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Dielectrophoresis-Mediated Electrodeformation as a Means of Determining Individual Platelet Stiffness

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Abstract

Platelets, essential for hemostasis, are easily activated *via* biochemical and mechanical stimuli. Cell stiffness is a vital parameter modulating the mechano-transduction of exogenous mechanical stimuli. While methods exist to measure cell stiffness, no ready method exists for measuring platelet stiffness that is both minimally-contacting, imparting minimal exogenous force and non-activating. We developed a minimal-contact methodology capable of trapping and measuring the stiffness of individual platelets utilizing dielectrophoresis (DEP)-mediated electrodeformation. Parametric studies demonstrate a non-uniform electric field in the MHz frequency range (0.2–20 MHz) is required for generating effective DEP forces on platelets, suspended in isotonic buffer with conductivity $\sim 100\text{--}200\ \mu\text{S}/\text{cm}$. A nano-Newton DEP force (0.125–4.5 nN) was demonstrated to be essential for platelet electrodeformation, which could be generated with an electric field with strength of $1.5\text{--}9\ \text{V}/\mu\text{m}$. Young's moduli of platelets were calculated using a Maxwell stress tensor model and stress-deformation relationship. Platelet stiffness was determined to be in the range of 3.5 ± 1.4 and $8.5 \pm 1.5\ \text{kPa}$ for resting and 0.4% paraformaldehydetreated cells, respectively. The developed methodology fills a gap in approaches of measuring individual platelet stiffness, free of inadvertent platelet activation, which will facilitate further studies of mechanisms involved in mechanically-mediated platelet activation.

Keywords

Platelets; Dielectrophoresis; Electrodeformation; Cell stiffness; Mechanotransduction; Electrodeformation; Young's modulus

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INTRODUCTION

Stiffness plays a major role in determining mechanical limitations of viscoelastic materials. When subjected to an external load, viscoelastic materials typically store the majority of the input energy through deformation, while partially releasing energy as heat.¹⁰ As input energy increases, and the ability of a given material to store energy is exceeded, materials begin to crack and fracture.^{33,48} As such, in mechanics, based on the Griffith–Irwin concept, this critical elastic energy release rate is inversely proportional to material stiffness.⁴⁹ Similar mechanical behavior applies to many natural materials and cells.^{12,47,51} By way of example, platelets when subjected to mechanical deformation *via* shear, reach a point where the ability of their membrane to withstand and store energy is exceeded, leading to shape change and fragmentation, with resultant platelet activation and initiation of thrombosis.^{47,51}

Platelet activation is a two-edge sword—essential to limit bleeding and repair wounds⁵⁹ while undesired or inadvertent platelet activation, as occurs with blood passage through atherosclerotic stenotic arteries, diseased valves or therapeutic devices—e.g. ventricular assist devices, mechanical heart valves and stents, results in thrombus formation, reduced blood flow, tissue ischemia, infarction and possible death.^{5,21} While numerous agents exist to pharmacologically limit platelet activation, present agents limit only biochemical activation pathways with little or no effect on shear or other mechanical activation.

Recent work by our group has demonstrated that modulation of platelet membrane fluidity limits platelet activation resulting from mechanical deformation and shear.^{55,56,65} It has been also reported by others that modulation of membrane fluidity may alter cell stiffness.^{15,54} Modulation of platelet stiffness may provide a new means for therapeutically altering the responsiveness of platelets to mechanical deformation and shear. In order to pursue this approach, and further develop agents of clinical value, a methodology is needed to accurately and non-destructively measure platelet stiffness.

Several methods have been described to measure single cell stiffness^{27,58} including: atomic force microscopy (AFM), molecular force spectroscopy, cytoindenter, flow cytometry, magnetic twisting cytometry, micropipette aspiration, microfluidics, magnetic tweezers, microplate manipulation, optical tweezers, and optical stretchers. In general these methods either require adhesion or fixation of the cell followed by some means of applied deformation—e.g. micropipette aspiration and optical tweezers,^{13,36} direct contact and deformation of cells and their surface—e.g. AFM and cytoindenter,^{22,36} or altered flow or passage through constrictive channels or orifices leading to applied deformation and shear—e.g. microfluidics and flow cytometry.^{17,28} These methods are best suited to measure the mechanical properties of adherent, anchorage-dependent cells e.g. fibroblasts or epithelial cells; or for free-floating, anchorage-independent, suspended cells that have limited shear sensitivity—e.g. lymphocytes or circulating tumor cells. As such, they are poorly suited to measure the stiffness of un-activated resting platelets due to their free floating nature, extreme sensitivity to activation upon significant attachment or tethering to a foreign surface, and sensitivity to shear and applied force as a means of activation. Hence an opportunity and need exists for development of a simple method capable of measuring stiffness of suspended, un-activated cells—i.e. platelets, that is free from significant cell

surface area contact, requirement for surface adhesion or significant applied shear or direct deformation.

Dielectrophoresis (DEP) is a technique in which neutral particles are polarized when subjected to a non-uniform electric field, leading to translational motion of the particles, e.g. their attraction or repulsion.^{2,40,42} DEP has been applied to cells and has proven effective as a means of inducing movement that has been utilized for cell separation and partitioning.^{19,41,43} To a limited extent to date, DEP has been utilized as a means of studying the mechanical behavior of individual cells.^{6,31} No studies have examined the utilization of this approach for platelets. In the present study we have adapted and modified DEP as a means of mechanically trapping and deforming platelets for the purpose of determining their mechanical properties. Here we utilize DEP as a means of cellular electro-deformation (EDF). DEP and EDF were selected specifically for platelets as they offer the potential advantage of yielding sufficient deformational force without the need for major platelet surface area contact, platelet substratum attachment, or induction of significant cell membrane indentation or damage. We hypothesized that trapping and subjecting individual resting platelets to an applied oscillating electric field, over a range of field strengths, would induce non-activating, quantifiable physical deformation, allowing determination of single platelet stiffness. Herein we have fabricated a micro-electromechanical chip, with multiple microelectrode arrays, capable of trapping and subjecting multiple platelets to an oscillating electrical field, to induce graded EDF. Using this construct we have devised a system to quantify the degree of EDF as a means of measuring individual platelet stiffness. We first characterized the microelectrode geometry and electrical parameters needed for generating sufficient DEP force to manipulate and deform platelets. The frequency dependence of EDF was then theoretically defined and experimentally verified. A Maxwell tensor stress model was then applied for estimating the magnitude of DEP force. The stiffness (Young's modulus) of platelets was then calculated and compared and contrasted with values reported in literatures. Finally, we confirmed that DEP and the EDF utilized did not lead to platelet activation.

MATERIALS AND METHODS

Platelet Isolation

Gel filtered platelets were prepared as previously described, from whole blood drawn from aspirin and non-steroidal anti-inflammatory medication free volunteers providing informed consent.⁵¹

Microelectrode Chip Fabrication

Parallel microelectrodes with gap separations of 10 μm , (Fig. 1(a)), were fabricated on a glass substrate by a lift-off process. A series of 500 Å titanium (Ti), 1500 Å gold and 500 Å Ti metal thin film were deposited onto a photoresist-patterned substrate by sputtering before resist development. A 127 μm -thick hollow polymer spacer was placed on top of the chip to create a fluid chamber and the chip was covered with a coverslip for microscopic observation. All chip glass surfaces were coated with Sigmacote (Sigma-Aldrich, MO, USA) and rinsed with D.I. water to prevent surface-induced platelet activation.

Electrodeformation Assay

To visualize platelets they were first exposed to Alexa Fluor 594 conjugated wheat germ agglutinin (1 $\mu\text{g/mL}$, 10 min, 37°C, Invitrogen, CA). Platelets were then diluted 100–200 fold (v/v) in isotonic buffer⁴⁴ containing 8.5% w/v sucrose and 0.3% w/v dextrose until the medium conductivity reached $\sim 150 \mu\text{S/cm}$, as measured by a Jenway 4520 conductivity meter (Jenway, Staffordshire, UK). 50 μL of the diluted platelets sample were pipetted into the fluid chamber of the microelectrode chip. An alternating electric current in a sine wave pattern was generated across the microelectrodes using a function generator (332220A, Agilent, CA.) connected to a wideband power amplifier (7600M, Krohn-hite, MA). The amplitude of applied voltage was monitor *via* digital oscilloscope (GDS-1102, GW Instek, CA). The sample chip was imaged *via* fluorescence microscopy (Eclipse E800, 100 $\times$ oil, Nikon, NY), images were captured *via* CCD (Sensicam 12bt CCD camera, Cooke Corporation, MI), processed and quantified using NIH Image J software. The experimental setup for electrodeformation is schematically outlined in Fig. 1(b).

Platelet Activation Assay

Platelet activation level was quantified *via* cell surface expression of P-selectin utilizing immunohistochemical staining.^{3,4,18} Platelets were paraformaldehyde fixed (3%, 20 min) rinsed and incubated (30 min., 37°C) with FITC Mouse Anti-Human CD62P (50 μL , 1:5 (v/v) dilution in PBS, BD Biosciences, CA) and imaged using fluorescence microscopy. Fluorescence intensity levels were measured following background subtraction. Mean and standard error were averaged for >70 platelets under each experimental condition. Platelets incubated with 0.125 μM thrombin (10 min, 37°C) and untreated platelets served as positive and negative controls. Activation of the positive and negative controls was also quantified *via* the chemically modified prothrombin-based platelet activation state (PAS) assay.²⁰

Mathematical Modeling

Mathematical modeling of the frequency dependency of the real part of the Clausius–Mossotti factor³⁴ was simulated by Matlab using parameters from the literature, listed in Table 1. The Clausius–Mossotti (CM) factor was defined by:

$$K(\omega) = \frac{\hat{\epsilon}_c - \hat{\epsilon}_m}{\hat{\epsilon}_c + 2\hat{\epsilon}_m}$$

where ϵ_c and ϵ_m are the relative permittivity of platelet cytoplasm and membrane.

2D mathematical modeling of the electrodynamic force, generated by non-uniform electric field, exerted on a single platelet surface was simulated by finite element analysis software, Comsol Multiphysics 4.4 (Comsol, Burlington, MA). The platelet was assumed as a linear, isotropic and lossy dielectric single shell sphere trapped on an electrode tip and submersed in a lossy dielectric medium. Relative permittivity of lossy dielectric ($\hat{\epsilon}$) was defined as:

$$\hat{\epsilon} = \epsilon' + i \frac{\sigma}{\omega}$$

where ε' is the real part of permittivity, σ is the electric conductivity and ω is the angular frequency. Electric field induced electromechanical properties were governed by the Maxwell equation:

$$\nabla \times H = \sigma E + j\omega D + J_e$$

with the boundaries condition on cell-medium interface of:

$$n \cdot (J_1 - J_2) = -\nabla_t \cdot d_m ((\sigma_m + j\varepsilon_0\varepsilon_m E) \nabla_t V)$$

The magnetic field intensity (H) was assumed as zero in the calculation. Electric field intensity (E) and displacement (D) are calculated by the gradient of potential (V):

$$\begin{aligned} E &= -\nabla V \\ D &= \varepsilon_0 \varepsilon_r E \end{aligned}$$

where ε_r , ε_0 and ε_m is the relative permittivity of the medium, vacuum and platelet membrane respectively, σ_m is the electric conductivity of cell membrane, d_m is membrane thickness, J_1 and J_2 are current density of internal and external boundaries, t is time, J_e is the external source current density and n is vector normal to surface (S).

Electrodynamic force (F) applied on the cell surface can be estimated by integrating the Maxwells's stress tensor (T) over the cell surface, given by:

$$F = d \int_{\partial\Omega} n \cdot T dS$$

and

$$T = \begin{bmatrix} \varepsilon_0 \varepsilon_r E_x^2 - \frac{1}{2} \varepsilon_0 \varepsilon_r (E_x^2 + E_y^2) & \varepsilon_0 \varepsilon_r E_x E_y \\ \varepsilon_0 \varepsilon_r E_x E_y & \varepsilon_0 \varepsilon_r E_y^2 - \frac{1}{2} \varepsilon_0 \varepsilon_r (E_x^2 + E_y^2) \end{bmatrix}$$

where Ω represent the cell body, d is the out-of-plane thickness, which assumed as the focal length of the microscope objective.⁷

Data Analysis

Student's t test was performed to assess the statistical significance of the experimental results. Values statistically significant at $p < 0.05$ were considered a difference.

RESULTS

Microelectrode Chip Design and Fabrication

Microelectrode chip design was developed *via* a modification of a castellated (square indentations or turret-like) microelectrode array geometry.^{46,61} Under positive DEP, particles are trapped in the regions of the strongest electric field.⁴⁶ As in Fig. 2, the triangular tip

design (Fig. 2c and 2e) enabled a more confined, single-point trapping position, compared to a castellated or parallel finger electrode layout.⁶⁷ This allowed more ready observation of single cell behavior, versus multiple cell trapping more likely with castellated or finger-like designs (Fig. 2b and 2d). One key parameter to achieve trapping is the magnitude of electric field, defined as the potential difference against separation distance across electrodes. Microelectrodes with gap distances of 10 μm were tested to provide sufficient field strength for trapping and deforming platelets under a potential of 90–150 $V_{\text{pk-pk}}$. To withstand such high input voltages, a titanium–gold–titanium (Ti–Au–Ti) sandwich structure was utilized to fabricate the chip. Titanium has relatively lower thermal conductivity and better biocompatibility²⁵ compared to most metals. Moreover, a thin layer of titanium dioxide film formed on top of the microelectrode helped prevent bubble formation or deterioration of the electrode from high voltages.¹¹ However, as titanium has a comparably low electrical conductivity against other metals, a gold layer was embedded as the middle layer to oppose this drawback. We found in our studies that our designed construct could be readily and reproducibly fabricated.

Dielectrophoresis-Mediated Platelet Trapping

DEP imparted *via* our fabricated microelectrode chips was found to readily and reliably capture and trap platelets. Capture of platelets on microelectrode tip edges was observed when a gradually increasing AC voltage from 90 to 150 $V_{\text{pk-pk}}$ at 1 MHz was applied. Theoretically, DEP is a lateral motion generated on uncharged particles due to polarization induced by non-uniform electric field.²⁶ The time averaged DEP force exerted on platelets was obtained by:

$$\langle F_{\text{DEP}}(t) \rangle \geq 2\pi\epsilon_r a^3 \text{Re}[K(\omega)] \nabla |E_{\text{rms}}|^2$$

where $|E_{\text{rms}}|^2$ is the time-averaged root-mean-square magnitude of electric field intensity and $\text{Re}[K(\omega)]$ is the real part of Clausius–Mossotti (CM) factor, which determines the magnitude and direction of DEP force.

Real CM factors of platelets in DI water, isotonic buffer, electrodeformation buffer (diluted platelet buffer in isotonic buffer) and platelet buffer were simulated in Fig. 3a using parameters from the literature, listed in Table 1. The imaginary relative permittivity of medium and cytoplasm was neglected in this calculation. Our simulations predicted positive DEP forces would be generated at frequency ranges of 1 k–10 MHz for DI water, 7 k–10 MHz for isotonic buffer and 20 k–20 MHz for electrodeformation buffer. Conversely, only a negative DEP force would be generated in platelet buffer under an applied frequency of 1 k–1 GHz. Further, our simulations predicted that adequate deformation would occur with only minimal cell contact with the electrode tip. As such, with an electrode tip contact area of 0.25 μm^2 , based on a generally rectangular tip configuration, with dimensions of 0.25 μm (height) $\times$ 1 μm (width), and an average platelet surface area of 20–30 μm^2 ,⁵³ only 0.83–1.25% of the platelet surface area is significantly engaged utilizing this methodology.

Frequency Dependence of DEP-mediated Electrodeformation

In frequency dependency studies, an applied voltage of $90V_{pk-pk}$ was utilized to deform trapped platelets. Applied frequencies were maintained higher than 0.5 MHz to avoid electrolysis, which had been observed at frequencies lower than 0.5 MHz. Platelet deformation along the axis of maximum extension was measured and normalized based on the original length. As shown in Fig. 3b, a decrease in deformation from 12.7 ± 7.52 to $-20.8 \pm 3.6\%$ was observed when the applied frequency increased from 0.5 to 10 MHz. Compare to the stimulated CM factor, shown in Fig. 3a, the frequency range of the positive DEP force was well predicted without any fitting parameters.

Voltage Dependence of Electrodeformation

According to the theory of DEP, the magnitude of the DEP force is proportional to the square of the applied voltages. In our studies, platelet deformations under sequential increase of applied voltage, from 0 to $90V_{pk-pk}$ at 15 V steps, were measured at a frequency of 1 MHz and the result is shown in Fig. 4a. Rapid deformations were observed immediately following application of voltage oscillations. Images of deformed platelets were captured in a duration ~ 5 s. Representative images of a single platelet deformed by the increased magnitude of applied forces were demonstrated in Fig. 4b, showing reversible deformations of 7, 10 and 16% at applied voltages of 45, 75 and $90 V_{pk-pk}$ respectively. To verify that the measured deformations were dependent on platelet stiffness, a low concentration of cell fixative, i.e. 0.4% paraformaldehyde, was utilized to stiffen the platelets. The measured deformations were increased from 0.73 ± 1.33 to $4.90 \pm 1.98\%$ when applied voltages increased from 15 to 90 V. Compared to untreated platelets, a significant drop in platelet deformation lengths was observed, with p values < 0.05 . Deformation was decreased by 2.75 times at $90 V_{pk-pk}$ applied voltage, with p value = 0.0002.

Young's Modulus Simulation

A COMSOL Multiphysics 2D electric current model consisting of three parallel microelectrodes, with identical geometry to those of the experimental chips, was created for simulating the electrodynamic forces exerted on the platelet surface. The general method utilized for estimating the forces was by integrating the Maxwells's stress tensor over the cell surface.^{7,60} Assuming the chip was grounded at the top and bottom edges while electric current was applied on the left and right, the electric parameters as listed in Table 1 and the out-of-plane thickness as 100 nm, approximately equaled to the focal length of the objective. The simulated results of electric field norm, electric potential distribution and time-averaged Maxwell surface tensor generated by a $90 V_{pk-pk}$ AC applied voltage at 1 MHz frequency was demonstrated in Fig. 5a.

By Maxwells's stress tensors integration, time-averaged electromagnetic force parallel to the microelectrode was calculated and listed in Fig. 5b. The magnitude of DEP forces increased by the square of electric field strength; from 0.125 to 4.5 nN when the applied voltage increased from 15 to $90 V_{pk-pk}$. Young's modulus (E), defined as the ratio of the stress ($\sigma = F/A$) to the amount of deformation ($\varepsilon = \Delta L/L$), of the platelets was obtained by the slope of linear fitted stress-deformation curve, shown in Fig. 5c. They were estimated be 3.5 ± 1.4 kPa for resting platelets and 8.5 ± 1.5 kPa for platelets treated by 0.4% paraformaldehyde.

The measured deformations were compared to the simulated deformation calculated by the estimated Young's moduli. In both conditions, resting and 0.4% paraformaldehyde treated platelets, the R^2 value between the two curves were >0.97 .

Platelet Activation Studies

We examined whether DEP-mediated electrodeformation would inadvertently activate platelets. As shown in Fig. 6a, P-selectin expression in thrombin-activated platelets was significantly higher than electrodeformed platelets and untreated platelets, with averaged fluorescence intensity levels of 1526.15 ± 75.04 vs. 309.04 ± 26.79 and 238.89 ± 21.64 , respectively. The activation level of untreated and thrombin-activated platelets was verified *via* use of the PAS assay and resulted in <1 and 24.7% activation, respectively. Compared to platelets activated by a known agonist, no major activation of platelet was observed with DEP-induced electrodeformation as employed in our assay. To further investigate the effect of electrodeformation on platelet activation, the morphology of platelets before and after a cycle of voltage dependent deformation was compared, as shown in Fig. 6b. No observable morphological changes were detected. Pearson correlation coefficient between two images was calculated using the Image J Co-localization Finder Plugin with a result of 0.859, indicating a high correlation between the two images. As such, morphologically, no significant platelet activation was observed.

DISCUSSION

In the present study we demonstrate that DEP may be adapted as a means of trapping and stabilizing individual, free-floating, resting platelets and subjecting them to electrodeformational forces. Further, by varying the field strength of the electrodeformational force, coupled with measurement of the extent of cell deformation, the overall stiffness of the platelet can be determined. The present method provides advantage in that cells are suspended and deformation occurs without full anchoring. As such, stiffness may be determined without contact of an external probe or test device, with minimal contact —i.e. $<1.25\%$, of the platelet surface area. This is of particular value for platelets, which are contact sensitive, for which mild mechanical perturbation above a threshold leads to activation, with shape change and initiation of thrombosis.

Over the past decade methods have been developed for determining the mechanical properties of a wide range of cells. These include: micropipette aspiration for endothelial cells and neutrophils;^{35,50} laser trapping for red blood cells;³⁸ AFM for cancer cells;⁶⁴ a microfluidic shear device for fibroblasts³⁰ and micropost arrays for smooth muscle cells.⁸ Platelets are unique amongst cells in that they are more sensitive to applied forces.^{23,24} Imparting a physical force, i.e. as with aspiration, or *via* shear in a microfluidic channel, or *via* direct contact with microposts, is sufficient to activate complex intracellular reactive elements leading to shape change and degranulation. The platelet then becomes a time-varying structure with dynamically changing mechanical properties, limiting the utility of the measurement. Platelets have also been shown to be sensitive to accumulated damage.^{37,52} Repeated measurements *via* membrane contacting probes and techniques ultimately may exceed a damage threshold, also leading to activation. Haga *et al.* utilized

micropipette aspiration to measure resting platelet mechanical properties and detailed how with increasing applied aspiration force platelet membrane fragments were noted to separate from the overall platelet body with ensuing platelet activation.¹⁴ Methods which rely on cell adhesion, i.e. AFM techniques and micropost arrays, are also not favorable for resting platelets. Commonly used cell adhesion approaches include surface coating with extracellular matrix proteins—e.g. fibrinogen, vitronectin or collagen; surface plasma treatment or use of glass substrates, all of which are known to induce platelet activation.^{9,16,29}

DEP and electrodeformation have been utilized to measure cell stiffness including cervical cancer lines,⁶ *Chinese hamster* ovary cells³¹ and *Brassica oleracea* protoplasts.⁶³ Despite this experience this technique has not been applied to date to platelets. Adopting this technique to resting unactivated platelets presents several challenges. First, there is the issue of platelet size. Platelets are an order of magnitude smaller than the other cells studied. Referring to the F_{DEP} equation, the generated forces exerted on platelet surfaces could be ~1000 times weaker than that on other cell types if the same microelectrode chip as utilized in these published DEP electrodeformation studies were to be utilized. Secondly, platelets could easily be activated by the electric pulse and glass substrate utilized in previously described microelectrode chips. In our method, gap distances between microelectrodes were fixed at 10 μm while AC applied electric voltages were in the range of 0–90V_{pk-pk}. This allowed the DEP force generated on platelet surfaces to be maintained in the nN range (Fig. 5b), which was demonstrated to be sufficient to reversibly deform platelets. The CM factor simulations (Fig. 3) predicted that a positive DEP force would be obtained when applied frequencies were in the MHz range with buffer conductivity maintained in between 100 and 200 $\mu\text{S}/\text{cm}$. This estimation generally matched with that observed with our experimental measurements (Fig. 3). Minor deviations, between the simulated curve and the experimental curve, are potentially caused by the variations between the predicted electric properties found in the literature (Table 1) and their actual values.

To characterize platelet stiffness quantitatively we utilized the described methodology to calculate Young's modulus. Platelet Young's modulus was derived from the measured deformation—stress relationship. Tensile stresses applied to platelet surfaces were calculated by mathematical simulation using the Maxwell stress tensor integration method (Fig. 5b). Extension deformations were experimentally measured (Fig. 5c). Our result estimated that the Young's modulus of resting platelets was between 3.5 ± 1.4 kPa, corresponding to Young's modulus determination as was previously reported.¹⁴ Although not directly comparable, the Young's modulus of activated platelets measured by AFM method was reported to be between 100 and 5000 Pa depending upon the measurement location.⁴⁵

Recently we have used a multiscale modeling approach to study the effects of platelet deformability on flowing platelet hemodynamics and its resulting membrane dynamic shear stress distribution that may induce platelet activation.⁶⁸ Rigidity, usually applied in platelet simulations because of their much higher stiffness as compared to RBCs, is likely to lead to an overestimation of their activation potential. By comparing rigid and deformable platelets simulated while flipping in Couette shear flow we demonstrated that deformability significantly influences the flow-induced shear stress levels on the platelet membranes. The stresses in the rigid model were approx. 2.6 times higher as compared to the deformable

model.⁶⁸ By removing the rigidity constraint for simulating mechanotransduction processes this model offers description of processes where, e.g., membrane stiffening plays a role^{1,32,39,62} such as membrane flexibility loss during adhesion because of the stiffening.^{1,32} The electrodeformation approach for measuring platelets stiffness *in vitro* will be further applied by us for validating the numerical predictions. It will also be applied to studies examining manipulation of platelet membrane fluidity and flexibility by pharmacological agents, as are presently ongoing.⁵⁵

Finally, a distinguishing feature of the DEP electrodeformation method is the lack of platelet activation observed. While there are reports in the literature that nanosecond pulse electric fields can activate platelets,⁶⁶ at the levels utilized by our method no morphological changes in platelets, before and after electrodeformation, were observed (Fig. 6b). Compared to platelets activated by the commonly known agonist, thrombin, platelet activation induced by electrodeformation was insignificant (Fig. 6a).

In summary, DEP-induced electrodeformation applied to platelets *via* specifically designed, triangular, single point microelectrode chips allowed for successful trapping and cyclic deformation of platelets. Applying a range of field strengths to trapped cells allowed for a range of deformations to be obtained, which could be captured and quantified for stiffness determination. Over the range of electric fields utilized no significant degree of platelet activation was detected. Utilizing the Maxwell stress tensor integration method applied force could be calculated and individual platelet stiffness determined from the stress-deformation relationship derived. The present method extends tools available for cell biology research and for studies of platelet mechanotransduction, as a technique that is free from confounding effects associated with significant cell contact, underlying substrate effects or repeated contact-mediated damage.

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FIGURE 1.

(a) The Ti–Au–Ti electrode deformation chip with the bright field microscopy image of the triangular tip designed microelectrodes in the insert. (b) Schematic diagram of the experimental setup for electrode deformation-based platelet stiffness measurement. Platelets sample were placed in a $127\ \mu\text{m}$ thick fluid chamber on top of the microelectrodes connected to an amplified AC power supply. Geometry of platelets was captured *via* computer controlled CCD camera-imaging unit.

FIGURE 2.

Simulated electric field strength in parallel (a), castellated (b) and triangular (c) microelectrode array geometries. (d & e) Enlargement of the major cell trapping regions on castellated (d) and triangular (e) designs to emphasize the cell-trapping patterns between two electrodes. Single cell trapping (orb with double arrow) and focusing is more likely to occur with the triangular design verse the castellated one. This claim is theoretically verified and experimentally proven. Red arrows are electric field vectors with magnitude proportional to the field strength.

FIGURE 3.

(a) Simulated Clausius–Mossotti (CM) factors as a function of AC field frequencies for human platelets suspended in different buffers. Medium conductivities of D.I. water, isotonic buffer, electrodeformation buffer and platelet buffer were measured as 1, 5.85, 144.23 $\mu\text{S}/\text{cm}$ and 11.14 mS/cm respectively. (b) Experimental platelet electrodeformation dependence against AC field frequencies. The applied electric potential was 90 V_{pk-pk}. Averaged deformations ($n = 12$) were plotted in black triangle symbol and the error bars indicated standard errors.

FIGURE 4.

(a) Platelet electrodeformation dependence versus applied electric field strength at 1 MHz applied frequency. The averaged deformation of untreated (control, $n = 13$) and 0.4% paraformaldehyde treated platelets ($n = 20$) are plotted with black diamond and grey square symbols, respectively, error bars indicate standard errors. The power fitted curve is shown as a black solid line. (b) Fluorescence image sequence demonstrating a single platelet deformed *via* a positive dielectrophoresis force generated by 0, 45, 75 and 90 V_{pk-pk} applied AC voltages. With increasing voltage platelet lengthening is detected. Lengths of the platelet were measured along the axis of maximum deformation (labeled by white solid line) and labeled by text. The frequency of the AC electric field was 1 MHz.

FIGURE 5.

(a) Simulated electric field norm (pseudo color surface), electric potential distribution (colored contour line) and time averaged Maxwell surface stress tensor (red arrow) across a pair of microelectrodes. (b) Table of calculated time averaged horizontal electromagnetic force (along x -direction of figure a) applied to a single platelet under varying electric field strength. The frequency of the AC electric field was assumed as 1 MHz. (c) Stress-strain curves of untreated control (black solid diamond) and 0.4% paraformaldehyde treated samples (grey solid circle); dotted lines are the linear fitted curves (In c: $n = 13$ for control and $n = 20$ for 0.4% PFA, error: standard error).

FIGURE 6.

Effect of electrodeformation on platelet activation. (a) P-selectin expression levels for untreated (control), electrodeformed and thrombin (0.125 μ M) treated (positive control) platelets were quantified via fluorescence. No significant activation was induced *via* the utilized DEP EDF protocol. Average and standard error were calculated from measurements of over 70 individual platelets. (b) Fluorescence image of electrodeformed platelets. No significant morphologic evidence of activation was detected.

TABLE 1List of electrical properties of platelets and electrodeformation buffer.^{7,57}

Parameters	Values
Real relative permittivity of medium, ϵ'_r	80
Real relative permittivity of platelet membrane, ϵ'_m	10
Relative permittivity of platelet cytoplasm, ϵ_c	40
Electric conductivity of platelet membrane, σ_m	0.00001 S/m
Electric conductivity of platelet cytoplasm, σ_c	0.1 S/m
Radius of platelet	2 μm
Platelet membrane thickness	10 nm