Article

Microconstriction Arrays for High-Throughput Quantitative Measurements of Cell Mechanical Properties

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ABSTRACT We describe a method for quantifying the mechanical properties of cells in suspension with a microfluidic device consisting of a parallel array of micron-sized constrictions. Using a high-speed charge-coupled device camera, we measure the flow speed, cell deformation, and entry time into the constrictions of several hundred cells per minute during their passage through the device. From the flow speed and the occupation state of the microconstriction array with cells, the driving pressure across each constriction is continuously computed. Cell entry times into microconstrictions decrease with increased driving pressure and decreased cell size according to a power law. From this power-law relationship, the cell elasticity and fluidity can be estimated. When cells are treated with drugs that depolymerize or stabilize the cytoskeleton or the nucleus, elasticity and fluidity data from all treatments collapse onto a master curve. Power-law rheology and collapse onto a master curve are predicted by the theory of soft glassy materials and have been previously shown to describe the mechanical behavior of cells adhering to a substrate. Our finding that this theory also applies to cells in suspension provides the foundation for a quantitative high-throughput measurement of cell mechanical properties with microfluidic devices.

INTRODUCTION

Mechanical properties of living cells are important for essential cell functions including cell contraction (1,2), crawling and invasion (3), differentiation (4–6), and wound healing and division (7,8). Moreover, alterations of cell mechanical properties have been linked to common human diseases such as cancer (9,10), inflammation and sepsis (11), asthma (2), malaria (10,12), and cardiovascular disorders. To measure cell mechanical properties, numerous techniques have been developed including atomic force microscopy (13), micropipette aspiration (14,15), particle tracking microrheology (16), and magnetic tweezer microrheology (17). However, these techniques suffer from low measurement throughput of ~10–100 cells/h. By contrast, microfluidic technologies can achieve a much higher throughput, for example by shear flow stretching (18,19) or by measuring the entry or transit time of cells through micronscale constrictions (microconstrictions). Such microconstriction setups have been used to investigate suspended erythrocytes (20), leukocytes (11), neutrophils (21), and invasive and noninvasive cancer cell lines (22–24). Although the cell entry time into microconstrictions correlates with cell stiffness and viscosity, it also depends on the externally applied pressure, cell size, and friction between the cell and the channel walls (25). Therefore, we believe that quantitative measurements of cell mechanical properties have thus far not been achieved with such setups.

In this article, we describe a quantitative, high-throughput method to measure the mechanical properties of cells in suspension (suspended cells or adherent cells that have been detached and brought in suspension) with a parallel microconstriction device. We use constrictions that are smaller than the nucleus of the cell and therefore deform and probe both the nucleus and the cytoskeleton, resulting in a bulk measurement of the whole cell. Our approach is to measure for each cell and each microconstriction not only the entry time, but also the cell size and the applied pressure. Using a high-speed charge-coupled device camera in combination with automated image analysis, we achieve a throughput of ~10,000 cells/h.

We find that the relationship among entry time, cell deformation, and driving pressure conforms to power-law rheology. Power-law rheology describes the mechanical properties of cells with only two parameters: cell elasticity (stiffness) and cell fluidity (the power-law exponent). Moreover, we find that elasticity and fluidity data from cells treated with a wide range of chemicals that alter the cytoskeletal (actin, microtubule) or the nuclear structure (chromatin packing) all collapse onto a master curve. This master curve establishes that the mechanical properties of cells in suspension are governed by only a single parameter, namely cell fluidity. Therefore, with only a single

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measurement, we can quantitatively characterize the mechanical state of each cell.

MATERIALS AND METHODS

Design of the device

The microfluidic device consists of eight parallel constrictions connected to a single inlet and outlet with a low-resistance pressure-equalizing bypass, similar to previously published designs (11,21) (Fig. 1 *a*). Cells in suspension first have to pass through a filter mesh before the flow is divided into eight parallel constriction branches. The height of the device is chosen to be in the range of the cell diameter (17 μm for K562 cells). The width and height of the constrictions are chosen to be smaller than the nuclear diameter. For the K562 leukemia cells used in this study, we empirically find a good compromise between high throughput (wide constrictions) and high sensitivity (narrow constrictions) for a constriction width of 5 μm and a height of 9 μm (Fig. S1 *a* in the Supporting Material). With these dimensions, an average entry time between 5 and 1000 ms can be achieved. The length and shape of the constriction (Fig. 1, *b* and *d*) is chosen so that the passage time is dominated by the time the cell needs to deform to the width of the channel. Once fully deformed, the cell slides through the constriction channel in $<$1.4 ms, which is the temporal resolution of the camera. Therefore, cell friction at the constriction walls can be neglected. The microfluidic device is mounted on a glass coverslip and imaged from below using an inverted microscope.

Devices are fabricated using standard PDMS molding techniques from a photolithographically developed SU8 master. Briefly, SU8-2025 Photoresist (MicroChem, Westborough, MA) is spin-coated onto 3″ Si wafers (Silicon Materials, Pittsburgh, PA) to form layers of 17 μm height. Due to light diffraction during ultraviolet exposure of the Photoresist through a chrome mask, the constrictions have a decreased height of 9 μm (Fig. S1 *a*). Devices are produced from a 10:1 ratio of elastomer/curing agent (Sylgard 184; Dow Corning, Midland, MI), which is mixed and poured onto the SU8 master. After baking for at least 2 h at 65°C, the device is peeled from the SU8, plasma-bonded to a microscope coverslip using air plasma generated by a plasma oxidizer (Zepto; Diener Electronic, Nagold, Germany), and further baked for 1 h at 65°C.

Cell culture

K562 cells (No. CCL-243; American Type Culture Collection, Manassas, VA) are cultured at 37°C and 5% CO_2 in Iscove's modified Dulbecco's medium (IMDM, Gibco Cat. No. 12440053; Life Technologies/Thermo Fisher Scientific, Waltham, MA) containing 10% FCS (fetal calf serum, Gibco, Cat. No. 16000-036) and 1% PSG (Penicillin-Streptomycin-Glutamine, Gibco Cat. No. 10378-016). MDA-MB-231 cells (No. HTB-26; American Type Culture Collection), U2OS cells (No. CRL-1573; American Type Culture Collection), and HEK293T cells (No. ACS-4500; American Type Culture Collection) are cultured in Dulbecco's modified Eagle Medium (Gibco Cat. No. 11960044), also containing 10% FCS and 1% PSG. Cells are passaged every third day. Nuclear staining is performed with DRAQ5 (Cat. No. ab108410; Abcam, Cambridge, MA) according to the manufacturer's guidelines. Whole cell staining for size measurements is performed with calcein (Cat. No. C0875; Sigma-Aldrich, St. Louis, MO). Life-death staining is performed with calcein and propidium iodide (Cat. No. P4170; Sigma-Aldrich).

Transfection

For the generation of K562 and MDA-MB-231 cells expressing eGFP-lamin A, we use lentiviral transduction. For producing lentiviral particles, HEK293T cells are cotransfected with the vectors pMD2.G, psPAX2, and pLVX containing the coding sequence of lamin A N-terminally fused to eGFP using lipofectamine LTX (Gibco Cat. No. 15338100). The cell culture supernatant is collected daily and replaced with fresh Dulbecco's modified Eagle's medium for the next four days. The collected medium containing assembled virus particles is pooled and filtered through 0.45 μm pores, supplemented with 8 μg/mL polybrene (Cat. No. 107689; Sigma-Aldrich) and added to K562 and MDA-MB-2331 cells for 18 h. Starting from Day 2 after lentiviral infection, cells are selected using 2.5 μg/mL puromycin. After 10 days, eGFP-lamin A-expressing K562 cells are sorted using a MoFlo Legacy cell sorter (Dako Cytomation, Carpinteria, CA).

For the generation of U2OS cells stably expressing the fluorescent F-actin marker LifeAct-TagGFP2, we transfect the expression vector using lipofectamine LTX (Gibco Cat. No. 15338100) according to the manufacturer's instructions. Starting from Day 2 after transfection, stably expressing cells are selected using 1 mg/mL G418 (Cat. No. 83768;

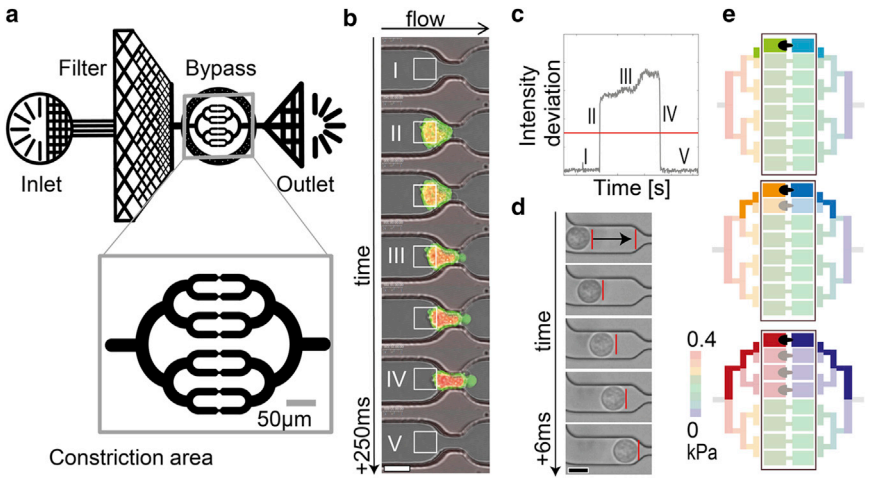

FIGURE 1 (*a*) Schematic of the microfluidic device with inlet, debris filter, constriction area surrounded by a bypass, and outlet. (*Inset*) Magnified view of the symmetric constriction area with eight parallel channels. (*b*) Sequential micrographs of a cell entry into a constriction. The actin cytoskeleton is stained with LifeAct (*green*); the DNA is stained with DRAQ5 (*red*). The white squares mark the regions of interest (ROI) for estimating the cell's entry time. Scale bar is 10 μm. (*c*) The standard deviation (SD) of the brightness within the ROI is used to detect the time points when the cell enters a microconstriction (rise of the signal) and when it has fully deformed to pass through the microconstriction (fall of the signal). The entry time is calculated by thresholding (*red line*). Roman numbers correspond to the numbered ROIs from (*b*). (*d*) By tracking the cell before it enters the constriction, we obtain the flow speed and thus can calculate the pressure drop over each constriction with Hagen-Poiseuille's law. Scale bar is 10 μm. (*e*) Changing occupational states in all constrictions are monitored continuously to calculate the pressure drop across each microconstriction during a cell's entry. The figure depicts three examples of how the pressure across the leftmost constriction (color-coded in *saturated colors*) changes when cells block the fluid flow through neighboring constrictions (the pressure in the remaining system is color-coded in *light colors*). The 256 different combinations of blocked and free microconstrictions give rise to six possible pressure drop combinations that can differ by up to a factor of 2. To see this figure in color, go online.