2024 IEEE 19th International Conference on Nano/Micro Engineered and Molecular Systems (NEMS) | 979-8-3503-5983-1/24/$31.00 ©2024 IEEE | DOI: 10.1109/NEMS60219.2024.10639841

Electro-deformation Spectroscopy of Biological Cells

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***Abstract*— Electro-deformation implemented in microfluidic platform has enabled a general method for biomechanical testing of human blood cells. However, electro-deformation spectroscopy (EDS), the detailed analysis of the frequency-dependent electro-deformation that can offer more information on cell electromechanics has yet to be explored. This paper reports the first EDS experiment, demonstrated using human and mouse blood specimens in a range of 100 kHz – 15 MHz. Preliminary results showed similarity and distinction between human and mouse specimens under different testing conditions. Using the standard values of dielectric properties for human cells, membrane shear modulus was determined and validated by comparing to the values reported in literature. The framework can potentially decouple the electrical and mechanical aspects involved in electro-deformation measurement. This scheme can be easily adapted to test blood cells and other deformable cell types in animal species and animal models of human diseases.**

Keywords—Electro-deformation spectroscopy, red blood cells, biomechanics, dielectrophoresis, animal models, human disease

I. Introduction

Electro-deformation refers to the phenomenon that deformable biological cells are stretched by the electric field due to interfacial Maxwell–Wagner polarization across cellular membranes[1]. Since its first demonstration in 1984 by Engelhardt et al[2], electro-deformation has received considerable interest and has been utilized as a useful strategy for biomechanical testing in a variety of biological samples, such as red blood cells[3], mammalian cells[4], cancer cells[5], and vesicles[6]. When implemented with microfabricated electrode arrays in microfluidic platforms, electro-deformation has shown several advantages that surpasses other conventional methods for cell biomechanical characterization[7]. Recently, new capability in fatigue testing of human red blood cells (RBCs) [3, 8, 9] has been demonstrated by modulating the electric signals with amplitude shift keying technique.

Animal models of human diseases are commonly used in biomedical research to investigate pathology and explore potential treatments in human[10]. The intrinsic biophysical characteristics, such as dielectric properties and mechanical properties of those cells can provide useful information to monitor disease progression and serve as biophysical markers to assess therapeutic efficacy. Electro-deformation-enabled cell biomechanics study has been largely focused on human cells, its utilization in animal cells seems to be less explored. In this study, we report the electro-deformation spectroscopy (EDS), namely, the detailed analysis of the frequency-dependence of cell electro-deformation. We first demonstrated the EDS using human RBCs, then extended to mouse RBCs. Preliminary results show useful kinetics information in a wide range of frequency spectrum, which help us to study specie-specific characteristics and further search for biophysical markers of species and diseases.

II. Electro-deformation Spectroscopy

A. Electro-deformation theory

Electro-deformation occurs due to interfacial Maxwell–Wagner polarization across cellular membranes[1]. The interaction of the electric field and the induced surface charges across cell membranes generates distributed forces on the two cell halves, which result in elongation of cells, known as electro-deformation. The time-averaged tensor is given by[11],

$$\langle \boldsymbol{\sigma}^{\mathrm{MST}} \rangle = \frac{1}{4} Re[\tilde{\epsilon}](\boldsymbol{E}\boldsymbol{E}' + \boldsymbol{E}'\boldsymbol{E} - |\boldsymbol{E}|^2 \boldsymbol{I}) \quad (1)$$

where $\tilde{\epsilon}$ denotes the complex electrical permittivity, $\boldsymbol{E}$ the electrical field, $\boldsymbol{I}$ the unit second-order tensor, and the product of two vectors results in the dyadic product.

Human RBC membranes exhibit a typical viscoelastic creep behavior due to electro-deformation. Detailed time-dependent deformation can be modeled by the Kelvin-Voigt solid model[12, 13]. The maximum electro-deformation when cell membrane reaches to equilibrium is determined by the effective electrical forces exerted on cell membranes and the membrane shear modulus,

$$T_s = 2\mu\gamma_s = \frac{\mu}{2}(\lambda_1^2 - \lambda_2^2) \quad (2)$$

where $T_s = (T_1 - T_2)/2$, is the membrane shear stress determined from the in-plane principal membrane stresses, T_1 and T_2; γ_s is the shear strain determined from the in-plane principal extension ratios λ_1 and λ_2. The shape of a deformed cell can be approximated as a triaxial ellipsoid shape, where the electro-deformation induced elongation occurs in the length axis of the ellipsoid. The extension ratio, λ can then be determined from the ratio of the initial axis over the transient

axis. Assumption of constant membrane area further simplifies the above equation through $\lambda_1\lambda_2 = 1$.

Electro-deformation occurs in companion with positive dielectrophoresis. The latter is well known as the movement of dielectric particle towards the higher field strength, due to the electrical force as a result of the interactions of the induced dipole and the electric field under specific frequencies[14],

$$\langle \boldsymbol{F} \rangle = \frac{\pi}{4} abc \cdot \epsilon_{\mathrm{m}} \cdot Re(f_{\mathrm{CM}}) \cdot \nabla \boldsymbol{E}^2_{\mathrm{rms}} \quad (3)$$

where a, b and c are the dimensions along the three axes of the ellipsoid, ϵ_{m} is the permittivity of the medium, and $Re(f_{\mathrm{CM}})$ is the real part of the Clausius-Mossotti factor.

The electro-deformation of cells is frequency dependent. Hence, EDS, the frequency-dependent electromechanics can be used as a new biophysical characterization of the coupling between cell mechanics and cell dielectric properties in biological cells.

B. Blood sample

Finger prick blood was collected from a healthy donor under approval of the Institutional Review Board (IRB) at Florida Atlantic University. Animal blood was collected from facial veins of anesthetized mouse under approval of the Institutional Animal Care and Use Committee (IACUC) at Florida Atlantic University. The EDS working medium was prepared with 0.3% (w/v) dextrose (Sigma Aldrich), and 8.5% (w/v) sucrose (Sigma Aldrich) in deionized water. The conductivity of the medium was adjusted to 400μS/cm and 1600 μS/cm using phosphate-buffered saline (Gibco® Life Technologies) and confirmed with a conductivity meter (Extech- EC500). The microchannel was primed using the working medium to keep wet before use. The whole blood specimen of 1μL was diluted directly in 500μL working medium for EDS measurement at room temperature.

C. EDS experiment

A schematic illustration of the EDS experimental setup is shown in Fig. 1. The microfluidic device consists of a pre-patterned indium tin oxide (ITO) electrode array (100 nm thick, 44 μm gap, 106 μm band) pre-patterned on a 0.7 mm thick glass substrate, and a 75 μm deep polydimethylsiloxane (PDMS) channel. The microchannel has an inlet and an outlet of 3 mm and 1 mm for loading samples. The thin film electrode array created an asymmetric electric field, allowing EDS measurement of tens of cells in a single field of view.

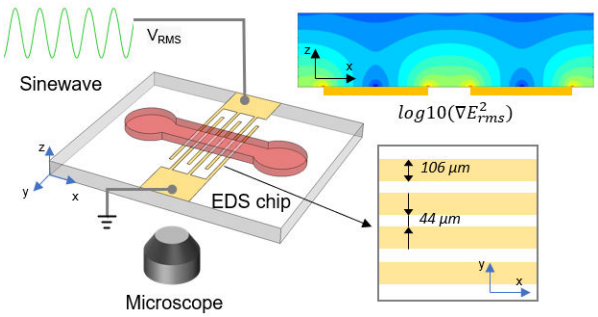

Figure 1. Schematic of the EDS setup. The interdigitated ITO electrodes create an asymmetric electric field (E-field) to induce cell electro-deformation, as shown by the contour plot of E-field gradient.

Sine wave signals ranging from 100kHz to 15MHz were created using a signal generator (SIGLENT SDG830) to enable EDS measurement. Voltage values in current study were root mean square values. Electrical frequency was sequenced from 15MHz with a decrement of 1 MHz till 1 MHz, then a decrement of 100 kHz till 100 kHz. EDS measurement was stopped at three scenarios, including (a) when hemolysis occurred, (b) when cells were repelled away from the electrode edges, known as negative dielectrophoresis, and (c) when cell deformation became negligible.

For the higher conductivity medium, 0.16S/m, 3 V was used for both human and mouse blood specimens. For blood specimens suspended in the lower conductivity medium, 0.04S/m, 1.5 V and 2 V were used for mouse blood cells and human blood cells, respectively. Each EDS was repeated once. Cell image was obtained under a 20× objective lens, using an Olympus IX73 inverted microscope with image contrast enhanced by adding a 414 ± 46 nm bandpass filter in the optical path. Microscopic video was recorded at 30fps and analyzed using ImageJ[15]. Cell image was fitted by an ellipse and deformation was quantified by the elliptical shape factor,

$$\mathrm{ESF} = \mathrm{a/b} \quad (4)$$

where a and b were major and minor axes of the ellipse in the view. The maximum ESF values were measured when the cell membranes are in equilibrium.

III. Results

In relaxation and prior to electro-deformation, both human RBCs and mouse RBCs were mostly circular, with averaged ESF values being 1.2 and 1.08, respectively. Human RBCs were slightly bigger than mouse RBCs with the equivalent diameter of 7.8 μm and 6.0 μm, respectively. Upon electrical excitation, RBCs in suspension moved towards electrode edges with higher E-field strength due to the effects of positive dielectrophoresis, meanwhile cell membranes were stretched uniaxially by electro-deformation, as shown in Fig. 2. The deformation of RBCs, as quantified by ESF showed nearly normal distribution.

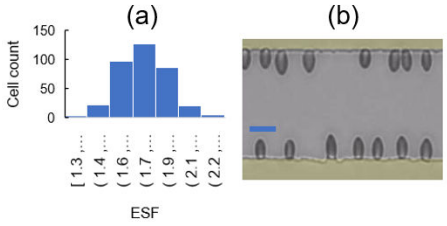

Figure 2. Electro-deformation of mouse RBCs with 3V 15MHz in 0.16 S/m medium. (a) Normal distribution of ESF values. (b) Representative image of uniaxially stretched RBCs. Highlighted areas represent the ITO electrodes. Scale bar - 10μm.

A. Frequency-dependence of electro-deformation

Both blood specimens exhibited frequency-dependence in electro-deformation. Fig. 3 compares the EDS profiles between

human and mouse RBCs measured with the 0.16 S/m medium. At 15MHz, human cells were stretched to 1.51±0.22 (n = 166) while mouse cells were stretched to a significantly higher extent ($p<0.001$), 1.83±0.16 (n = 361). When frequency reduced to 1MHz, the ESF values of mouse cells decreased gradually. When the electrical frequency reduced to 800kHz, a fraction of cells was no longer stretched and thus not included in EDS analysis, as shown by the red arrows in Fig. 3(d). Those cells inclined outwards, which could be due to the drag forces from other electrokinetic forces, such as electrothermal flow[16]. When frequency decreased further, cells started to move away from electrode edges, known as negative dielectrophoresis. In contrast, human RBCs exhibited different behavior, ESF values slightly increased as frequency decreased from 15MHz and reached a peak around 3MHz. As frequency further decreased to 100kHz, ESF values reached a plateau. No obvious hemolysis was noted in human cells.

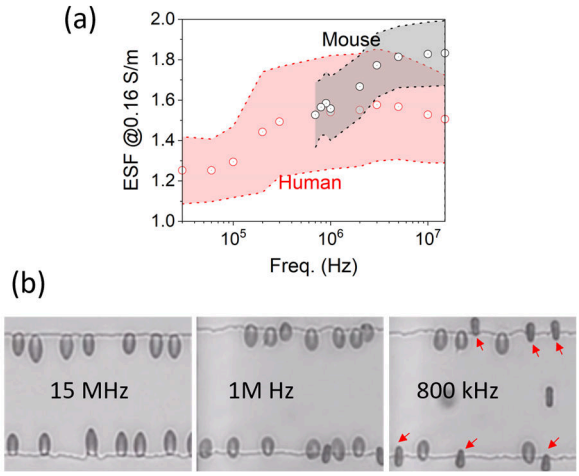

Figure 4. EDS of mouse RBCs and human RBCs using 0.16 S/m medium. (a) Electro-deformation spectral difference between human and mouse RBCs with standard deviation represented as shading. (b) Representative images of mouse cell electro-deformation under 15 MHz, 1 MHz, and 800 kHz.

B. Effects of medium conductivity

The conductivity of surrounding medium affects the interfacial polarization across cell membranes, thus the electrical forces exerted on the cell membranes. A reduction in medium conductivity tends to increase the cellular polarizability. When the medium conductivity decreased from 0.16 S/m to 0.04 S/m, a lower voltage shall be used to avoid extremely large deformations in cell membranes, limiting the accuracy in the ESF measurement using ellipse fitting. Therefore, a lower voltage, 2 V was used to measure the EDS of human cells in this lower conductivity medium. In addition, mouse RBCs have relatively smaller size as compared to human cells, the magnitude of the resultant electrical forces was expected to be greater. Thus, an even lower voltage,1.5V was used to measure mouse cell EDS. Accordingly, to compare between the different medium conductivities and species, electro-deformation was normalized by dividing the ESF values to squared voltage, as the magnitude of the electrical forces is proportional to the gradient of field strength square,

$$ESF_{norm} = ESF/V^2 \quad (5)$$

Fig. 4 shows the remarkably different EDS profiles between human and mouse specimens as well as between two different medium conductivities. Generally, mouse RBCs had a greater deformation than human cells, which are likely due to a higher gradient of field strength via the smaller sizes and/or higher membrane elasticity as compared to human cells. In lower medium conductivity of 0.04 S/m, both cell types showed similar trend in ESF_{norm}, where the maximum deformation was achieved between 3 MHz and 6 MHz and decreased greatly beyond 1 MHz. On the other hand, both cell types exhibited much less deformation in the higher medium conductivity of 0.16 S/m. The difference in the ESF_{norm} trend was also marked between human and mouse specimens. The truncate frequency when electro-deformation became less important were likely near the cross-over frequencies of dielectrophoresis.

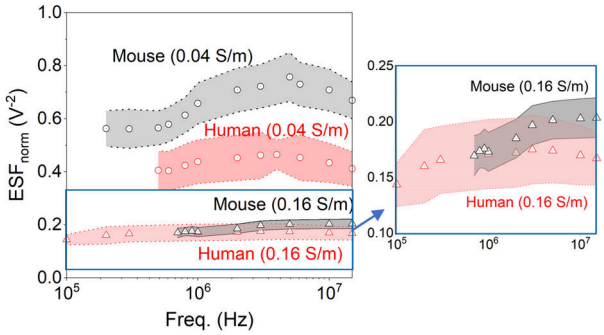

Figure 3. Effects of medium conductivity on the EDS profiles of human and mouse RBCs. Circles and triangles represent the normalized ESF values obtained using 0.16S/m and 0.04S/m media, respectively. Shading represents the standard deviation. Inset shows the 0.16 S/m data.

C. Decoupling analysis of EDS

The magnitude of cell electro-deformation depends on the dielectric properties of cells and membrane elasticity. Decoupling dielectric and mechanical properties from EDS characterization is possible by a combination of numerical analysis of the time-averaged tensor and the viscoelastic model of cell membranes as described in Equations (1, 2). Alternatively, Equation (3) could be used to determine the equivalent electrical forces as an approximation of the Maxwell stress tensor method[17]. Assuming the membrane shear modulus of human RBCs are given, value of the Clausius-Mossotti factor, $Re(f_{CM})$ can be extracted from the EDS obtained in 0.04 S/m medium.

Fig. 5a shows the major and minor axes from the ellipse fitting of cell electro-deformation. Data represented by the solid symbols within the frequency range of 800 kHz -15 MHz were considered when cells deformed primarily due to electro-deformation effects, thus were used to extract $Re(f_{CM})$. Fig. 5(b) compares the $Re(f_{CM})$ profiles with membrane shear modulus of 2.0, 3.0, and 5.4 μN/m, respectively. The solid and dashed curves represent the $Re(f_{CM})$ profiles calculated by MyDEP software[18] using the standard dielectric properties of human RBCs[19] and cell dimensions measured at maximum

electro-deformation, ESF = 1.81 and minimum electro-deformation, 1.64, respectively. Under the assumption of consistent dielectric properties of human cells, matching the Re(f_{CM}) profiles between EDS and dielectrophoresis approaches, the membrane shear modulus of current specimen is determined to be 3.0 μN/m, which agrees with the standard values, 5.4±3.4 μN/m[20].

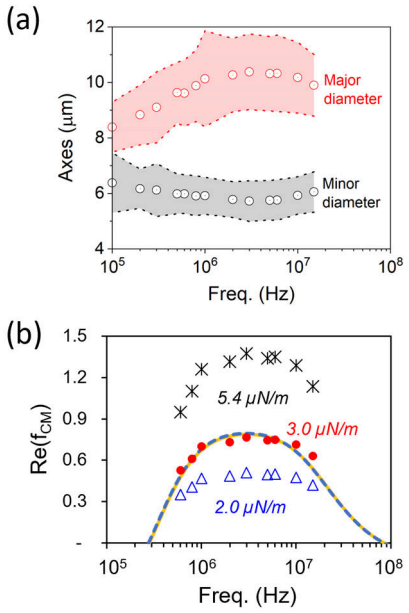

Figure 5. Membrane shear modulus determined by decoupling analysis of EDS for human RBCs in 0.04 S/m medium. (a) Major and minor axes of cells. Error bar – standard deviation. (b) Comparison of Re(f_{CM}) obtained from dielectrophoresis (solid and dashed curves) and EDS (symbols) with different values of membrane shear modulus.

IV. Conclusion and Future Work

This paper reported a novel EDS approach, which provides an interesting biophysical characterization of frequency-dependent cell electromechanics. Other electrokinetic forces, such as the shear flow arising from electrothermal effects, especially in conductive medium, could generate viscous drag that potentially interferes with cell deformation when the dielectrophoretic force is not dominant at low frequencies. Hence, EDS characterization is ideally restrained to the frequency range coinciding with positive dielectrophoresis and a low conductivity medium that is less likely to cause significant electrothermal effects. To fully separate the potential electrothermal effects from electro-deformation, numerical analysis can be performed[21]. The EDS framework can be further extended to study blood cells in animal models of human diseases.

Acknowledgment

Authors would like to thank the support from the National Science Foundation under Grants 1941655 and 2032730, as well as Florida Atlantic University I-SENSE pilot grant.

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