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Quantification of inter-erythrocyte forces with ultra-high frequency (410 MHz) single beam acoustic tweezer

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Abstract

Efforts on quantitative measurements of the interactive forces of red blood cells (RBC) have been pursued for many years in hopes of a better understanding of hemodynamics and blood rheology. In this paper, we report an approach based on an ultra-high frequency (410 MHz) single beam acoustic tweezer (SBAT) for quantitative measurements of inter-RBC forces at a single cell level. The trapping forces produced by this ultra-high frequency (UHF) SBAT can be quantitatively estimated with a micropipette. Since the focal beam diameter of the 410 MHz ultrasonic transducer used in this SBAT was only 6.5 micrometer (μm), which was smaller than that of a RBC ($\sim 7.5 \mu\text{m}$), it was made possible to directly apply the beam to a single RBC and measure inter-RBC forces against the pre-calibrated acoustic trapping forces as another example of potential cellular applications of the SBAT. The magnitude of these forces was found to be $391.0 \pm 86.4 \text{ pN}$. Finally, it is worth noting that unlike several other methods, this method does not require the measuring device to be in contact with the cells.

Keywords

Ultra high frequency ultrasonic transducer; Single beam acoustic tweezer; Acoustic trapping force; Red blood cell; Micropipette aspiration technique

I. Introduction

RBC aggregation affects blood circulation and tissue perfusion including microcirculatory blood flow dynamics and venous flow resistance. Abnormal RBC aggregation has been linked to diseases such as circulatory disorders; malaria, sickle cell disease,³² and system lupus erythematosus (SLE).²⁷ RBC agglutination may be caused by intermolecular attractive forces of electrostatic nature e.g., Van der Waal forces and hydrogen bonds. In previous studies, two different models have been proposed to account for RBC aggregation: the Cross-Bridge model and Depletion model. The Bridge model proposed that fibrinogen in plasma and the macromolecules non-specifically were absorbed onto the RBC membrane and formed bridges between adjacent cells.^{5,7} Fibrinogen mediated aggregation of RBCs

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could induce reversible rouleaux formation, and RBC aggregation was found to be directly proportional with the concentration of fibrinogen.^{6,25} The depletion theory proposed that the surrounding macromolecules around RBCs were expelled from the region among RBCs, and the concentration of macromolecules depleted in this region compared to the bulk phase¹ resulted in an osmotic pressure difference leading to solvent displacement in the bulk phase that in turn caused an attraction force among RBCs.^{26,31}

In-depth studies of RBC aggregation were carried out and allowed for monitoring the state of blood microcirculation and for characterizing and analyzing RBC aggregates including size and strength as a diagnostic tool.^{2,14,29} Moreover, a knowledge of inter-RBC forces is needed to allow a better understanding of the mechanism of red blood cell aggregation. A knowledge of inter-RBC forces is needed to allow a better understanding of the mechanism of red blood cell aggregation. In the last few decades, the mechanics of RBC interaction at the cellular level have been studied by different technologies: scanning electron microscopy (SEM),¹¹ micropipette aspiration technique (MAT),⁴ atomic force microscopy (AFM),³¹ and optical tweezer (OT).³ The interactive energy density among RBCs in autologous plasma was measured using a MAT and was found about a few $\mu\text{J}/\text{m}^2$.⁴ The AFM allowed the measurement of the depletion induced adhesive force between two RBCs which was found to be in the range from 14 to 170 pN.³¹ However, both methods were required direct contact with the objects to measure the force, disturbing the object on which the measurement was made. More recently, many aspects of RBC behaviors have been studied with optical tweezers, which is a non-contact tool as well. Maklygin et al. fabricated double beam optical tweezers to measure the interactive forces between RBCs in an aggregate.²⁴ Khokhlova et al. measured aggregation forces and aggregation velocities in RBC pairs obtained for healthy and SLE blood samples using double-beam optical tweezers.¹² Moreover, Lee et al. compared between RBC aggregating and disaggregating forces in both autologous plasma¹⁹ and in protein solutions²⁰ to study dynamic RBC interactions depending on the environment conditions. Aggregation forces measured by optical tweezers were found to be in the range only from 8.4 to 30 pN.^{12,19,20,24} However, in cases where RBCs interacted with forces stronger than the maximal force that could be generated by optical traps limited to dozens of pNs, the optical trap force could no longer induce further disaggregation, hence making it impossible to measure those forces. In approximately 90% of the experiments, the RBCs were either connected by small tethers or still in contact with each other, meaning that they were not totally separated.^{12,13} Moreover, an additional concern of the optical trapping approach is that the laser sources may easily introduce thermal effects, resulting in damages to the sensitive RBC membrane surface.

As an alternative tool for measuring inter-RBC forces, the single beam acoustic tweezer (SBAT) may have the following advantages over optical tweezers: 1. Stronger trapping forces in a range from pN to nN depending on the driving conditions^{17,18,22,23} 2. There are no mechanical contact and no labeling, thus less likely to lead to damages on objects on which measurements are to be performed 3. It may be used for in vivo experiments with a larger depth of penetration in light opaque medium such as blood. For these reasons, to date, the SBAT have been used to study the mechanical property of the cancer cell membrane⁹ and intracellular calcium signaling properties of cancer cells.¹⁰ Previous studies were carried out with transducers at frequencies under 200 MHz, which produced a spatial resolution in

the range from tens to hundreds of micrometers. This is because the fabrication of a UHF transducer is challenging and time consuming process requiring the precise control of fabrication parameters. In this study, we designed and fabricated 410 MHz ultrasonic transducers, which had a beam width of only a few micrometers, allowing more precise control of a microparticle, a nanoparticle, or a single cell. In addition, a calibration system based on MAT for the trapping force of the SBAT at UHF was recently developed.²³ The 410 MHz SBAT was used to trap and detach RBC from a RBC aggregate. The intercellular adhesion forces between two RBCs were estimated against the pre-calibrated trapping force using a MAT. The SBAT at UHF with an ultrasonic beam width smaller than the diameter of the RBC is a key advantage over previous SBATs. The small beam width allowed trapping of a single RBC preventing any significant disturbance to the adjacent RBCs or aggregates.

II. Materials and Methods

A. UHF Transducer fabrication

Following steps were taken to fabricate a 410 MHz lithium niobate (LiNbO_3) press focused ultrasonic transducer.³⁰ Lithium niobate (LN) single crystals (36° rotated Y-cut) were purchased from Boston Piezo-Optics (Bellingham, MA). LN was preferred for the design of a large aperture device and it also offers a high sensitivity due to its desirable properties of a good electromechanical coupling coefficient ($k_t \sim 49\%$), a low dielectric permittivity ($\epsilon^s \sim 39$), and a high longitudinal sound velocity ($c \sim 7340$ m/s). This transducer consisted of three layers: a matching layer, a piezoelectric element, a backing layer as shown in Figure 1a. The optimized aperture size and the thickness of a LN and the parylene matching layer thickness were designed by A Krimholtz, Leedom, and Matthaei model software (PiezoCAD, Sonic Concepts, Bothell, WA). The backside of the LN was sputtered with a 1500 angstrom ($\text{\AA}$) chrome and gold layer. A conductive silver epoxy (E-SOLDER 3022, Von Roll Isola, USA) backing layer with an acoustic impedance of 6 MRayl was casted onto the backside of LN, centrifuged at 3000 rpm for 15 min, and cured overnight at room temperature. The acoustic stack including the piezoelectric material and the backing layer was pasted on the glass with wax. The backing layer was lapped down to its designed thickness of 1 mm first and then, LN was lapped down to $7.1 \mu\text{m}$ with an extreme care, and diced into the size of $250 \mu\text{m} \times 250 \mu\text{m}$ using a dicing saw (Tcar 864-1, Thermocarbon, Casselberry, FL). The acoustic stack was placed in a brass housing concentrically and the gap between the stack and the housing was filled by an insulating epoxy (Epo-Tek 301, Epoxy Technologies, Billerica, MA). A single silver coated copper wire was connected to the backing layer using conductive silver epoxy. The transducer was assembled in a high frequency Sub Miniature version A (SMA) electrical connector. Mechanical press focusing was performed using a heated bearing ball to obtain an f-number 1.3. This bearing ball of 0.6 mm diameter was highly polished to make a smooth surface of the LN. The electrodes were sputtered with a layer $1500\text{\AA}$ chrome and gold layer on the front surface of the piezoelectric layer to make the ground connection between the elements and the brass housing. A $1.1 \mu\text{m}$ thick parylene layer was vapor-deposited using a PDS 2010 Labcoater (SCS, Indianapolis, IN) on the front face of the transducer. This parylene coating served as the matching layer as well as protection layer. A cross-sectional view of the transducer and a photo of final transducer are shown in Figure 1a and 1b.

B. Transducer performance

The pulse–echo response and the frequency spectrum of the transducer are shown in Figure 1c and 1d. The center frequency was 410 MHz and the -6 dB fractional bandwidth was 31%. Transducer was connected to a JSR (Pittsford, NY) model DPR 500 pulser/receiver and excited by electrical impulses at 500 Hz repetition rate at 50 Ω damping. The reflected waveform was received by a 500 MHz bandwidth receiver with high pass filter of 5 MHz and low pass filter of 500 MHz and was digitized at 2 G sample/s. The lateral beam profile was measured with a wire target. The movement of transducer was controlled by a three-axis positioning system (Inchworm, Burleigh Inc, Fishers, NY) with a digital-controlled system. A pulser/receiver (JSR ultrasonics DPR 500) excited the signal to a 2.5 μ m tungsten wire (California Fine Wire, Grover Beach, CA). The wire was immersed in deionized water and scanned in lateral directions at a resolution of 0.5 μ m. The echo signal reflected from the wire was used to construct the beam profile to determine the size of beam in the lateral direction. Figure 1e shows the relative magnitude as a function of lateral distance, and the -6 dB beam width was measured to be 6.5 μ m similar to the theoretically calculated lateral beam resolution (4.8 μ m = f-number * wavelength).

C. Blood sample preparation

Fresh blood was obtained from ten healthy donors by a fingertip needle prick (Institutional Review Board study ID, UP-16-00713). RBCs from each of ten donors were washed three times with isotonic phosphate buffered solution (PBS) by centrifuging at 3000 g for 5 min, and the separated plasma was stored for later re-suspension. 1 μ L of RBCs was diluted in 100 μ L PBS. Microspheres of 3.78 μ m diameter (Bangs Laboratories Fishers, IN) were centrifuged in distilled water at 3000 g for 5 min. Three times washed microspheres were re-suspended in 0.1 % poly-L-lysine solution (Sigma-Aldrich, St Louis, MO) and stored at room temperature for 4 hours for coating. The coated microspheres were washed one time and diluted to $2.5 \times 10^4/\mu$ L in PBS. 100 μ L poly-L-lysine coated microsphere suspension was mixed with 100 μ L diluted RBCs suspension so that the ratio of RBCs and microspheres was 2:1, and waited for 1 hour resulting in strong adhesive interaction between RBCs and coated microspheres.¹⁶ After that, RBCs and microspheres were suspended in 1 mL autologous plasma for cell aggregation. The resultant solution was added into a petri dish with an acoustically transparent mylar film (thickness of 20 μ m) as the bottom surface. The poly-L-lysine coated microspheres made a strong attachment with mylar film as well as RBCs aggregation. Subsequently, 0.1 % serum bovine albumin (BSA) solution was added to the suspension in order to prevent RBCs from sticking to the mylar film.

D. RBC experiments

The experimental arrangement for SBAT is shown in Figure 2. Sinusoidal burst signals at 410 MHz, generated by a function generator (Stanford Research Systems, Sunnyvale, CA) and amplified by a 50 dB power amplifier (525LA, ENI, Rochester, USA) were used to drive the transducer. The motion of RBCs was observed using an inverted microscope (Olympus IX-71, Center Valley, PA) and recorded via a CMOS camera (ORCA-Flash2.8, Hamamatsu, Japan). How a single RBC can be manipulated by the SBAT is shown in Figure 3. A single RBC could be trapped by the SBAT at 410 MHz with a duty factor of 1%, a pulse repetition

frequency (PRF) of 1 kHz, and a peak-to-peak input voltage (V_{pp}) of 7.94. The black arrow in Figure 3a–3c shows a single RBC movement along the SBAT direction.

Figure 4 illustrates experimental and schematic steps of measuring inter-RBC forces. The poly-L-lysine coated microsphere was anchored to the bottom of the petri dish as well as attached to one RBC of an aggregated RBC pair. Two RBCs (labeled as RBC-1 and RBC-2 in Figure 4) were aggregated by autologous plasma. The focal point of the trap center was initially placed at 10 μm from the center of the RBC-2 as shown in Figure 4a via a three axis motorized linear stage (SGSP 20, Sigma KOKI Co., Japan). The transducer was turned off at first and turned on with an input voltage starting from 0 V_{pp} with a duty factor of 1%, a PRF of 1 kHz. As the input voltage of the transducer was increased with a step size of 0.31 V_{pp} , the RBC-2 was deformed and gradually towed into the acoustic trap as shown in Figure 4b–4e. The input voltage was increased until the trapping force was strong enough to disaggregate the two RBCs as shown Figure 4f. Microsphere was immobilized, holding the RBC-1 during the SBAT experiment due to its poly-L-lysine coating. The distance between the center of the RBC-2 and the trap center when the disaggregation occurred was recorded using a camera and analyzed by an image analysis software (Metamorph, Molecular device, Sunnyvale, CA).

E. Trapping force calibration using a MAT

Calibration of the trapping force from the SBAT was made with a micropipette aspiration technique (MAT) which was described in details previously.²³ The micropipette was upgraded by reducing the size of inner diameter into 0.5 μm . The main advantage of this technique is its capability of measuring the trapping force ranging from pN to nN depending on transducer's driving conditions. Micropipette devices were made with the targeting inner diameter size by a glass capillary with filament (GD-1, Narishige, NY) and a vertical micropipette puller (PC-10, Narishige, NY). A pressure controller (ez-gSEAL 100B, Neo biosystem, CA) was used to accurately generate the suction pressure in a range from 0 to 33.3 kPa, controlled by an ez-Gseal control software (NBSC Controller, Neo biosystem, CA). As shown in Figure 5a, the schematic diagram of the experimental arrangement, the SBAT and the micropipette controlled by two XYZ positioners, respectively, were used to manipulate the RBC in a petri dish. A bent steel connector was connected the pressure controller with the micropipette lining at 0 degree.

The procedure of the calibration experiment is shown in Figure 5b. Figure 6 shows microscopic images illustrating the change of RBC motion during calibration. A randomly selected RBC in the dish was trapped and located at 10 μm away from the micropipette tip by the SBAT so that the trapping action of the SBAT was not affected by the presence of the micropipette. Red dot circles in Figure 6 indicate the ultrasonic trap at focus. The trapping forces of the SBAT acting on the RBC were under the same driving conditions of the SBAT as those of the inter-RBC force experiment: a duty factor of 1%, a PRF of 1 kHz, and the peak-to-peak voltages 7.94 V_{pp} . The suction pressure was increased from 0 Pa at the step size of 13.3 Pa until the RBC was completely attached to the tip of the micropipette as shown in Figure 6e. As the suction pressure was increased, the acoustic trapping force was equally balanced out by the suction force of the micropipette to displace the RBC from the

center of acoustic beam to the micropipette tip. Suction forces were recorded when the RBC was located at four designated displacements from the trap center (1.0 μm , 2.0 μm , 3.0 μm and 4.0 μm). It is worthwhile to note that as the suction pressure exceeded a certain level, RBC was completely sucked into the micropipette.

F. RBC viability test

A viability assay was carried out with Calcein AM, (Invitrogen Corp., Grand Island, NY) after the cell trapping experiment. Calcein-AM is a vital dye that rapidly enters live cells converting to green fluorescent. To assess the cell viability, Calcein-AM was prepared as a stock solution of 1 mM in dimethylsulfoxide stored at room temperature. At a final concentration of 10 μM of Calcein-AM it was added into the sample chamber containing the RBC. Fluorescence imaging of the RBC was obtained by a microscope with an excitation of 488 nm and an emission of 532 nm. The intensity of green fluorescence was normalized before (control) and after trapping (10, 20 and 30 min), and statistical analysis including mean and standard deviation with the sample size of $n=10$ were calculated. After all, the two-tail paired t-test with a p-value < 0.05 was examined to check their significance level of difference.

III. RESULT

As shown in Figure 4, blue closed circles and red circles represent the center of the RBC-2 and the trap area respectively. Trap center was originally located 10 μm from the blue closed circle in Figure 4a. As the input voltage of the transducer was increased from 0 V_{pp} while other parameters were fixed i.e., a duty factor of 1% and a PRF of 1 kHz, the RBC-2 was gradually moved closer to the focal zone. When two RBCs started to be disaggregated as shown in Figure 4e and the distance between the RBC-2 and the trap and the applied voltage were found to be $3.76 \pm 0.84 \mu\text{m}$ and $7.94 \pm 2.35 V_{pp}$ respectively (sample number of 10). The acoustic trapping force to disaggregate two RBCs equal to the inter-RBC force (Trapping force = Inter-RBC force). In order to find the trapping force at the displacement, 3.76 μm from the trap center, a calibration experiment was performed.

Since the force generated from a micropipette ($F_{\text{micropipette}}$) was equally balanced out by could be calculated from the equation of the suction the acoustic trapping force (F_{trapping}), F_{trapping} force at the micropipette tip below;²⁸

$$F_{\text{micropipette}} = \pi R_p^2 \Delta P \quad (1)$$

where R_p is the inner radius of a micropipette, is the aspiration pressure in a micropipette. The force measurement range of the pressure controller was from 0 to 6.54 nN based on the inner radius of the micropipette (0.25 μm in this study) and the suction pressure (i.e., 0 to 33.3 kPa). According to the Hooke's law, the trapping stiffness could be calculated by the equation given below;¹⁷

$$F_{trapping}=kx+y \quad (2)$$

where y is the y -intercept and x is the displacement of RBC from the trap center. An ultra-high frequency SBAT generates a highly focused acoustic trap to induce the RBC toward its trap center. Depending on the displacement of the trapped RBC from the trap center, the trapping force changes like an elastic spring. The trap stiffness (k), an elastic spring constant of the trap¹⁸ provides the $F_{trapping}$ in the ultrasonic trap zone. The k was calculated to be 102.9 pN/ μ m through the slope of linear regression by plotting $F_{trapping}$ – displacement from the trap center curve. Therefore, the trapping forces on the RBC could be calibrated a function of the displacements in Figure 7. Since the final displacement was measured as 3.76 μ m between the RBC-2 and the trap center, the final trapping force was estimated to be 391.0 ± 86.4 pN (sample number: 10) from the graph shown in Figure 7.

Figure 8 shows the RBC's viability before (control) and after (10, 20, and 30 min). After trapping force applied to the RBC, fluorescence intensity of Calcein AM was measured in Figure 8a. All of the intensity was normalized in Figure 8b and compared with the control group using two tailed t- test with a p -value < 0.05 . No significant difference between them was observed as the p -value was 0.58 which was greater than 0.05. As a result, we may conclude that the trapping force produced little effect on the RBC under the given acoustic driving conditions.

IV. DISCUSSION

This study demonstrated that the capability of a SBAT at ultra-high frequency for measuring inter-RBC forces. Previously, several cellular SBAT applications were demonstrated. The SBAT was used to study the mechanical properties of human breast cancer cells including MCF-7,⁹ SKBR-3,¹⁰ and MDA-MB-231.⁸ Also, the SBAT was shown to be capable of manipulating cell-sized microparticles inside of an excised blood vessel.²¹ Although previous versions of SBAT's capability of measuring cell deformability was previously demonstrated⁹, it was neither possible to obtain absolute values of elastic constants of cells including RBC nor possible to measure the interactive forces between cells due to the frequency of the SBAT. This is because the beam width of an ultrasonic transducer is inversely proportional to the center frequency. If the beam size is larger than the dimension of a RBC, the radiation force generated by the transducer might disturb the adjacent RBCs as well making the interactive force measurements unreliable. Therefore, fabricating UHF transducer, which can generate acoustic beam size less than the dimension of a RBC (~ 7.5 μ m), was a key to allow this experiment to be successfully carried out. The 410 MHz ultrasonic transducer with a beam width, 6.5 μ m fabricated for this work allows the trapping of a RBC without producing disturbance to adjacent RBCs. Fabrication of the 410 MHz transducer was challenging because the piezo-electrical material had to be lapped down to the targeted thickness, 7.1 μ m, with an extreme care as shown in Figure 1a. In addition, the extremely small thickness of the piezoelectric material makes it very easy to be broken during the mechanical pressing focus using a heated bearing ball.

Previously with a SBAT 200 MHz the relative deformability of cancer cells was measured, but no quantitative absolute estimate of any cellular elastic constants could be obtained.^{9,10} One of the reasons is that the measurement of the acoustic trapping force at frequency over 60 MHz is not possible due to the technical constraints of hydrophone and system.¹⁵ Recently, the trapping force of 110 MHz transducers was successfully calibrated using a micropipette aspiration technique. By applying the MAT with a reduced inner diameter of the micropipette, the trapping force of the 410 MHz transducer was found to be in the order of a few hundred piconewtons. As shown in Figure 7, the calibrated trapping forces were ranged from 100 to 500 pN and the trap stiffness, k was calculated to be 102.9 pN/ μm . Consequently, the inter-RBC force was found to be 391 pN by interpolating from the pre-calibrated trapping force.

RBC aggregation and disaggregation forces were measured to be in the range of a few pN by optical tweezers previously.^{12,19,20,24} However, due to the limitation on the maximal trapping force produced by the optical tweezers, measurement could not be made if RBCs aggregation forces were stronger than the optical trapping force. Since they consider only the cases where successful break-ups of an aggregate were achieved in their experiments, aggregation forces measured by optical tweezers might be underestimated. This may be the primary reason that the aggregation force, measured by the SBAT is much stronger than that measured by optical tweezers. Since the SBAT has the advantage of being capable of generating larger trapping force from pN to nN depending its driving conditions, this method may be a viable alternative to optical tweezers.

In addition, between two RBC aggregation models, namely, the Cross-Bridge model and the Depletion model, we considered the Bridge model which is the fibrinogen mediated aggregation of RBCs and is favored for a longer contact time between two RBCs. This model indicates that the aggregation forces are dependent on the contact time of two RBCs during the first few seconds.³ The AFM measured the depletion-induced adhesive forces between RBCs which were found to be in the range of 14 to 23 pN and 43 to 169 pN for dextran 70 and 150 respectively.³¹ The main reason of these weaker aggregation forces measured by the AFM compared to our findings was probably that the actual contact time between RBCs was minimized to a few seconds in order to measure the interaction force based on purely depletion-induced adhesive forces. On the other hand, the Cross-Bridging model allows much longer contact time than that of the AFM, which results stronger aggregation forces between two human RBCs. In conclusion, we found from the first set of experimental measurements of the fibrinogen mediated aggregation force with SBAT that the inter-RBC forces are in the range of hundred pNs.

V. CONCLUSION

In this study, the inter-RBC force was directly measured based on the pre-calibrated trapping force produced by a 410 MHz LiNbO₃ focused transducer. A 410 MHz transducer is capable of generating a focused beam with a width smaller than the dimension of a RBC. As a result, it does not cause any disturbance to the neighboring RBC during the trapping experiments. An aspiration micropipette approach was used to calibrate the trapping force on a single RBC produced by the 410 MHz transducer. In the near future, efforts will be made to use

this technology to assess the interactive forces between other types of cells including the adhesive forces between white blood cells and the endothelial cells of the blood vessel surface.

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Figure 1.

Profile of a single element 410 MHz focused transducer (a) Cross sectional view of the transducer (b) Photograph of the fabricated transducer (c) Receive-echo response. (d) Frequency spectrum. (e) Lateral profile.

Figure 2.

Schematic diagram of experimental systems for single RBC trapping with SBAT. RBC was trapped by a 410 MHz transducer driven a duty factor of 1%, a PRF of 1 kHz, and a peak-to-peak input voltage 7.94 of V_{pp} .

Figure 3. Microscopic images of the single RBC trapping using the 410 MHz transducer. The black arrow represents the direction of transducer movements.

Figure 4.

Experimental images and schematic diagram for measurement of the inter-RBC forces. (a) A poly-L-lysine coated microsphere was attached to the bottom of the dish as well as a RBC aggregate. The transducer was fixed at one position and input voltage was increased starting from 0 V_{pp} . (b-d) As the transducer gradually increased the input voltage, the RBC-2 is pulled into the acoustic trap by deforming. (e) at input voltage 7.94 V_{pp} , the RBC-2 is completely detached from the RBC-1. (f) microscopic images of disaggregated RBCs. Red circles, yellow solid circles, and blue solid circles represent the acoustic trap zone, the center of acoustic trap and the center of RBC-2 respectfully. $n=10$

Figure 5.

Calibration acoustic trapping force using a MAT. Schematic diagram of the experimental arrangement. The RBC was trapped by a 410 MHz transducer driven at a duty factor of 1%, a PRF of 1kHz, and a peak-to-peak input voltage of 7.94 V_{pp}. The micropipette was set up at the side of the RBC and the suction force was increased starting from 0. The RBC is moved to the tip of micropipette as the two forces, the suction force and the trapping force are balanced out.

Figure 6.

Microscopic images of calibrating trapping force. Red circles represent the acoustic trap zone. (a) the RBC was trapped by the transducer and located 10 μm from the micropipette tip. (b-e) The suction force from the micropipette was turned on and was increased until the RBC was attached to the tip. (f) The RBC was completely attached to the micropipette tip and started to deform. (g) The RBC was completely sucked into micropipette and disappeared.

Figure 7.

Calibration graph for the 410 MHz transducer driven at a duty factor of 1%, a PRF of 1 kHz, and a peak-to-peak input voltage of 7.94 V_{pp} showing the relation between the trapping force and the displacement from the trap center and the RBC center. n=10

Figure 8. Trapping force effects on RBC viability. (a) Fluorescence image before trapping (control) and after trapping (10, 20, 30 min). (b) Normalized cell viability. The fluorescence intensity was normalized and compared with the control group. n=10