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Microfluidic analysis of red blood cell deformability

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ABSTRACT

A common indicator of rheological dysfunction is a measurable decrease in the deformability of red blood cells (RBCs). Decreased RBC deformability is associated with cellular stress or pathology and can impede the transit of these cells through the microvasculature, where RBCs play a central role in the oxygenation of tissues. Therefore, RBC deformability has been recognized as a sensitive biomarker for rheological disease. In the current study, we present a strategy to measure RBC cortical tension as an indicator of RBC deformability based on the critical pressure required for RBC transit through microscale funnel constrictions. By modeling RBCs as a Newtonian liquid drop, we were able to discriminate cells fixed with glutaraldehyde concentrations that vary as little as 0.001%. When RBCs were sampled from healthy donors on different days, the RBC cortical tension was found to be highly reproducible. Inter-individual variability was similarly reproducible, showing only slightly greater variability, which might reflect biological differences between normal individuals. Both the sensitivity and reproducibility of cortical tension, as an indicator of RBC deformability, make it well-suited for biological and clinical analysis of RBC microrheology.

1. Introduction

The deformability of red blood cells (RBCs) is critical for their transit through the smallest capillaries and is a potent indicator of the health of the cell. Cellular deformability is diminished in RBCs during cellular senescence (Waugh et al., 1992), environmental stress (Antonelou et al., 2010) and micronutrient deficiency (Paterson et al., 1994, 1987; Yip et al., 1983). Furthermore, reduced deformability is common to numerous rheological pathologies, such as malaria parasite blood-stage infection (Glenister et al., 2002; Guo et al., 2012b; Herricks et al., 2009a, 2009b; Hosseini and Feng, 2012; Miller et al., 1972; Mills et al., 2007; Shelby et al., 2003; Suresh et al., 2005), sickle-cell disease (Brandão et al., 2003; Clark et al., 1980; Nash et al., 1984), thalassemia (Advani et al., 1992), elliptocytosis and spherocytosis (Clark et al., 1983; Wandersee et al., 2004). Diminished RBC deformability may be a consequence of disease related cell stress, since reduced RBC deformability can arise from cytoskeletal crosslinking following oxidative stress (Scott et al., 1993, 1992), membrane lipid peroxidation (Dobretsov et al., 1977; Gutteridge, 1995) and vesiculation (Wagner et al., 1987). However, a decrease in RBC deformability may also contribute to the pathology of rheological diseases, as RBCs must undergo significant cell deformation to enter the microvasculature where they play a critical role in respiratory gas exchange. Together, the association between RBC deformability and disease pathology makes the measure of cellular deformability an important biomarker for rheological dysfunction.

The primary challenge associated with measuring cell deformability is that it has a complex rheological property that relies on numerous parameters (cell volume, surface area, cytoplasmic viscosity, and membrane elasticity) (Musielak, 2009). Cell deformability can be inferred from the cellular response to the flow of fluid, such as adsorption or flow rate of the cell suspension through a microfilter (Reid et al., 1976; Worthen et al., 1989) or cell elongation under constant shear stress by rheometry or ektacytometry (Bussolari et al., 1982; Fischer et al., 1978). Filtration strategies are simple to perform but they lack adequate measurement sensitivity while rheometry and ektacytometry have suitable sensitivity for clinical application but are technically difficult to perform and require labor-intensive instrument maintenance between measurements (Groner et al., 1980). Furthermore, both of these strategies only assess the bulk mechanical properties of the cell population and extremely stiff RBC subpopulations may confound measurement of cell deformability.

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Alternatively, the RBC deformability can be accessed from the measurement of single cells. Micropipette aspiration involves partial or complete aspiration of a cell into a glass micropipette and relating the suction pressure to the length of cellular protrusion into the micropipette (Evans and La Celle, 1975; Glenister et al., 2002; Nash et al., 1989; Paulitschke and Nash, 1993). This method measures the shear elastic modulus of the cell membrane, a contributing factor to the deformability of the cell. Another single cell technique, the optical tweezer, uses laser beams to apply a controlled displacement to measure the force-displacement curve between two locations on the RBC membrane (Mills et al., 2007, 2004; Suresh et al., 2005). A third strategy, atomic force microscopy, measures the repulsive force between the surface of a cell and a flexible cantilever (Chen et al., 2009). While single-cell analysis provides precise measurements, these techniques are laborious and only measure the properties of individual cells.

Microfluidic technologies offer the potential for high-throughput single-cell analysis. The most established techniques are adaptations of the cell transit analyzer (CTA) that infers RBC deformability from the time for individual cells to transit micropore structures (Moessmer and Meiselman, 1990). CTA has detected differences in deformability of RBCs from healthy and diseased individuals (Baskurt et al., 1996; Koutsouris et al., 1989; Scott et al., 1993, 1992). Adaptations of this approach measure RBC deformation in capillary obstructions and tapered constrictions (Shelby et al., 2003), transit through constrictions (Gifford et al., 2006, 2003; Herricks et al., 2009a, 2009b), pressure drop while transiting constrictions (Abkarian et al., 2006), and elongation via fluid shear stress (Forsyth et al., 2010; Katsumoto et al., 2010; Lee et al., 2009). Some common limitations of these approaches are that their measures of RBC deformability do not account for variation in cell size, nor do they account for friction between the cell surface and the vessel walls (Zheng et al., 2012).

We present a strategy to infer RBC deformability based on the critical pressure required to deform a RBC through a microscale pore. The RBC may be regarded as a Newtonian liquid drop because it is anucleate and the membrane elasticity, not cytoplasmic viscosity, is the primary determinant of cell deformability (Evans and Hochmuth, 1976). Using the Law of Laplace, we calculate the RBC cortical tension and use this to assess cell deformability. We provide empirical evidence for this simplistic model of a RBC by presenting a microfluidic strategy capable of highly robust and sensitive measurement of RBC cortical tension.

2. Materials and methods

2.1. Sample preparation

Blood was sampled from donors by finger-prick, following informed consent, using a sterile lancet. RBCs were washed in Phosphate Buffered Saline (PBS; $CaCl_2$-free and $MgSO_4$-free; Invitrogen, Carlsbad, CA), by centrifugation 700*g* for 5 min, and diluted to a hematocrit of 0.005 l/l. Washed RBCs were incubated for 30 min (25 °C) with 0–0.003% glutaraldehyde (GA; Alfa Aesar, MA). After incubation, the RBC suspension was washed three times in PBS and resuspended in 0.2% Pluronic™ F-127 (Invitrogen) in PBS, where indicated. Microfluidic analysis was performed immediately.

2.2. Microfluidic device design and operation

The microfluidic device consists of a control layer, that controls valve constriction, and a flow layer, that consists of microchannels and a funnel constriction and was fabricated as described previously (Guo et al., 2012a). The flow channel was pre-incubated for 30 min with a PBS, supplemented with 5% Pluronic™ F-127 and 5% bovine serum albumin (BSA; Sigma-Aldrich, St Louis, MO). The control channels were filled with de-ionized water.

Cells were infused into the flow layer inlet, with valves 1 and 2 closed (Fig. 1A). Narrowing of the microchannel from 200 μm to 50 μm (Fig. 1B) increases the flow speed and spacial resolution between individual cells, such that only one cell transits the funnel region at one time. As the cell enters the constriction region (Fig. 1C), valves 1 and 2 are closed and valves 3 and 4are opened to precisely control

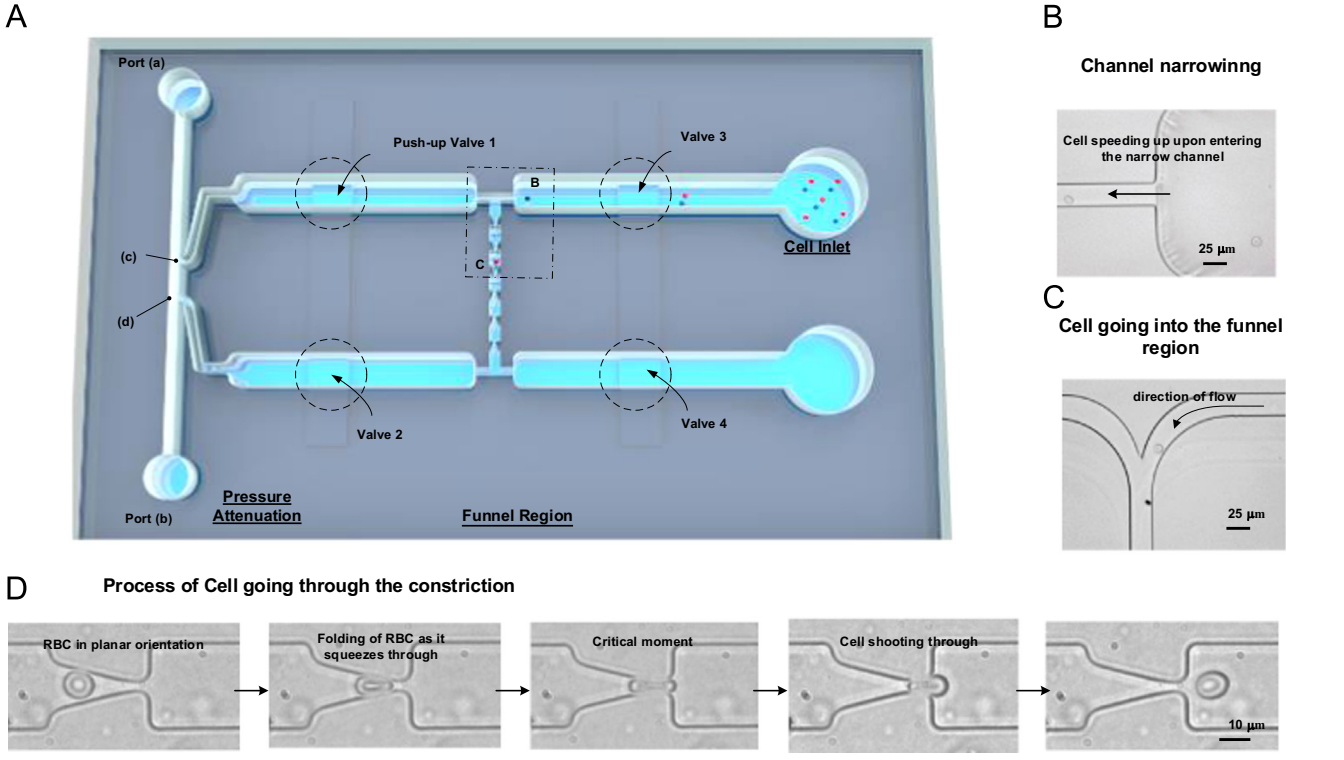

Fig. 1. Design and operation of the microfluidic device for determination of cell deformability. (A) The flow layer of the microfluidic device consists of a pressure attenuator, to precisely control the applied pressure, and a funnel chain, where cell deformation is observed and recorded. Micrographs of cell inlet show that (B) cells accelerate through the narrow channels of the device and (C) are conducted to the funnel chain. (D) Panel of micrographs showing the deformation of a single red blood cell as it passes through the microfluidic funnel.