

High-speed microfluidic differential manometer for cellular-scale hydrodynamics

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We propose a broadly applicable high-speed microfluidic approach for measuring dynamical pressure-drop variations along a micrometer-sized channel and illustrate the potential of the technique by presenting measurements of the additional pressure drop produced at the scale of individual flowing cells. The influence of drug-modified mechanical properties of the cell membrane is shown. Finally, single hemolysis events during flow are recorded simultaneously with the critical pressure drop for the rupture of the membrane. This scale-independent measurement approach can be applied to any dynamical process or event that changes the hydrodynamic resistance of micro- or nanochannels.

pressure measurement | microcirculation | hemolysis | red blood cell | membrane properties

Fluid motions at the micrometer scale are at the heart of many recent developments in microfabrication (1), separation processes (2), cellular-scale identifications (3), DNA sequencing (4), protein crystallization (5) and many basic transport pathways in plants (6), in the microcirculation (7), and specific to industrial processes. The main characteristics of these advances lie in the manipulation and understanding of the dynamics of “soft” objects such as polymers (8) (e.g., DNA), drops (9, 10), microemulsions (11), microfoams (12), cells (13), vesicles and microcapsules (14). In fact, the interaction of the flow with these deformable entities is a tool to further investigate the details of their mechanical properties and their structural features (e.g., the entropic elasticity of a polymer, the viscoelastic properties of a capsule, or the rheology of the liquid film between microbubbles in a foam). For the case of strong confinement offered by microchannels, the flow and shape of any close-fitting soft object is controlled by a competition among the properties of the objects, the fluid pressure, and the viscous stresses acting on the boundaries that resist the motion. The hydrodynamic resistance resulting from this fluid–structure interaction is reflected in a dynamical variation of the pressure drop along the channel during the flow and hence represents a crucial parameter to be measured.

Nevertheless, rapid variations of pressure are very difficult to measure at the micrometer scale and below. Indeed, the difficulties do not originate from the lack of precision sensors commercially available or those described in the research literature (15). The problem is a subtle mix of pragmatism and technological limits. In addition to issues of dead volumes in standard pressure-measurement techniques (16–18) and those associated with interfacing microelectromechanical system devices to standard pressure gauges (19), existing techniques are simply difficult to implement [lasers, quadrant diodes, deformable membranes, multistep process of production (16–18)] and are unable to measure at millisecond rates the pressure changes in micrometer-scale flows. For instance, when a single red blood cell (RBC) enters a channel of $5 \times 5 \mu\text{m}$, the volume variation produced by a flow at physiological speeds of a few millimeters per second is $\approx 100 \text{ fL}$ in a few milliseconds, which represents a typical pressure-drop variation of tens to hundreds of pascals. Such rapid pressure measurements at the cellular scale, crucial to future device and microcirculatory advances, are not available

at this time. Here we report a technique that overcomes these limitations and demonstrate the ability to measure rapid variations of pressure drop between two points in a microfluidic device. The technique needs no external elements and is easy to implement with soft lithography. The basic principle should naturally allow similar measurements of pressure variations in nanochannels.

We chose to illustrate the flexibility of our approach by focusing on blood cells, which allows us to provide insights into outstanding problems in hemodynamics. Indeed, there is a long history of the study of cells at the micrometer scale for assessing mechanical properties (20) and cell shape (21) and for applying these ideas for understanding microcirculatory diseases (22, 23). In fact, the main approach for characterizing the influence of possible diseases on a suspension of cells is the filtration technique (23–25). Briefly, the cells flow through a multipore polycarbonate membrane, and the measured pressure-drop versus flow-rate relation (or the mean passage time of the cells) serves as an index of the deformability state in a population of healthy or sick cells (23, 26). Even if the potential of this technique is for the analysis of large samples, the resultant data reflect only the average properties: the technique is not able to resolve information at the scale of a particular pore or a single cell. Recent advances in microsystems technology allow direct observation of the flow of cells in microchannel arrays similar to the filtration geometry (27, 28); for example, area and volume measurements for cell populations have been reported. However, the fact remains that the most basic mechanical measurements that are characteristic of the physical state of individual cells in physiological flow conditions have not been accomplished.

We propose several illustrations of our differential micromanometer that address these issues and indicate further avenues for studies of dynamics and hemorheology. We measure (i) the pressure-drop variation associated with the motion of single and multiple RBCs and white blood cells (WBCs) in a microchannel, (ii) the additional pressure drop associated with the flow of drug-treated cells, and (iii) single hemolysis events.

Measurement Principle

To measure simultaneously the dynamical deformation of the cells and the variation of the pressure drop produced by their motion in the channel, we developed a device with twin channels: a test channel (Fig. 1*A Upper*) and an identical control, or “comparator,” channel (Fig. 1*A Lower*), both of which produce downstream two parallel and adjacent streams of fluid. To maintain a stable interface, the two fluids are miscible, and the liquid flowing through the control channel is dyed to visualize the interface downstream. Hence, the principle of the measurement lies in the use of the second control channel to detect any variation of pressure in the test channel when a cell, or another object, is flowing through it. In effect, for a given applied pressure difference across the device, the

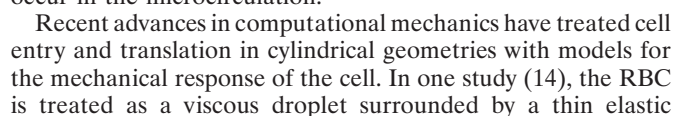
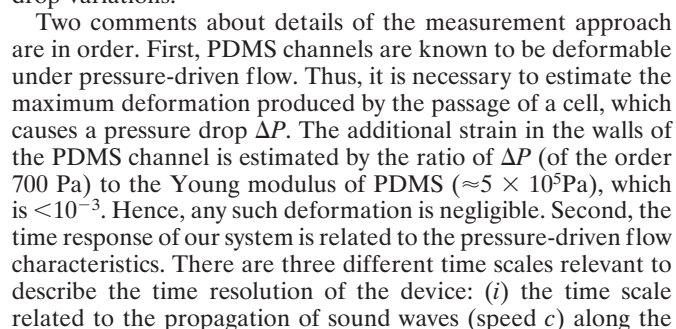
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Abbreviations: RBC, red blood cell; WBC, white blood cell; PDMS, poly(dimethylsiloxane).

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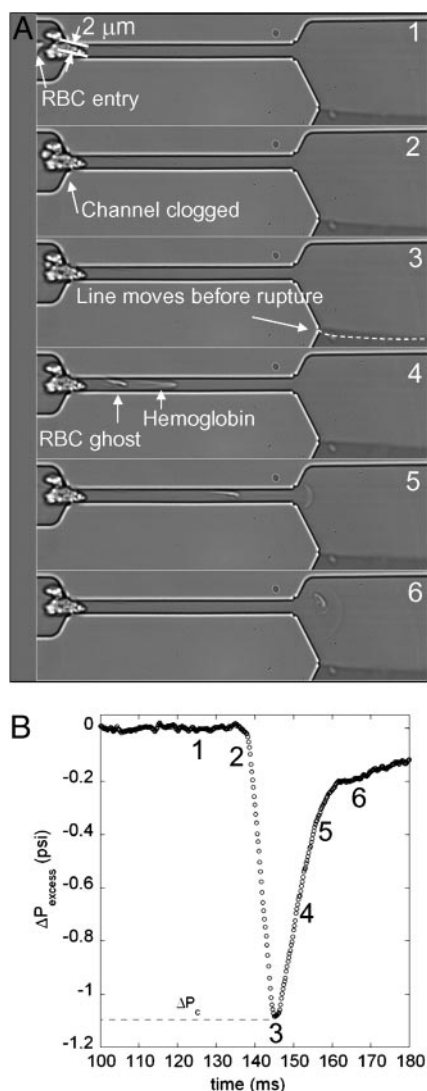


Fig. 4. Hemolysis of an RBC passing through a narrow constriction formed by some particulate matter that clogs the entry of the upper channel. The equivalent size of the entry pore is $2\ \mu\text{m}$.

membrane of two-dimensional modulus E_S . The dynamical response of these systems depends on the capillary number, which is a dimensionless parameter $\varepsilon = \mu V_0 / E_S$, where μ is the viscosity of the outer fluid and V_0 is the mean velocity of the fluid in the channel. For example, the maximum additional pressure drop ΔP_{add} during the flow is calculated to be $\Delta P_{\text{add}} = O(10-100)\mu V_0 / R_i$ for $10^{-3} < \varepsilon < 0.05$, where R_i is the radius of the circular capillary (see figure 14 in ref. 14). Using the measurements shown in Fig. 3, our results give $\Delta P_{\text{add}} = 16 \mu V_0 / R_i$, which is in good agreement with the order of magnitude from the computational model. Finally, we note that the computational models provide ΔP_{add} as a function of the position along the channel, and our results are in qualitative agreement. A detailed

comparison of simulation and experiment would require the same geometry and should, in principle, allow extraction of the mechanical properties.

The interactions of cells, and their number density, in the microcirculation impact the overall pressure drop in a tissue and is still not well understood (31). Next, we report in Fig. 3 results that suggest a way to study these hydrodynamic interactions of cells through the measurement of the pressure drop for the flow of one, two, and five cells translating through a microchannel (cells are closely spaced, similar to a rouleaux). The pressure drop systematically increases as the number of cells increases, but the results are not simply proportional to the number of cells. This qualitative response is typical of confined geometries with suspended particles spaced closer than the microchannel width.

Pressure-Drop Change due to Membrane-Modified Cells. Next, we consider the change in the hydrodynamic resistance that occurs when the mechanical properties of the cells are modified. In Fig. 3, we compare a single healthy cell with a glutaraldehyde-treated cell, which is known to be stiffer (25): the pressure drop is enhanced after treatment with glutaraldehyde, and the stationary shape of the cell is obtained at later times. Thus, we conclude that our approach allows differentiation of cells with different mechanical properties or geometrical features, which may provide a simple biomedical tool for clinical hemorheology and pharmaceutical testing.

Hemolysis. As a final example that illustrates the insights that can be obtained with our microfluidic differential manometer in Fig. 4, we visualize a cell blocking the entrance to a channel (Fig. 4A42) and the subsequent hemolysis event (the cell membrane ruptures) (Fig. 4A44–A46). When the blockage event begins, the pressure drop increases linearly over <10 ms and reaches a maximum value of ≈ 1.1 psi when hemolysis happens. We then see the ghost of the RBC (Fig. 4A44–A46) as well as the hemoglobin solution, which follows the parabolic velocity distribution. This critical value of stress that is necessary for hemolysis is in good agreement with the approximate value of $4,000$ Pa ≈ 0.6 psi found with static micropipette experiment on preswollen RBCs (32). It is interesting to note also that malaria-infected RBCs have increased rigidity, which is associated with organ failure. Microfluidic approaches have been used recently to examine qualitatively the flow-induced hemolysis (or “pitting”) of malaria-infected cells (7), and our methodology provides a quantitative approach for more in-depth studies of these systems.

In summary, we have provided an approach for time-dependent pressure-drop measurements at the micrometer scale with millisecond resolution. We have shown how insights into mechanical processes can be obtained at the scale of individual cells. These ideas naturally apply to other soft objects and to nanoscale flows.

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