Extreme Mechanics Letters 1 (2014) 35–41

Contents lists available at ScienceDirect

Extreme Mechanics Letters

journal homepage: www.elsevier.com/locate/eml

Quantitative biomechanics of healthy and diseased human red blood cells using dielectrophoresis in a microfluidic system

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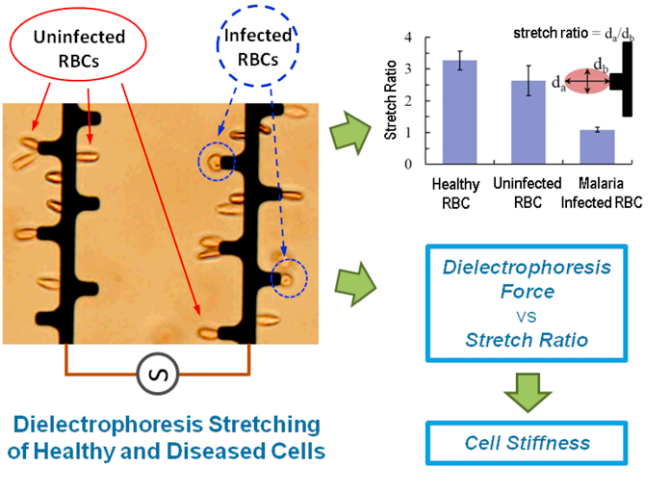
HIGHLIGHTS

- A dielectrophoresis microfluidic method is established for quantitatively characterizing the mechanical properties of a large number of biological cells.
- Experiments clearly distinguish uninfected and healthy red blood cells from those infected by Plasmodium falciparum malaria parasites.
- The characterized deformability for uninfected and healthy red blood cells compare well with those derived from independent single-cell biomechanical tests that entail much greater cost, set-up time and complexity and much more limited scope and portability.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 October 2014
Received in revised form 14 November 2014
Accepted 15 November 2014
Available online 9 December 2014

Keywords:
Dielectrophoresis force
Cell deformability
Malaria

ABSTRACT

We present an experimental method to quantitatively characterize the mechanical properties of a large number of biological cells by introducing controlled deformation through dielectrophoresis in a microfluidic device. We demonstrate the capability of this technique by determining the force versus deformation characteristics of healthy human red blood cells (RBCs) and RBCs infected *in vitro* with *Plasmodium falciparum* malaria parasites. These experiments clearly distinguish uninfected and healthy RBCs from infected ones, and the mechanical signatures extracted from these tests are in agreement with data from other independent methods. The method developed here thus provides a potentially helpful tool to characterize quickly and effectively the isolated biomechanical response of cells in a large population, for probing the pathological states of cells, disease diagnostics, and drug efficacy assays.

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http://dx.doi.org/10.1016/j.eml.2014.11.006
2352-4316/

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1. Introduction

Studies of the mechanics of single biological cells and cell populations offer useful insights into the mechanistic origins of human diseases, and pave the way for novel disease diagnostics, drug efficacy assays, and therapeutics [1–6]. Given the relatively simple cytoskeletal arrangement and nucleus-free structure of RBCs, the mechanics and biorheology of healthy and diseased RBCs, and their connections to blood disorders, have been topics of investigation for decades [4,7–9]. Numerous techniques are available for studying single cell biomechanics [3,5,8]. Key methods include micropipette aspiration [10,11], atomic force microscopy [12,13], optical tweezers [14–16], diffraction phase microscopy [17–19], and magnetic twisting cytometry [20]. Although these techniques have varying levels of force and displacement resolutions for probing cellular and subcellular components in living cells, they often suffer from low throughput, cumbersome experimental set-up and data interpretation, limited portability, and/or applicability only to specific testing and geometry conditions [3–5,21]. Microfluidic platforms for assessing cell biorheology, on the other hand, offer the means to probe large cell populations in high throughput [22–25], but are often limited in their flexibility to quantitatively determine specific cell mechanical properties using label-free methods (i.e., methods without using any biochemical or immunological tagging techniques).

There have been many successful efforts in utilizing dielectrophoresis (DEP) method to separate different cell populations from heterogeneous samples [26–30]. The principle of dielectrophoresis (DEP) and the ensuing electro-deformation of cells [31–33] have been employed in conjunction with microfluidic systems for biomechanical measurements of cancer cell lines [34,35] and mammalian cell lines [33,36]. But to our knowledge, DEP has not been used for quantitative, high throughput characterization of well-defined specific biomechanical properties (such as RBC membrane shear modulus) for large numbers of single cells, and to systematically invoke these biomechanics markers to distinguish between healthy and pathological states of human cells. Here we present a method that can rapidly assess the biomechanical characteristics of multiple cells simultaneously within a microfluidic chamber by recourse to DEP-induced force and electro-deformation.

In order to demonstrate the capability of this method to simultaneously test the biomechanical characteristics of many cells, we choose healthy human RBCs. We further validate this method for disease diagnostics by comparing the biomechanics of healthy RBCs with RBCs infected *in vitro* by *Plasmodium falciparum* human–malaria parasites (*Pf*-iRBCs). This choice was also motivated by the availability of results on cell mechanical properties of healthy RBCs and *Pf*-iRBCs from prior experiments conducted with other independent experimental techniques such as micropipette aspiration [37–39], optical tweezers [16,40] and microfluidics [23,24,41]. In this manner, the new results of this work could be systematically compared with other baseline work from a number of prior research studies. Although this paper focuses only on healthy RBCs and *Pf*-iRBCs, the technique presented here is conceptually applicable to study cell biomechanics in the context of other RBC-related diseases as well as to other cell-lineages.

The present work deals with a DEP method that is suitable for label-free discrimination of uninfected RBCs from *Pf*-iRBCs at its early stage, i.e. ring stage, which is the only stage found in circulating blood for diagnosis. To differentiate ring stage *Pf*-iRBCs from uninfected RBCs is critical and more challenging for the existing mechanics-based diagnostic approaches, since the modifications in mechanical properties for host cells at this stage are still relatively mild. Our method is capable of evaluating cellular mechanical properties by trapping and stretching as many as 700 RBCs per mm^2 in less than a second. We present experimental observations that reveal strong DEP trapping of healthy RBCs and uninfected RBCs, and weak DEP response of *Pf*-iRBCs over a broad range of electric frequencies.

2. Materials and method

2.1. The DEP method

DEP refers to the force exerted from the induced dipole moment on a particle by a non-uniform electric field. The DEP behavior of a particle is determined by the applied electric field, and the Clausius–Mossotti (CM) factor expressed by

$$\beta = \frac{\varepsilon_{\mathrm{p}}^* - \varepsilon_{\mathrm{m}}^*}{\varepsilon_{\mathrm{p}}^* + 2\varepsilon_{\mathrm{m}}^*}, \tag{1}$$

where the subscripts p and m stand for particle and medium, respectively, $\varepsilon^* = \varepsilon - i\sigma/\omega$ with ω being the angular field frequency, $i = \sqrt{-1}$, and ε and σ being the permittivity and conductivity, respectively, of the dielectric. The time-averaged dielectrophoresis force (DEP force) is expressed by [42]

$$\langle \boldsymbol{F}_{DEP} \rangle = 2\pi\, \varepsilon_{\mathrm{m}} r^3 \mathrm{Re}\,(\beta)\, \nabla \boldsymbol{E}_{\mathrm{rms}}^2, \tag{2}$$

where r is the radius of a spherical particle, Re( ) is the real part and $\boldsymbol{E}_{\mathrm{rms}}$ is the root mean square (rms) value of the electric field $\boldsymbol{E}$. Re(β) ranges from −0.5 to 1. When Re(β) is positive, the polarized particle moves toward the electric field maxima, under so-called positive DEP (p-DEP); when Re(β) is negative, the particle moves away from the electric field maxima, under so-called negative DEP (n-DEP). These two cases are demonstrated in Fig. 1(A).

2.2. Microfluidic device

The microfluidic device for DEP testing (Fig. 1(B) and (C)) consists of an interdigitated electrode structure and a 50 μm deep microchamber. The Ti/Au electrode of 10 nm/100 nm thickness was deposited on thin glass substrate (700 μm) using E-beam vaporization and lift-off process. The microfluidic channel was fabricated using polydimethylsiloxane (also known as PDMS) casting protocols and bonded to the glass substrate. Alternating current (AC) voltage of 3.5 V_{rms} at a frequency between 500 kHz and 50 MHz was applied to the electrode using