability for the single-cell deformation measurement, there are very few DEP studies about using exact parameters to describe the mechanical properties of single cells, such as elastic modulus and shear modulus (Engelhardt and Sackmann, 1988; Haque et al., 2015). Besides, there is a lack of comprehensive models for quantitatively profiling of cells stretched by DEP, which may result in the inaccurate qualification of single-cell mechanical properties, such as the overestimation of shear modulus under large deformation (Qiang et al., 2018). Therefore, we will not discuss the DEP in detail here.

With great advances in measurement techniques and instruments, researchers have achieved the measurement and manipulation of cells with forces at the piconewton level and displacements at the nanometer scale (Bao and Suresh, 2003). For example, MTs and OTs enable piconewton force measurements when cells are subjected to deformation in the range of 5–10^5 and 0.1–10^5 nm. AFM can detect forces ranging from piconewton to micronewton when cells are deformed in the range of 0.5 to 10^4 nm (Neuman and Nagy, 2008). MMs stand out due to their high-throughput capability for measuring single-cell mechanical properties in the order of 10^3–10^4 cells/s, which can greatly reduce the burden of cytopathologists and accelerate the sample testing processes. Moreover, some of the above-mentioned methods have also been modified to improve their performance and applications in probing specific cellular mechanical properties. For example, modified atomic force microscopy (mAFM) with specific tips has been proposed to characterize intracellular and nuclear mechanical properties that would otherwise be challenging to achieve with the standard AFM (Liu et al., 2015).

Based on measurement locations, methods used to measure the mechanical properties of cells can be categorized into two groups: methods for measuring the mechanical properties of single cells at local regions (including cell surface and cell interior) and whole-cell scale (see Fig. 2). In methods that measure the mechanical properties of single cells at local regions, AFM (including mAFM), MTC and MTs can measure the mechanical properties both on cell surface and cell interior, but other methods like MA can only provide measurements on the cell surface, while PTM and OTs are both limited to the intracellular measurements. Methods for measuring the mechanical properties of single cells at the whole-cell scale include AFM, AMs, MMs, MA, MTC, OTs, OS and PPT. Please note that we mainly focus on passive mechanical properties of single cells, active mechanical properties, such as muscle cell traction and cardiomyocyte beating are not discussed in this review article.

2. Measurement methods for mechanical properties of single cells

In this section, different methods for measuring single-cell mechanical properties will be introduced with working principles, technological characteristics and examples in detail. Each method will be discussed based on the measurement locations (i.e. at the cell surface, cell interior or whole cell). Some methods are capable of measuring at different locations, such as AFM, MA, MTC, MTs and OTs, while some methods can only perform the measurement at a single position, for example, PTM can only perform the intracellular measurement and MMs, PPT, OS and AMs can only deform the whole cell to obtain the mechanical properties.

2.1. Atomic force microscopy

In AFM, a micro-fabricated flexible cantilever beam with a tip is used to indent a cell. The deflection of the cantilever beam, which represents the cell deformation is measured by a laser. The capabilities of AFM have been enhanced significantly in terms of multi-parameter, multi-frequency and high-speed measurements since its invention in 1986 (Dufrêne et al., 2017). For example, high-speed AFM (HS-AFM) can shorten the image acquisition time by 1000 folds, allowing the dynamic mechanical phenotypes of single cells or even single molecules. When using AFM to measure the mechanical properties of single cells, the effect of probe geometry must be taken into consideration, as probes with different geometric parameters, such as shapes and radii may provide different moduli for a given sample. For example, the elastic modulus of MCF-7 cells obtained with a conical probe is about 9 times higher than that obtained with a spherical probe under the same experimental conditions (Wu et al., 2018). In another research, AFM with spherical tips could get more accurate values of cell elasticity, but pyramidal tips under medium loads (i.e. 3nN) could result in extraneous contact between AFM tip and cell surface, overestimating the elasticity values (Harris and Charras, 2011). Furthermore, different contact positions of probes could also result in different measurement results. For example, the elastic modulus of the region near the nucleus is different to that of the cell edge region (Aryaei and Jayasuriya, 2013; Berdyyeva et al., 2004).

AFM is commonly used to measure the mechanical properties at cell local regions, such as cell surface and nucleus. AFM-based broad modulus range nanomechanical mapping method (Fig. 3a) can achieve the quantification of elastic moduli in the range of 1 kPa to 20 GPa at

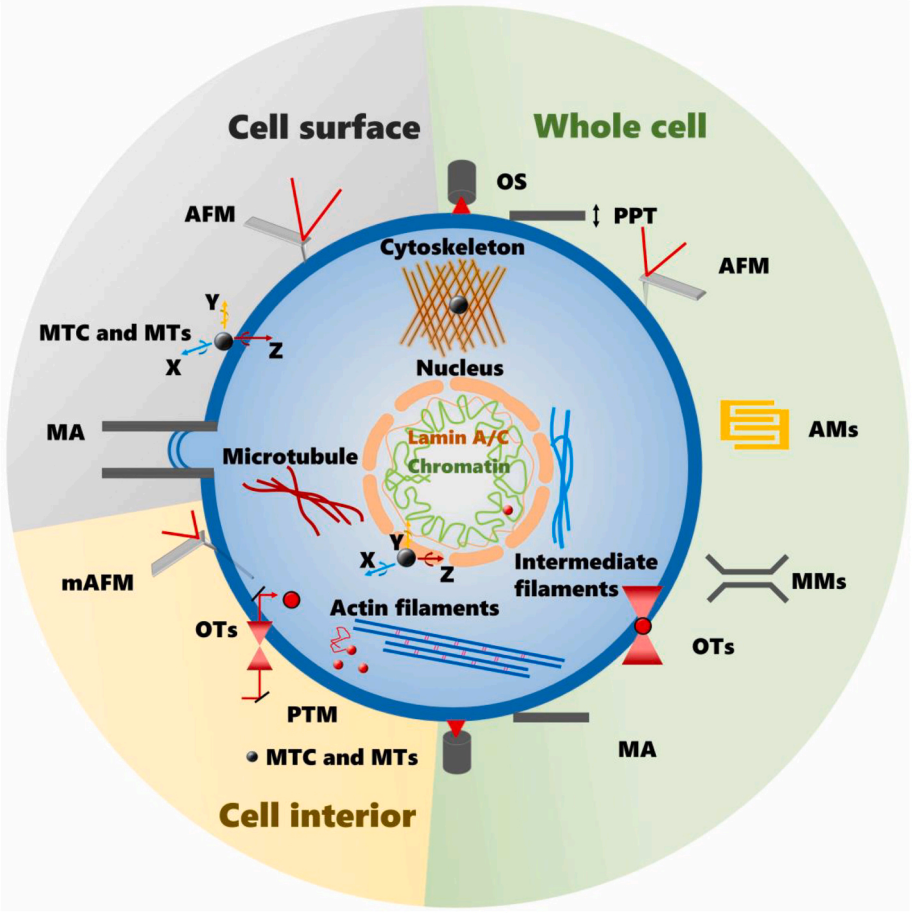

Fig. 2. A summary of various methods for measuring single-cell mechanical properties based on different measurement locations: cell surface (gray), cell interior (yellow) and whole cell (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sample surface by directly measuring the force and indentation depth during the acquisition of force-distance curves. This method can characterize the surface of Escherichia coli (*E. coli*) cells and quantify the elastic modulus of flagella with an average width of 29.37 ± 14.73 nm, which shows great potentials in probing single-cell mechanical properties for a broad range of applications, such as investigation of mechanoresponse to stimuli and characterization of mechanical dynamics of heterogeneous surfaces (Meng et al., 2017). Hosokawa et al. (2011) used AFM to quantify the impulsive forces induced by a femtosecond (fs) laser pulse. When a fs laser pulse focused in the vicinity of a targeted cell, shockwaves and stress waves acting as impulsive forces on the cell are produced at the laser focal point (Emmony et al., 1976; Yasukuni et al., 2017). Conventional AFM measurement usually utilizes the probe of AFM to indent cells, however, Hosokawa et al. found that the force applied on cells can be replaced with the impulsive force induced by fs laser in a non-contact manner. This method has been used to measure the required impulsive force to break the intercellular adhesion of HL-60 leukocytes attached to human umbilical vein endothelial cells (HUVECs) and Madin-Darby canine kidney (MDCK) cells monolayer as well as quantify the adhesion strength between mast cells and neurite cells with a throughput of >100 cells/h (Iino et al., 2016). Moreover, the capability of this non-contact method for quantifying mechanical interactions in both animal and plant cells, e.g. zebrafish embryonic epithelia and palisade mesophyll cells, has been demonstrated (Oikawa et al., 2015; Yamada et al., 2017b).

Modified AFM with special tips has been used to measure the mechanical properties of intracellular organelles. Current technologies to modify AFM tips can be categorized as follows: (1) focused ion beam (FIB) method and (2) integration of nanowires or nanotubes on traditional AFM tips (Liu et al., 2015). The modified AFM with a special needle tip (Fig. 3b) was developed to measure the mechanical properties of the cell nucleus in situ without disturbing the activities of living cells. The results revealed that the isolated nucleus of fibroblast-like valve interstitial cells (VICs) had significantly lower Young's moduli than the intact nucleus in situ, and the VICs cultured on a rigid substrate exhibited higher Young's moduli than the VICs on soft one. Besides, the less metastatic RT4 cells had higher membrane/cytoplasm and nuclei stiffness than T24 cells in the higher metastatic stage (Fig. 3b right) (Liu et al., 2014). Given that AFM with modified tip has great potential to penetrate the cell membrane without disturbing the viability of living cells, this method is likely to help discover the mechanical phenotypes of intracellular organelles.

Besides measuring the mechanical properties of cell local regions (e.g. cell surface and nucleus), AFM can also be used to measure the mechanical properties of whole cell with selected cantilever having appropriate stiffness (i.e. 0.01–0.06 N/m) and probe geometry (i.e. a spherical tip of approximately 5 μm) (Yim et al., 2010). AFM is usually combined with some advanced theories, such as Hertz contact theory, exponential equation, and parallel-spring recruitment model to comprehensively describe the whole-cell mechanical properties (Jaasma et al., 2006). Pogoda et al. (2012) demonstrated the depth-sensing analysis of mechanical properties of living fibroblasts and found that Young's modulus values of whole cell decreased with the increase of indention depths. They also performed experiments to distinguish two human melanomas (i.e. WM35 and A375) based on the proposed depth-sensing theory and presented the capability to identify cancer cells with indentation larger than 500 nm.

AFM is a classical method for measuring mechanical properties of

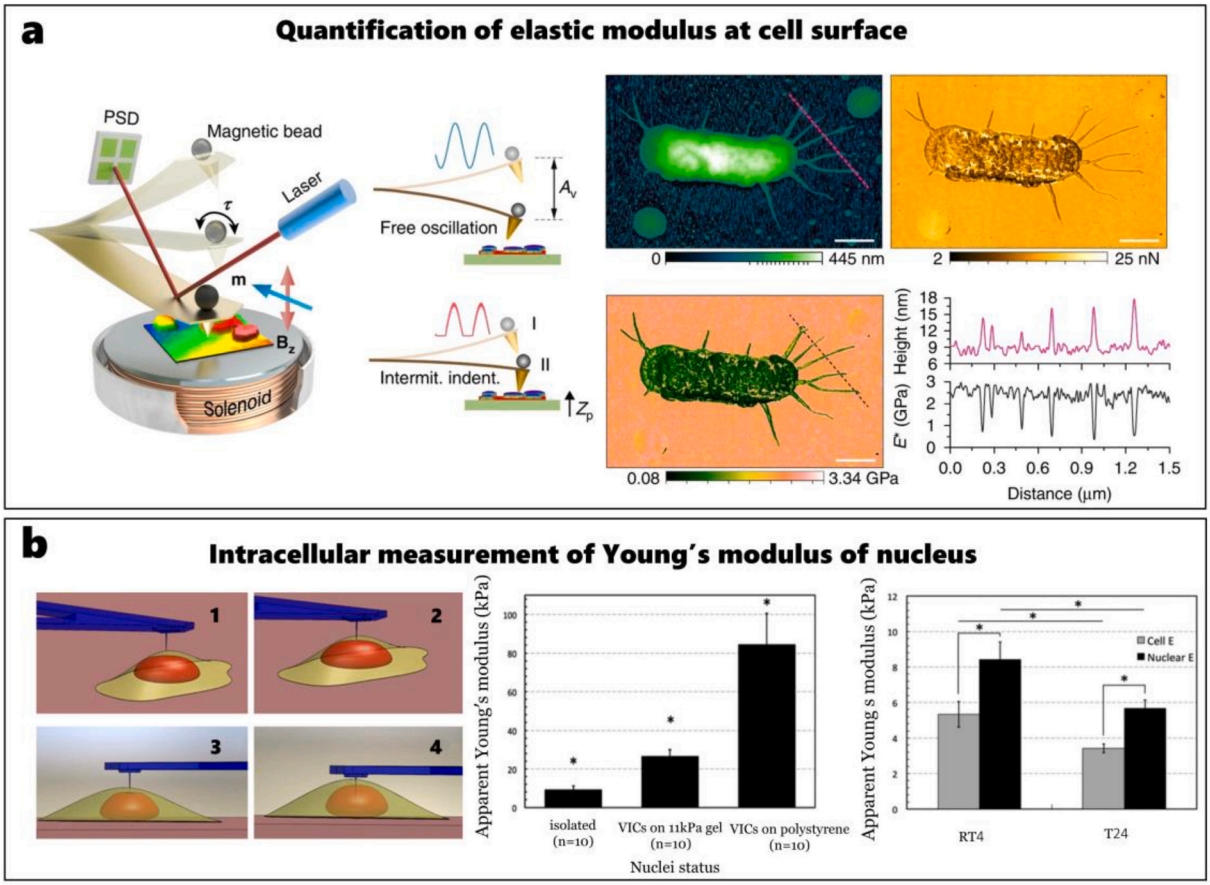

Fig. 3. Atomic force microscopy for measuring the mechanical properties of single cells. (a) Schematics of broad modulus range nanomechanical mapping method and its application for measuring topography, adhesion force and elastic modulus of *E. coli* cells. Scale bar is 500 nm. Reproduced with permission from (Meng et al., 2017). (b) Schematics demonstrating different locations of AFM needle tip: 1. contacts the cell membrane (yellow), 2. penetrates the cell membrane before contacting the nuclear membrane (red), 3. deforms the cell nucleus, 4. penetrates both the cell membrane and nuclear membrane. The results of measured Young's modulus of isolated and intact nuclei of VICs on both soft and stiff substrates, as well as those of cell membrane/cytoplasm and nuclei of RT4 and T24 cells are presented and compared. Reproduced with permission from (Liu et al., 2014). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

single cells at different locations, such as cell surface, interior and whole cell and it is often used to verify the measurement results obtained with other methods. With the further development of AFM-based methods, especially in throughput improvement and multi-parameter measurement, we believe that AFM will continue to play an important role in probing the mechanical properties of single cells.

2.2. Microfluidics methods

MMs are distinct for assessing cell deformability, a typical single-cell mechanical property, due to ultra-high throughput (10^3–10^4 cells/s). In general, there are three main microfluidics-based methods (Fig. 4) for measuring cell deformability: (1) micro-constriction, (2) extensional flow and (3) shear flow. The micro-constriction has a smaller size than that of target cells and is able to quantify cellular deformability using the passage time of cells when they passing through. The shear flow in a long and narrow channel can deform cells and the deformation of cells is recorded with the optical imaging method. The extensional flow deforms cells in a cross-slot microfluidic device and it also needs the assistance of optical microscope to record the deformation of cells. It has been discovered that the extensional flow based deformability cytometry method provides the highest strain rate (10 kHz) and applied stress (nearly 10 kPa) among the three methods mentioned above. Urbanska et al. performed a study to compare the performance of these three methods for measuring cell deformability. The results indicated that both micro-constriction and shear flow based methods are suitable for actin cytoskeleton related mechanical properties measurements, while the extensional flow method is applicable to measure the cellular components (i.e. nucleus) related mechanical properties (Urbanska et al., 2020).

A study based on the use of micro-constriction arrays (Fig. 4a) quantified the mechanical properties of suspended K562 leukemia and NIH 3 T3 cells and demonstrated the dose-response relationship between protein expression and cell mechanical properties, cell elastic modulus increases and cell fluidity decreases with the rising level of lamin A (Lange et al., 2017). Gossett et al. (2012) used an extensional flow based method (Fig. 4b) to characterize the deformability of leukocytes and malignant cells in pleural effusion with a throughput of 2000 cells/s. This method predicted the disease status of patients with cancer and immune activation with high sensitivity (92%) and high specificity (86%), which shows great potentials in practical clinical applications. The shear flow is integrated with deterministic lateral displacement (DLD) to measure the size, deformability and shape of red blood cells (RBCs) in a high throughput manner (Fig. 4c). With an appropriate design of post and array geometry, this method could be employed to obtain more information of cell mechanical properties, such as elasticity and viscosity (Beech et al., 2012).

Compared to other methods, MMs can achieve high-throughput measurements in contact and non-contact manners, moreover, MMs are easily integrated with other methods (e.g. MA and OTs) to achieve

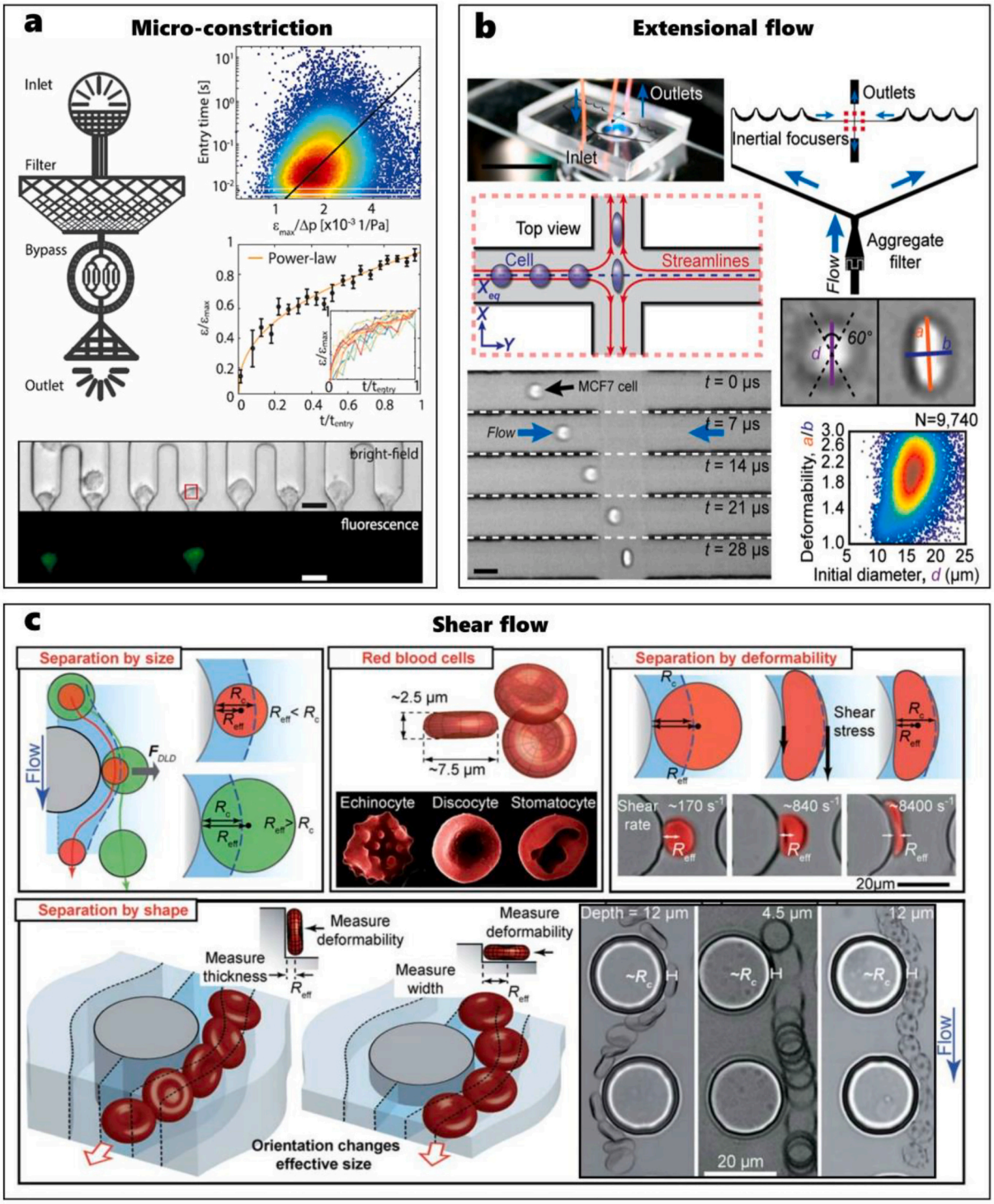

Fig. 4. Microfluidics methods for measuring the mechanical properties of whole cells. (a) The high-throughput microfluidic micro-constriction device for measuring mechanical properties of K562 leukemia and NIH 3 T3 cells. Structure of the microfluidic device (top left), bright-field and fluorescence images of the constriction region (bottom), bivariate kernel density estimation of entry time vs $\varepsilon_{max}/\nabla p$ (ε_{max} is the maximum cell deformation and ∇p is the driving pressure) of K562 leukemia cells and normalized strain evolution of NIH 3 T3 cells (top right) are demonstrated. Scale bar is 20 μm. Reproduced with permission from (Lange et al., 2017). (b) High-speed automatic microfluidic deformability cytometry based on extensional flow. Structure and schematics of the microfluidic deformability cytometry (top), high-speed microscopic images of cells in the extensional flow region (bottom left), parameters extracted from the deformed cell (centre right) and density scatter plot of size and deformability of measured cells (bottom right) are presented. Scale bar is 40 μm. Reproduced with permission from (Gossett et al., 2012) (c) Measurements of size, deformability and shape of red blood cells with shear flow integrated with deterministic lateral displacement (DLD) and its applications for cell separation. Scale bar is 20 μm. Reproduced with permission from (Beech et al., 2012). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

accurate manipulation of single cells and high-throughput measurements of cell mechanical properties, which will be discussed in Section 3.

2.3. Micropipette aspiration

When using MA to measure single-cell mechanical properties, the friction between the cell membrane and micropipette walls can be ignored (Evans and Yeung, 1989). Under the effect of micropipette induced suction pressure on the cell surface, cell deformation along with its geometrical changes are measured to determine the elastic or viscoelastic properties. The pressures of different magnitudes are required to characterize the mechanical properties of different cell components, for example, threshold pressures about 1 Pa and 1 KPa (or several hundred Pa) are required to measure cell membrane and cell cytoskeleton, respectively (Rowat et al., 2006). Besides, MA can measure the mechanical properties of cell nucleus (Pajerowski et al., 2007; Rowat et al., 2005; Swift et al., 2013). It needs to consider the effects of surface energy when using MA to measure soft biological materials, e.g. tumours and embryos. This is because the surface energy may decrease the normal surface displacement and internal pressure if the micropipette radius or aspiration length is comparable to the elastocapillary length, resulting in the overestimation of the elastic modulus (Ding et al., 2018).

Hogan et al. (2015) proposed an MA-based method to investigate cellular adhesion force at the single-cell level. The force required to detach a cell from the substrate could be quantified with a single interference reflection microscopy (IRM) image of the cell and the area covered by the adhesive bonds. By integrating with a theoretical model that describes the polymerization and depolymerization of actin, MA allows us to investigate the mechanical properties of membrane and

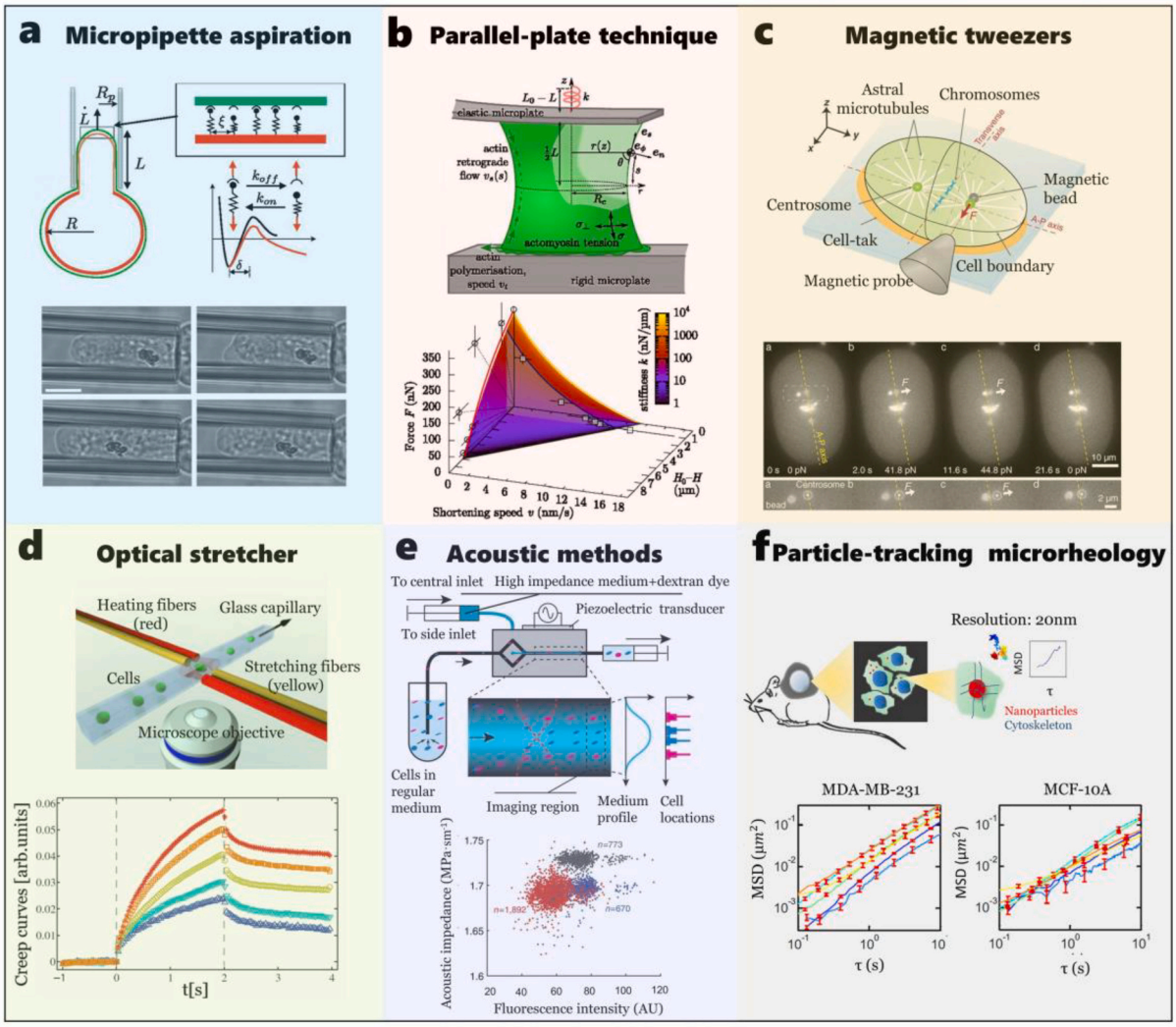

Fig. 5. Micropipette aspiration (MA), parallel-plate technique (PPT), magnetic tweezers (MTs), optical stretcher (OS), acoustic methods (AMs) and particle-tracking microrheology (PTM) for measuring mechanical properties of single cells. (a) MA manipulation of *E. histolytica* to demonstrate the dynamical organization of cytoskeletal cortex. Schematics of MA and cell model (top) and snapshots of the cell tip inside the micropipette (bottom) are presented. Scale bar is 10 μm. Reproduced with permission from (Brugués et al., 2010). (b) Model of PPT measurement system and cell (top) and the force-velocity-length phase portrait of the experiment investigating the dynamics of actomyosin (bottom). Reproduced with permission from (Étienne et al., 2015). (c) MTs for measuring force-generating machinery during mitosis. Experimental conditions (top) and video images (bottom) of spindle displacement with force and close-up of the displacement are demonstrated. Reproduced with permission from (Garzon-Coral et al., 2016). (d) OS with heating fibers (top) for measuring thermorheology of living cells and measured evolution of creep curves (bottom). Reproduced with permission from (Kießling et al., 2013). (e) Measurement setup of IAF (top) and measured acoustic impedance (bottom) with respect to the fluorescence intensity of pre-enriched monocytes (blue), lymphocytes (red) and neutrophils (dark gray). Reproduced with permission from (Augustsson et al., 2016). (f) Schematics of PTM for intravital measurement (top) and measured mean squared displacement (MSD) of MDA-MB-231 and MCF-10A cells (bottom). Reproduced with permission from (Wu et al., 2020). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cytoskeleton cortex of Entamoeba histolytica (*E. histolytica*) (Fig. 5a). The results revealed that cell morphological changes lay the foundation for cell motility (Brugués et al., 2010). Moreover, MA can also suck whole cell into micropipette bore and deform the whole cell to measure the viscoelastic properties in a similar process. Hochmuth (2000) investigated the solid and liquid behaviours of neutrophils and chondrocytes by aspirating whole cell into a micropipette, and discovered that the neutrophils behaved like liquid with the surface tension of 30 pN/μm, while the chondrocytes behaved as solid with an elastic modulus at the level of 0.5 kPa.

MA can measure the mechanical properties of different cell organelles (e.g. membrane and nucleus) and whole cell with accuracy that is comparable to AFM. Moreover, it is able to integrate with MMs to achieve relatively high-throughput measurements, reducing the complexities of apparatus and measurement procedure.

2.4. Parallel-plate technique

In the PPT, a cell is placed between two parallel plates: one is flexible, and the other is rigid (Bufi et al., 2015a). The stiffness of the rigid plate is around 1000-fold higher than that of the flexible plate, and each plate is calibrated against a reference plate with known bending stiffness. PPT is suitable for measuring whole-cell mechanical properties by inducing dynamic mechanical deformation of cells, which has advanced the development of theoretical models of single-cell mechanics (Desprat et al., 2006; McGarry, 2009; Ronan et al., 2012; Thoumine and Ott, 1997). Wu et al. (2018) measured MCF-7 cell mechanical properties using PPT, and the obtained viscous, elastic and shear modulus were 340 ± 40 Pa, 950 ± 150 Pa, and 380 Pa, respectively, at the frequency of 1 Hz, which were comparable to the measurement results obtained by AFM with dull probes and MTC. A model depicting the dynamics of actomyosin cortex (Fig. 5b) revealed that the instantaneous changes of environment stiffness can lead to the intrinsic mechanical responses of actomyosin cortex. This model characterizes the maximum force that cells can exert and the highest speed that cells can contract, which are validated with PPT experiments results (Étienne et al., 2015).

PPT is suitable to measure Young's modulus, deformability, relaxation and creep functions of single cells with high accuracy, but the high-throughput measurement is difficult to achieve with this method.

2.5. Magnetic twisting cytometry and magnetic tweezers

MTC deforms cells by using magnetic beads, which rotate under the influence of a magnetic field (Hoffman et al., 2006; Wang et al., 1993). MTC can apply forces on multiple beads to enable the measurement of cell local mechanical properties at multiple locations, including cell surface and cell interior. When measuring the mechanical properties inside cells, the motion of magnetic beads in cells is monitored by a high-resolution image system and the experiments must be conducted under well-controlled conditions. MTC has been used to probe the dynamic responses of cellular components, such as cytoskeleton (Laurent et al., 2003a), membrane (Puig-de-Morales-Marinkovic et al., 2007) and cytosol (Laurent et al., 2003b; Laurent et al., 2002a).

MTs have similar working principles to MTC, which has been used for various applications, such as investigation of vinculin function in F9 embryonal carcinoma cells (Alenghat et al., 2000), breakdown of endothelial barrier function with MDA-MB-231 cells and endothelial cells (Mierke, 2011), high-throughput gene transfection and screening of heterogeneous leukemic cells (Chang et al., 2015) and measurement of viscoelastic responses of NIH3T3 mouse embryonic fibroblasts (Bonakdar et al., 2016). However, an apparent disadvantage of MTC and MTs methods is the limited force that can be applied on the magnetic beads (Assi et al., 2002; Walter et al., 2006). To this end, a modified MTs were developed, which could apply a force up to 100nN on 5 μm magnetic beads to deform cells (Kollmannsberger and Fabry, 2007). Moreover, the modified MTs could be applied to measure the local viscoelastic response of soft materials in the nonlinear regime and used to investigate force-regulated processes and mechanotransduction in living cells.

The three-dimensional (3D) MTs system has been developed to achieve the measurement of intracellular components, such as cytoplasm and nucleus. The first 3D intraembryonic magnetic beads navigation, with forces up to 120pN (at the resolution of 4 pN), revealed that the viscosity of cytoplasm was higher than water by eightfold and the middle regions of the inner cell mass were more deformable than the periphery regions in the mouse embryo cells (Wang et al., 2018). In 2019, with an optimized 3D MTs system, they achieved 3D manipulation of submicrometer magnetic beads inside T24 cells by applying a stable force for more than 30 min (with a positioning error of 0.4 μm), and uncovered that nucleus' major axis was much more difficult to deform than the minor axis in T24 cells (Wang et al., 2019). MTs can study the force-generating machinery (Fig. 5c) that maintains the spindle at the cell center during mitosis in *Caenorhabditis elegans* embryos, which unveiled that this machinery with high stiffness is capable of suppressing thermal fluctuations to locate the mitotic spindle precisely (Garzon-Coral et al., 2016).

Both MTC and MTs can measure the mechanical properties of cell surface and cell interior. With the advances of controlling technology of magnetic beads, these two methods can be used to characterize cell mechanical properties more comprehensively (e.g. simultaneous measurement of mechanical properties of cell surface and interior), which might provide new insights into single-cell mechanical phenotypes.

2.6. Optical stretcher and optical tweezers

OS uses two diverging beams to trap and deform cells. Depending on the size and index of refraction, the forces applied on glass or latex beads fall in the range of piconewton to nanonewton with the laser power changes from a few mW up to 1.5 W (Guck et al., 2001; Kas and Guck, 2000), which is sufficient to stretch whole cells. With the increase of laser power, the magnitude of forces applied to cells could also increase and induce greater deformation of cells. However, too powerful laser beam might modify cell mechanical properties or damage cells. Bellini et al. (2012) designed and fabricated a monolithic OS by patterning optical waveguides in glass with a fs laser, and used it to investigate the effect of temperature on the depolymerization of microtubules at 0 °C (Yang et al., 2015a).

Due to the nature of laser heating, OS has also been used to study the effects of heating on the mechanical properties of single cells. Modified OS (Fig. 5d) produces transient changes of temperatures in milliseconds, contributing to the concept of thermorheology, which was proposed to describe the intrinsic properties of cells in response to temperature changes (Kießling et al., 2013). Chan et al. (2014) used OS to investigate the effects of heating on the mechanical properties of suspended HL60 cells. The obtained results showed that the compliance of HL60 cells scaled linearly with temperature below a critical point (i.e. 52 ± 1 °C), but cells showed an active contraction in the direction of maximal stress when the temperature was higher than the critical point, due to the activation of TRPV2 ion channels. The volume variations of isolated nuclei of HL60 cells under heating effects were also studied with OS. Chan et al. demonstrated that the volume of isolated nuclei was highly temperature-dependent and the isolated nuclei could be regarded as highly charged polymer gels with thermoresponsive properties (Chan et al., 2017).

OTs utilize a focused laser to create a 3D light gradient, which could apply attractive and repulsive forces on a bead or cell (Dao et al., 2003). However, the force applied by OTs is limited to the magnitude at the piconewton level, which is not large enough to deform the whole nucleated cell but is suitable to measure the properties of actin filaments. The viscoelasticity of adherent alveolar epithelial cells could be measured with OTs by placing microbeads on F-actin, and measurement results were comparable to those obtained by MTC (Laurent et al., 2002b). In addition to actin filaments related mechanical properties of

cell surface, OTs can also measure intracellular mechanical properties by trapping and oscillating beads inside a cell. Hoffman et al. (2006) measured the frequency-dependent shear modulus of mammalian cells with internal laser tracking microrheology (LTM) and the digital deflection rate of random beads could be 50 kHz. Wei et al. (2008) used OTs to manipulate internal lamellar bodies in alveolar epithelial type II cells to measure viscoelastic moduli, and the obtained mean value of the power-law exponents agreed with the intracellular measurement results obtained by Hoffman et al.

OS and OTs can measure cell mechanical properties without direct contact of cells and are able to integrate with MMs to achieve high-precision and relatively high-throughput manipulation and measurement of single cells. Therefore, they show great potentials for the development of automatic platforms for culture, manipulation and mechanical phenotype of single cells.

2.7. Acoustic methods

AMs have been widely used to separate cells based on size and compressibility using standing surface acoustic waves (Ding et al., 2014). However, the measured compressibility parameters should be transformed into conventional viscoelastic parameters to describe single-cell mechanical properties. Recently, some researchers have achieved noninvasive measurements of cell stiffness with acoustic fields (Tan et al., 2011; Yang et al., 2016b). An isoacoustic focusing (IAF) based method (Fig. 5e) is capable of achieving size-independent acoustic-mechanical phenotypes of cells, such as monocytes, lymphocytes, neutrophils, BA-F3 and MCF7 cells, because different types of cells have different values of effective acoustic impedance ranging from 1.55 MPa s m^{-1} to 1.75 MPa s m^{-1} (Augustsson et al., 2016). Kang et al. (2019) utilized the size-normalized acoustic scattering (SNACS) to quantify the mechanical properties of whole cell during a cell cycle, where a suspended microchannel resonator (SMR) was used as the acoustic energy source. This method allows us to understand how evolutionary cells preserve their mechanical intactness and could be applied to measure delicate and transient dynamic alterations of cell mechanical properties.

The last decade has witnessed exponential advancements of photoacoustic imaging technology for applications in biological and medical fields. The photoacoustic microscope is a powerful tool to quantitatively investigate cell physical and biomechanical properties (Strohm et al., 2016), such as temperature (Gao et al., 2013a, 2013b), oxygen saturation, size, morphology of single RBCs (Wang et al., 2013) and to detect melanoma cells in flowing bovine blood in vitro as well as erythrocytes, leukocytes and platelets in the flowing blood of rat mesenteric microvessels in vivo (Wang et al., 2011; Zharov et al., 2006). When illuminated by a laser pulse, RBC assimilated optical energy and delivered an ultrasonic pressure wave (photoacoustic wave), where the power spectrum carries distinct information about the size and morphology of the irradiated RBCs. Moreover, the alterations of RBCs morphology could also be quantified by the power spectrum, enabling healthy RBCs to be distinguished from abnormal ones with irregular shapes (Strohm et al., 2013).

AMs not only can achieve the mechanical phenotypes of single-cells in a non-contact manner with high throughput but also can be used for cell separation based on cell mechanical properties, such as deformability. But the measured parameters (e.g. compressibility) by AMs are needed to be transformed into conventional viscoelastic parameters to describe the mechanical properties of cells.

2.8. Particle-tracking microrheology

In PTM, sub-micrometer fluorescent beads are injected into the cell components (e.g. cytoplasm and nucleus) and the thermally driven motion of the beads is measured (Wirtz, 2009). Unlike other methods (e.g. AFM) that require the probe to be in continuous contact with the cell during the measurement, PTM assesses the mechanical properties of cell components based on the physical interactions between the standalone beads and the intracellular structures, which enables the measurements of single-cell mechanical properties in three dimensions (Moeendarbary and Harris, 2014). Savin and Doyle (2005) analyzed and validated the potential static and dynamic errors with purely viscous fluids when using PTM to characterize the mechanical properties of cells and biological fluids. The static errors were related to the inaccurate measurements of immobilized particles' positions while the dynamic errors were likely caused by the particle movement in limited exposure time for visualization. This theoretical research was meaningful by calculating the mean-squared displacement with a high spatial resolution (i.e. 10 nm) using PTM, enabling to obtain more accurate cell mechanical properties. By integrating PTM with intravital microscopy (Fig. 5f), Wu et al. investigated the cytoplasmic intracellular properties of MDA-MB-231 and MCF-10A cells under intravital microenvironmental circumstances in living mice with high temporal (<100 ms) and spatial resolutions (<15 nm) (Wu et al., 2020).

PTM is a passive method for measuring the mechanical properties of single cells and it is a promising tool to achieve the mechanical phenotypes of single cells in vivo without the influence of cell viability.

3. Integrated measurement methods for mechanical properties of single cells

Microfluidics methods are usually integrated with other techniques, such as MA, OTs and OS, to perform high-throughput and high-precision measurements of single-cell mechanics.

Microfluidic MA devices have achieved the measurements of different types of single-cell mechanical properties, such as whole-cell deformability, Young's modulus and nuclear deformability with simple implementation and significantly improved throughput, making them as promising tools for the clinical applications and fundamental cell biology research (Davidson et al., 2019; Lee et al., 2016; Lee and Liu, 2015; Li et al., 2019). Guo et al. (2012) proposed a microfluidic MA device with less specialized apparatus to measure the deformability of different types of cells, such as passive human neutrophils, human lymphocytes, RT4 cells and L1210 mouse lymphoma cells with high-resolution pressure control (0.3 Pa).

Since MMs can introduce and position cells in microchannels easily and OTs can manipulate single cells with high precision, the integration of microfluidics and OTs makes the best use of respective advantages of two methods and provides the capability to culture and manipulate cells for various applications. For example, a microfluidic OTs device has been validated as a feasible platform to investigate the mechanotransduction and tensile stiffness of single endothelial cells, by introducing and locating single cells at designed wells (MMs) and loading mechanical stimuli on the ECM-integrin-cytoskeleton linkage (OTs) (Honarmandi et al., 2011). In addition, microfluidic OTs device can measure the deformation of RBCs with speeds of >20 cells/s, providing an alternative for high-throughput measurement of single-cell mechanical properties (Sawetzki et al., 2013).

The integrated microfluidic OS has been used to measure the single-cell mechanical properties with throughputs of 50–100 cells/h (Lincoln et al., 2007) and realize the multi-parameters characterization of single cells (e.g. shear modulus, steady-state viscosity and relaxation time) (Huang et al., 2020). Moreover, an integrated microfluidic OS device has been demonstrated as an automatic platform for single-cell mechanical phenotypes, where the target cells are delivered and pre-positioned at the defined region in a microchannel and then stretched by OS without the manual intervention in the whole process (Lai et al., 2008). In addition to relatively high throughput measurements, the microfluidic OS system is also capable of measuring the subtle alterations of mechanical properties of human breast epithelial cells from normal to cancerous and even metastatic condition, where optical deformability is regarded as an inherent biomarker for fundamental cell biology research

and disease diagnostics (Guck et al., 2005).

4. Applications of single-cell mechanical properties

This section introduces typical applications of single-cell mechanical properties, including cell separation, disease diagnostics, immune status analysis and drug screening in the biological and medical fields.

4.1. Cell separation

The mechanical properties of cells are considered as potential label-free biomarkers for separating cells from heterogeneous populations, which is an important step for the subsequent disease diagnosis and therapy applications. Microfluidics technique is one of the most promising methods for cell separation based on their mechanical properties, such as deformability, size and shape, due to its high throughput and capability of simultaneous multi-parameter measurements of cell mechanics.

The microfluidic OS can achieve cell separation based on the viscoelastic properties of cells, which was validated by the traditional fluorescence-activated cell sorting (FACS) (Faigle et al., 2015). Separation based on the mechanical properties (e.g. deformability) of human melanoma cells with different metastatic potentials was achieved in an integrated optofluidic device fabricated by fs laser, where the cell viability is maintained in the whole procedure (Yang et al., 2015b). The integration of real-time fluorescence and deformability cytometry (RT-FDC) with standing surface acoustic waves (SSAW) and deep neural network (DNN) enables cell separation based on deformability, which shows great potentials in research and clinical applications, such as label-free separation of retinal precursor cells for transplantation in regenerative medicine (Nawaz et al., 2020). Moreover, optimized structures of microfluidic devices, such as DLD can achieve cell separation based on different mechanical properties, including deformability, shape and size. Microfluidic ratchets with a matrix of tapered constrictions have achieved label-free separation of circulating tumour cells (CTCs) from leukocytes and erythrocytes based on cell deformability in oscillatory flow (Park et al., 2016).

In addition to the relevant applications of cell mechanical properties for cell separation, theoretical studies have advanced to classify cells based on their mechanical properties. Using the mechanical properties of single cells as the primary inputs, neural network modeling is capable of classifying heterogeneous populations cells, such as zonal chondrocytes, chondrosarcoma cells and mesenchymal cells into subpopulations with high accuracy (Darling and Guilak, 2008). The integration of measurements of cell mechanical properties with advanced calculation models, like neural network modeling, is an ideal approach to separate cells in mixed populations, which is limited by current measurement technologies (Minelli et al., 2017).

4.2. Disease diagnostics

When a cell changes its functions or became physiologically altered, the subsequent reconstruction of cytoskeleton leads to the changes in cell intrinsic properties, such as nucleus-cytoplasm ratios and nuclear envelope shape, which have been regarded as important biomarkers for identifying and staging malignancy (Radhakrishnan et al., 2017). Tumour cells have to spread from the primary tumour and colonize distant sites during cancer progression and metastasis, in which the deformability of tumour cells plays an important role. One prevailing hypothesis is that the more metastatic tumour cells are more easily to be deformed, which promotes their invasion and motility to complete the metastasis process (Reinhart-King, 2016). Measurement of cell deformability is an effective method to distinguish metastatic cells and their healthy counterparts. Overall, the measurement of cell mechanical properties allows label-free, non-destructive and sensitive investigation of cell interior processes and potentially the diagnosis and treatment of disease (Guck, 2019).

The chromatin and nuclear envelope A-type lamin proteins are disturbed in many human diseases, such as heart disease, progeria and cancer. A pipette-based nucleus micromanipulation study demonstrated that chromatin controls the response to extensions smaller than 3 μm and euchromatin/heterochromatin levels regulate nuclear stiffness, while lamin A/C level determines the strain stiffening of nucleus under large extensions. The proposed framework can be used to investigate the differential effects of chromatin and lamin A/C in regulating the mechanical properties of nucleus and the influence of their changes on disease, thus exhibiting great potentials in the disease diagnostics (Stephens et al., 2017). The alterations in the mechanical properties of myeloid cells and blood cells developed from a common myeloid progenitor are related to diseases, for example, myeloid cells are stiffer for patients with acute myeloid (Bashant et al., 2020). Cell-by-cell morpho-rheological (MORE) analysis can achieve the identification of mechanical properties of all major blood cells and the characterization of their pathological changes in vitro in a continuous and label-free manner with a throughput of 1000 cells/s, which can be applied to the diagnosis, prognosis and treatment monitoring of different diseases, such as hematological diseases, inflammatory, infectious and metabolic disorders (Toepfner et al., 2018).

High throughput methods for mechanical phenotyping of single cells are highly desired in the field of disease diagnostics. The measurement at a rate of thousands of cells per second or more, which is comparable to the traditional flow cytometry, has been achieved in this decade. Henry et al. (2013) developed the deformability cytometry (DC) method to diagnose malignant pleural effusions with a throughput of 1000 cells/s, which is applicable to prescreen samples to lessen the burden of cytopathologists and advance clinical decision-making. The pinched-flow hydrodynamic stretching of single-cells yielded a throughout of 65,000 cells/s, which could be used to detect rare cells in clinically body fluids for disease diagnostics (Dudani et al., 2013).

4.3. Immune status analysis

The measurement of mechanical properties of immune system cells, including lymphocytes, neutrophils, and monocytes/macrophages, is an effective method to analyze the status of immunity.

Mechanical properties of lymphocytes with the relatively large nucleus, such as B cells, T cells and natural killer cells, are found to change when the immune system is activated. For example, the spontaneously activated lymphocytes of diabetic mice are stiffer than the control cells (Perrault et al., 2004). MMs have been used to measure the deformability of activated lymphocytes from patients with chronic lymphocytic leukemia (CLL). The results revealed that the lymphocytes of CLL patients are less deformable than the lymphocytes of the control group, which is different to the previous findings that the deformability of metastatic cells (i.e. breast cancer cells) increases with the progression of metastasis (Zheng et al., 2015).

The activated neutrophils can form the neutrophil extracellular traps (NETs), which are involved in the progression of infection, sepsis and autoimmune diseases. In the formulation process of NETs, the size and shape of nucleus change distinctly (Hakkim et al., 2011). After two or three hours of stimulation, the nuclear membrane disappears and the decondensed chromatin contacts directly with components of cytoplasm (Fuchs et al., 2007). These morphological alterations can be measured easily with methods introduced above to analyze immune status, which finally promote the diagnosis and treatment of related diseases, such as sepsis.

Macrophages play important roles in cleaning and disinfecting infected and injured sites as well as maintaining tissue homeostasis (Jain et al., 2019). The process for macrophages against invading microorganisms needs the remodeling of the actin cytoskeleton (Rougerie et al., 2013). A study by Man et al. revealed that the infection of *Salmonella* activated the NLRC4 (NLR family CARD domain-containing protein 4)

inflammasome, an important host defense mechanism. This resulted in the reduction of cellular movement by the stiffening of macrophages and infection susceptibility by reorganizing the cytoskeleton (Man et al., 2014).

4.4. Drug screening

It is a commonly used method for treating cancers or other diseases by employing selective drugs to modify the mechanical properties of cytoskeleton and nucleus and the subsequent cellular behaviours. If cells are sensitive to drugs, cells will show obviously differential behaviours in mechanical properties in pretreatment and posttreatment. Therefore, the measurement of mechanical properties at the single-cell level can advance our understanding of the pathological processes of diseases, allowing the screening of drug sensitivity and the discovery of new drugs. Many researchers used exogenous drugs to change the mechanical properties of cells and then monitored the effects of drug treatment with common measurement methods. For example, the effect of a pharmaceutical formulation (i.e. OTC-Ossitetraciclina liquida 20%) on the mechanical properties of K562 cells was investigated with MA (Di Cerbo et al., 2018) and the fact that atorvastatin treatment could soften human RBCs was found with OTs (Sheikh-Hasani et al., 2018).

Complement-mediated cytotoxicity (CMC) is activated after bonding drugs to tumour cells and can result in the lysis of tumour cells, which is an important mechanism to kill tumour cells in vitro with rituximab. Quantitative analysis of mechanical properties alteration of single tumour cells during the CMC process is important to the development of cancer therapy with antibody-based CMC (Li et al., 2014). The combination of suitable drug-carriers with different action sites to study the different cytoskeleton perturbing mechanisms can lead to the identification of more promising drugs. Taranejoo et al. (2016) used chitosan (CS) as the carrier and albendazole (ABZ) as the microtubule-targeting agent (MTA) to assess the mechanical properties of a cancer cell line (SW48) with MA. They revealed that the viscoelastic parameters, such as elastic constants and viscosity coefficient, altered greatly under the combined effects of CS/ABZ. The research on cell vitality elucidated that CS/ABZ exhibited enhanced anticancer efficacy for two cancer lines (SW48 and MCF10CA1h).

The mechanical properties of single cells are also important in regenerative medicine applications, because the mechanical properties of mesenchymal stem cells, including elastic modulus, instantaneous modulus and apparent viscosity are related to their lineage differentiation abilities (González-Cruz et al., 2012). Measurement of cell mechanical properties, such as stiffness and nuclear membrane fluctuations, provides a promising method to identify more therapeutically active mesenchymal stromal cells for stem cell related therapies (Lee et al., 2014). Moreover, it is well known that external mechanical loads can affect the responses to injuries (Vining and Mooney, 2017). The mechanical unloading of bone in the microgravity environment can reduce the growth and regeneration of tissues by inhibiting the genes for early differentiation of hematopoietic and mesenchymal stem cells (Blaber et al., 2014). Under the effects of low-magnitude and high-frequency mechanical stimulation, pediatric cancer survivors suffering from the low bone mineral density can improve the peak bone mass in their youth with or without the combination of other therapies (Mogil et al., 2016). The measurements and assays of single-cell mechanical properties pave the way to understand the mechanisms of how mechanical loading regulates stem cells, which can promote the development of regenerative medicine.

5. Disscussion and furture perspectives

This review introduces and categorizes ten commonly used methods for measuring single-cell mechanical properties based on different measurement locations: cell surface, cell interior and whole cell (Table 1). Four methods (AFM, MA, MTC and MTs) can measure the mechanical properties at cell surface, PTM, mAFM, MTC, MTs and OTs can perform the intracellular measurement, and AFM, MA, MMs, PPT, OTs, OS and AMs are able to characterize the mechanical properties of whole cell. In addition to the measurement locations, the working modes, such as active and passive actuation, contact and non-contact measurement are briefly discussed. PTM is the only passive method for measuring single-cell mechanical properties, while the other nine methods are all active methods, which require the assistance of external actuation. Moreover, AFM, MA, PPT, MTC, MTs, PTM are contact measurement methods while OS, OT, AMs are non-contact measurement methods. MMs can work with three main approaches: micro-constriction, extensional flow and shear flow. The micro-constriction approach is a contact-based measurement while the extensional flow and shear flow approaches are non-contact. The displacement, force and throughput ranges of different methods for measuring the single-cell mechanical properties are different (Loh et al., 2009; Neuman and Nagy, 2008; Van Vliet et al., 2003) (see Fig. 6). Please note that the ranges of force, displacement and throughput demonstrated here are just overall magnitudes of selected methods introduced above, which may not be achievable with a single device at the same time, for example, AFM cannot work simultaneously with the largest force and the highest resolution displacement. It is apparent that AFM can be utilized extensively in different displacement and force ranges, while MTC has the smallest working ranges (Fig. 6a). MA and MTs have a similar working range of displacement, but MA can apply larger forces on cells (Fig. 6b). OTs provide the highest force resolution and OS is suitable for applications with a medium force range. For the throughput (Fig. 6c), another important characteristic for measuring single-cell mechanics, MMs provide the highest throughput of 10^3–10^4 cells/s, AMs can measure single-cell mechanical properties at a throughput of 10^2–10^3 cells/s. MA and OTs can complete the measurements at the rate of less than 100 cells per second. Moreover, efforts have been made to enhance the throughput of methods that are not suitable for high-throughput measurement at the current stage, such as AFM with multiple probe arrays (Favre et al., 2011; Kawakatsu et al., 2002; Rangelow et al., 2007), high-speed AFM (HS-AFM) (Ando, 2018).

5.1. High throughput

High throughput is a desired capability for measuring single-cell mechanical properties in disease diagnosis and other clinical applications, especially when samples contain several thousand to million cells. High-throughput processing enables the screening and assays of a large number of cells to be conducted in a short time, which can lower the possibility of physical changes of cells during measurements, thus improving the testing accuracy. Since the testing results can be obtained as early as possible, it allows patients to receive medical care, diagnoses or treatments promptly. The AFM-based method has been optimized to improve its throughput for single-cell mechanical properties measurements. The dynamic variations of viscoelastic properties of living eukaryotic cells can be quantified at a throughput of approximately 7680 pixels in 10 s, which enabled real-time measurements of morphological and subcellular nanomechanical variations and the characterization of cell dynamic mechanical properties, e.g. the dynamics of the cytoskeleton (Cartagena-Rivera et al., 2015). Microfluidics may be the method having the highest throughput for single-cell mechanical properties measurements. The developed microfluidic instruments provided label-free physical measurements of single cells with massive throughput (e.g. 2000 cells/s (Gossett et al., 2012)). In addition, microfluidic devices of optimized materials can offer new advanced characteristics for measuring single-cell mechanical properties. For example, microfluidic devices made of ultra-thin glass sheets are transparent and physically and chemically stable, which allow high-resolution and real-time observation of cell deformability (Yalikun et al., 2016; Yalikun and Tanaka, 2017; Yalikun and Tanaka, 2016). With further advances in instrumentation and integration with other on-

Table 1
Summary of methods for measuring single-cell mechanical properties at different locations.

Locations	Methods	Cell types	Advantages	Disadvantages	Force	Throughput	References
Cell surface	AFM	epithelial cells	elasticity measurements, error sources analysis;	small force range;	pN-μN	1-20 cells/h	(Harris and Charras, 2011)
		MEF cells	nanoscale measurements;	single frequency, model dependency;			(Hecht et al., 2015)
		HL60 cells, HUVEC	noncontact intercellular adhesion strength measurements;	complicated experimental setup;			(Hosokawa et al., 2011)
	MA	endothelial cells	cell-substrate adhesion force quantification;	require further validation;	pN-μN	1-10 cells/s	(Hogan et al., 2015)
		mesenchymal stem cells	effects of different GFPs on cell mechanics;	increase of susceptibility to membrane bled;			(Sliogeryte et al., 2016)
	MTC	MCF-7 cells	high accuracy;	additional contact between magnetic beads and cell surface;	pN	0.5 cells/s	(Wu et al., 2018)
		HL-1 cardiomyocytes	1kHz measurement range and capabilities of working in MTC mode and MT mode;	single and low frequency measurement;			(Chen et al., 2016b)
		monocytic cells	simultaneous measurement of stiffness of monocytic cells in multi-direction;	no quantification of local detachment of membrane from the underlying cytoskeleton;			(Irmscher et al., 2012)
	MTs	F9 cells	large forces up to 100nN, high timing and force accuracy;	low throughput;	fN-pN (100nN)		(Kollmannsberger and Fabry, 2007)
		MEFs, NIH3T3, F9 cells, MDA-MB231, 786-O and A125 cells	measurement of time- and force-dependent viscoelastic properties of adherent cells under large forces;	phenomenological relationship rather than a constitutive theory;			(Kollmannsberger et al., 2011)
		rat brain cells	statistical study of mechanics of brain cells on different substrates;	viscoelasticity parameter is not consistent to previous studies;			(Chen et al., 2016a)
Cell interior	mAFM	RT4, T24 and fibroblast-like cells	direct intracellular measurement;	low-throughput, cell damage;	0-80 nN		(Liu et al., 2014)
		fibroblast cells	quantitative strength of cell cytoskeleton, manipulation of intracellular structure;	cell damage;			(Machida et al., 2010)
	MTC	macrophages	a wide range of particles diameters and specific surface area;	cell damages;	pN	0.5 cells/s	(Möller et al., 2005)
	MTs	mouse embryo cells	first 3D intraembryonic navigation;	complex setup structure;	fN-pN		(Wang et al., 2018)
		T24 cells	3D manipulation, precise spatial and temporal control;	limited force range;			(Wang et al., 2019)
		macrophages	compact size, temperature control;	no quantification of viscoelastic properties;			(Hosu et al., 2003)
	PTM	swiss3T3 fibroblasts cells	rate-dependent and ultrastructural measurement;	high cost, complicated computation;		30 cells/h	(Tseng et al., 2002)
		COS7 cells	noninvasive quantification of dynamic changes of cell viscoelasticity;	high cost and complex experiment setup;			(Yamada et al., 2000)
Whole cell	AFM	MC3T3-E1osteoblasts cells	theoretical models comparison;	restricted deformation range;	pN-μN	1-20 cells/h	(Jaasma et al., 2006)
		living fibroblasts, WM35 and A375 cell	larger indentation depths induced stiffness values decrease;	need further verification of universality;			(Pogoda et al., 2012)
		MIN6 cells	quantification of calcimimetic R568 effects on whole-cell elasticity;	small experimental sample;			(Siamantouras et al., 2014)
	AMs	L1210 cells	measure delicate and transient dynamic alterations;	limited time resolution;	pN	10^2-10^3cells/s	(Kang et al., 2019)
		RBCs	size and morphology measurement;	limited frequency range;			(Strohm et al., 2013)
		BAF3 and MCF-7 cells	evaluate cell mechanics and disease progress;	measured parameter should be linked to common cell mechanics parameters;			(Augustsson et al., 2016)
		MCF-7, HEPG2, HT-29	non-contact measurement;	complicated setup and measurement process;			(Hartono et al., 2011)
	MMs	K562 leukemia cells	reveal dose-response relationship between proteins expression and cell mechanical properties;	require multi-parameters;	pN	10^3-10^4 cells/s	(Lange et al., 2017)
		RBCs	high efficiency, measure heating effect;	complicated fabrication;			(Lee et al., 2009)
		HeLa cells	relativity high throughput;	need further calibration for clinical application;			(Adamo et al., 2012)
		cancerous and benign brain cells	investigation of cell size, hydrodynamic resistance, cell velocity and shape deformation;	no direct measurement of micromechanics of tumor cells			(Khan and Vanapalli, 2013)
	MA	blood granulocytes	a wide range of pipets size and suction pressure;	no characteristic ratio of flow resistance in cortex;	pN-μN	1-10 cells/s	(Evans and Yeung, 1989)
		chondrocytes	measurement of Poisson's ratio;				(Trickey et al., 2006)

(*continued on next page*)

Table 1 (*continued*)

Locations	Methods	Cell types	Advantages	Disadvantages	Force	Throughput	References
				absence of direct osmotic stresses;			
	OTs	alveolar epithelial cells	oscillatory and extracellular measurement;	no accurate measurement of subtending half-angle;	fN-pN	10-10^2 cells/s	(Wei et al., 2008)
		fibroblasts, neurons and astrocytes cells	consider the effects of bead cell thickness and bead immersion degree;	narrow frequency range;			(Ayala et al., 2016)
		breast cancer cells	reveal effects of neighboring cells on cell stiffness;	measured stiffness is sensitive to microenvironment;			(Yousafzai et al., 2016)
		human embryonic stem cells	measurement of dynamic and static micromechanical properties;	cell differentiation stage-specific measurement;			(Tan et al., 2012)
	OS	HL60 cells	robust, durable, transparent;	need further integration;	pN-nN	10-100 cells/h	(Bellini et al., 2012)
		breast epithelial cells	thermorheology;	cell damage by heating;			(Kießling et al., 2013)
		HL60 cells	heating effects on nuclei volume;	limited temperature range;			(Chan et al., 2017)
		RBCs, murine osteogenic cells	model extension and application;	heating influence on cells, requirement of refraction index of cell;			(Ekpenyong et al., 2009)
		erythrocytes	high-throughput, single-beam;	heating influence on cells;			(Sraj et al., 2010)
	PPT	MCF-7 cell	elastic and viscous moduli, relaxation and creep functions;	single frequency measurement;		6 cells/h	(Wu et al., 2018)
		human immune cells	measurement of viscoelastic modulus changes under inflammatory conditions	time-consuming measurement process			(Bufi et al., 2015b)

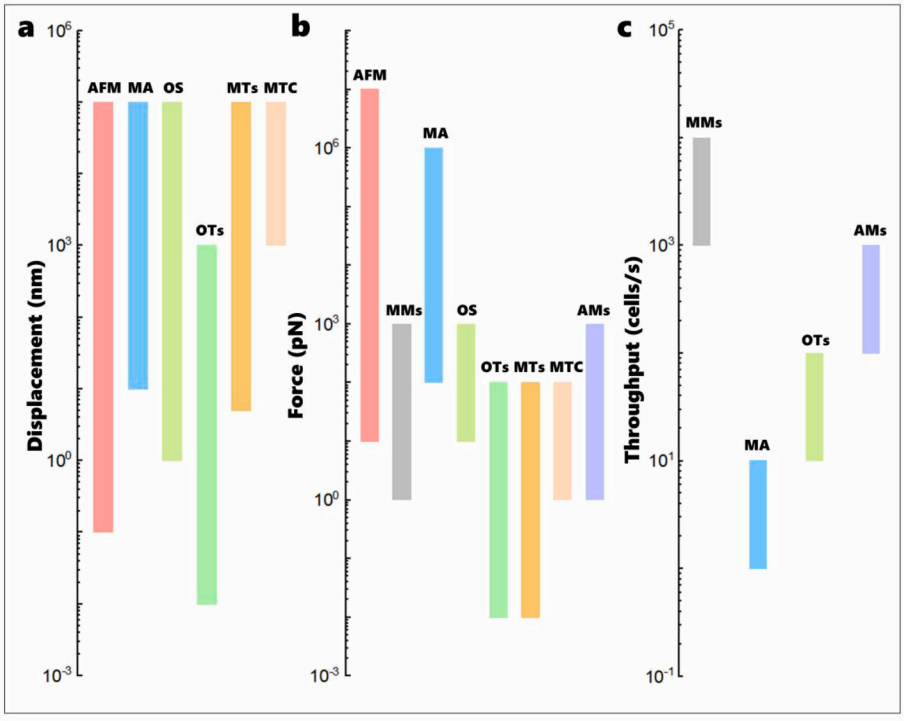

Fig. 6. Illustration of the ranges of (a) displacement, (b) force and (c) throughput for common methods used for measuring single-cell mechanical properties. Please note the ranges shown here are the overview of the described methods, which might not include the specific modification of a certain method.

chip components, such as rotating, focusing, sorting and image-based detection (Huang et al., 2018; Nitta et al., 2018; Pan et al., 2017; Shen et al., 2019; Tu et al., 2017; Zhang et al., 2020), improved practical routines for single-cell manipulation and characterization in clcinical and research applications will be achieved by microfluidics.

5.2. Automation

Currently, most methods for the characterization of single-cell mechanical properties need manual operations, which require professional skills and may cause undesired errors. Automation is one of the best ways to solve this problem, and some preliminary studies on automatic measurements of single-cell mechanical properties have been reported. Tan et al. (2010) proposed a method using robotic manipulation technology with OTs to measure the mechanics of RBCs in different osmotic conditions, which might promote pathological analysis and therapeutic development of human diseases. Thakur et al. (2014) presented a method for automated micromanipulation of cells

with indirect pushing using OTs. This method could investigate cell migration by settling cells in arrays without exposure to a laser beam. Gou et al. (2014) established a robot-aided OTs system to measure cell protrusion force and revealed that the protrusion force of Jurkat cells in response to a chemoattractant was about hundreds of piconewtons. This enables the description of cell migration and paves the way for proactive control over cell movement. A combined system with OTs and robot-integrated microfluidic chip has enabled the measurement of mechanical properties (Young's modulus) of single Synechocystis cells, where the OTs trap and transport the cells and the robot-integrated microfluidic chip with the pushing and sensing probes can deform the cells and transduce the deformation of cells to force (Chang et al., 2018). We note that there is an increasing number of studies on automatic manipulation and characterization of cells with OTs (Būtaitė et al., 2019; Xie, 2019; Xie et al., 2018; Yang et al., 2020), and the automation of other techniques is expected to be achieved in the foreseeable future.

5.3. Integration

Simultaneous measurement of cell surface and intracellular mechanical properties is an intriguing field to be explored, which can enable the comprehensive understanding of cell status and facilitate the disease prescreening. In theory, both MTC and OTs can simultaneously measure the mechanical properties of cells at different locations. However, to the best of our knowledge, there is no report demonstrating the simultaneous measurement of cell surface and interior mechanical properties with MTC or OTs, mainly due to technical issues. For example, both MTC and OTs use beads as probes to deform cells, however, it is difficult to calibrate the measurement system, such as the trap stiffness of OTs, when the measurements are simultaneously performed at different locations (cell surface and interior), because the beads work in different mediums (e.g. water solution for a bead outside a cell and cytoplasm for a bead inside a cell) (Arbore et al., 2019). Also, the integration of different techniques would be a solution to achieving this. For example, AFM and femtosecond laser can measure the mechanical properties of single cells without indenting cells, and PTM can measure the intracellular mechanical properties by the thermally induced motion of fluorescent beads. The low heating production characteristics of femtosecond laser ensure the normal measurement process of PTM, and the throughputs of AFM and PTM are on the same scale, so that there is good compatibility when integrating AFM and femtosecond laser with PTM. The technical details are still needed to be confirmed with experimental calibration and validation. An integrated OTs-AFM platform has been demonstrated for characterizing mechanical properties of single cells. There are two typical modes of the developed OTs-AFM platform: (1) OTs can work as a high-precision nanomanipulator and AFM can visualize the sample zone with high resolution; and (2) AFM with a colloidal cantilever works as a manipulator and OTs act as a force and spatial sensor. Moreover, this platform shows the possibility to trap and manipulate intracellular objects with OTs and measure the cell surface with AFM(Pierini et al., 2016). We expect that the integration of different methods will enable more accurate, sensitive and robust measurements of cell mechanical properties in the future, even though there are some technical challenges (e.g. compatibility and operation) needed to be solved.

6. Conclusion

In this review, we summarized ten different methods used for measuring the mechanical properties of single cells based on the different measurement locations (e.g. cell surface, cell interior and whole cell). For each method, we not only introduced the measurement principles but also elucidated the unique features of each method. Moreover, we discussed applications of mechanical properties of single cells in various fields, such as cell separation, disease diagnostics, immune status analysis and drug screening. We expect that with further improvement in terms of throughput, automation, and integration, more accurate and comprehensive mechanical phenotyping of single cells will be achieved.

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