Laser Controlled Cavitation Deformation in Red Blood Cells

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Abstract

In this research work, we studied μ TAS (μ total analysis system) method to measure the deformability of red blood cells. We took the sample of RBCs (red blood cells) in a micro fluidic based chamber, then allowed low intensity laser of infra red region, mostly at wavelength 1064 nm, to fall on the sample. This generated a cavitation bubble in the fluid because of evaporation mechanism involved. We tightly hold the slides containing blood sample so that the displacement of RBCs (red blood cells) stay confined in the viewing area of the microscope. Firstly the deformation due to expansion of cavitation bubble was sighted and then came the restoring of shape of RBCs (red blood cells) which was possible due to a focused and pulsed laser. The RBCs restored their shape after some time and was viewed with the help of a video recorded by a high-speed camera. In this way the plastic character and elastic properties of RBCs (red blood cells) was measured.

Keywords: μ TAS, Rheology, Cavitation Bubble

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INTRODUCTION

LASER technology has been under research for decades and because of its low intensity therapy utilized in medical applications. As a matter of fact optical properties of light interact tissues in different manner, and in this regard lasing is to let a tissue absorb laser light. Some parts of the laser light are scattered some are absorbed and others reflected but, the incident light must be analyzed for the sake of safety of the patient. The color of tissue is the wavelength of light it reflects, so color of blood appears red because it reflects wavelength of red portion of the visible light spectrum. Therefore when blood tissues interact with laser they have some influence in them. When laser light falls on an absorber, energy gets deposited in that absorber. If in a given volume enough energy get deposited a phase change occurs. If laser light encounters a liquid absorber phase change yield an unstable vapors bubble called cavitation bubble. Human RBCs are disc shape with diameter ranging from 7.5 to 8.7 μ m and 1.70 to 2.20 μ m thick. The RBCs have an extensible membrane covering whose surface area to volume ratio is very high and supports high revokable whippy strain. RBCs deformation is a necessary feature as required for microcirculation of blood at capillary level. Here we are aimed to study deformability of red blood cells as a key role measuring the viscoelastic properties of red blood cells. Historically, the pioneer measurement on static viscoelastic properties of RBCs was done by using the technique of micropipette suction Evans et al. (1973-1976). Since history to the prevailing time, there are variety of ways have been used to talk about the mechanical properties of RBCs (red blood cells) G. Bao et al. (2005). Some of these include micropipette aspiration E. A. Evans et al. (1973), twisting micro magnetic beads M. Puig-de-Morales Marinkovick et al. (2007). But all these methods add difficulty and limitations to the experiments and yet its time consuming to probe each cell at a time. In this extension, to avoid all the obstacles, we demonstrate a more generalized and easy method to treat many RBCs

(red blood cells) at a time inside a chamber with the help of a single laser induced cavitation bubble Pedro A. Quinto Su et al. (2010). We name this technique as LCCD for laser controlled cavitation deformation. The laser induced energy will provide the stress to change the shape of the cells with the help of a cavitation bubble. Then we measure the strain as a function of time and obtain a power law in time for three different categories of RBCs i.e. untreated and treated RBCs with two different reagents. In this way we study the elastic properties for RBCs with the help elastic restoring term (E) and damping viscous term (η). The dynamics of shape recovery is studied by a characteristic decay time.

In humans, RBCs job is to transport oxygen and carbon dioxide and other micronutrients. This exchange of matter occurs at micro level. So during microcirculation of blood, RBCs have to shift their shape from disc to bullet to facilitate transportation. This led happens because of the lipid bilayer membrane of RBCs which provide enough elasticity for the cells to change their shape. When pathogens like bacteria and viruses get involved in human body, they affect the functioning of various tissues and organ and make them ill. Elastic properties of red blood cells (RBCs) play a vital role in the indication of various disease J. Sleep et al. (1999). For example a known virus plasmodium causes malaria in humans. When it gets invaded in blood, it makes the membrane of RBCs (red blood cells) rigid so the diseased cells lose their elasticity and have a higher young's modulus value and come to their default shape in a shorter time than healthy cells G. B. Nash et al. (1989). To account the future stories out of the manuscript, one important application could be in biogenetics i.e. the gene and DNA study to speak roughly as very detailed and precised experimental setup is required to give results about internal activities of building blocks of life. Also could be a good method to study White blood cells deformations like leukemia. Another great use of this technique would be in study of cancer of liver or kidney. Also could play a vital role in the determination of intracellular activities of different cell organelles like Golgi bodies and Mitochondria.

Thalassemia could be diagnosed and treated well with low laser therapy.

MATERIALS AND METHODS

Erythrocyte preparations and enzyme treatment Human erythrocytes were drawn into tubes containing sodium citrate (BD vacutainer). 500 ml erythrocytes were resuspended in 1× PBS (phosphate buffered saline) with 0.1% BSA (bovine serum albumin) at 50% hematocrit in 1.5 ml Eppendorf tubes. 10 mU ml⁻¹ Neuraminidase (Calbiochem, 1 U ml⁻¹) was added and the sample incubated while rotating at 37 °C for 1 hour. Alternatively, 1 mg ml⁻¹ wheat germ agglutinin was added and the sample was incubated at room temperature for 30 min. The treated erythrocytes were pelleted and washed 3 times with 1×PBS containing 0.1% BSA. The cells were then ready for use. The RBCs were diluted 1:1000 in 1×PBS with 0.1% BSA and applied on the microscope cover slips. The cells were used within a few hours after extraction from a healthy donor. The experimental setup is similar to the ones used in previous studies that used laser-induced cavitation in combination with microfluidic devices. The laser pulse is focused at the bottom of the wide microfluidic chamber (see Materials and methods, Fig. 1B) containing the cells by a 60/0.9 NA water immersion microscope objective in a microscope.

As a result of the high intensities reached at the focal volume an optical breakdown is created which leads to a rapidly expanding bubble; the bubble expands to a maximum radius and then collapses under the pressure imbalance of the surrounding liquid. The fast dynamics of the bubble expansion and collapse induces flows with average velocities of several meters per second. After the bubble collapses, the flow stops immediately and RBCs are deformed. (fig. 1C). Then, the cells start to recover their shape (Fig. 1D). The cells are deformed due to the complex flow field that varies with height and distance from the center of the bubble. The flow velocity reaches the maximum value at the center of the micro chamber (in height h , see Fig. 1B, side view) and is proportional to $1/r$ (due to the quasi-two-dimensional geometry of the chamber), where r is the radial distance from the position of the bubble center. Hence, cells that are closer to the center are stretched more than the ones that are farther away. During optical breakdown a shock wave may be emitted. Previous work has shown that single shock waves even at high amplitude do not affect the viability of cells. In particular, strong shocks from a lithotripter²⁷ with a peak positive pressure of several hundred bars do not cause temporary or permanent cell membrane poration. These results were confirmed in a study on optical breakdown induced cell lysis²⁸ where the cavitation dynamics and not the shock wave is made responsible for the observed membrane rupture. The possible heat effects that can arise from this technique have been shown to be negligible for similar laser energies and pulse durations.

Cells very close to the bubble centre become lysed due to the fast and shearing flow. These cells become ghosts within a time scale of tens of milliseconds. Hence in the experiments reported here only cells which remain intact are analyzed. Two types of deformations are found: stretched and folded cells. The stretched or elongated cells recover their shape faster than the folded ones. This is because the stretching modulus is larger than the bending modulus, as it was observed by Evans et al. In this study we only concentrate on the analysis of stretched cells (Fig. 1D). The shape recovery process (strain decay) is studied with a high-speed camera recording at 54 000 frames per second for a time $T \approx 0.2$ s. Thus we are not able to resolve the initial deformation of the RBCs as it is too fast for this recording speed (Fig. 1C, expansion and collapse). During the experiments, every time after a bubble has been created, the stage supporting the microchamber is translated a few millimetres before a new high-speed recording is taken. Thereby we take care that the cells are only exposed once to the impulsive forcing.

Fig 1A. Experimental Setup to Generate The Flow For Stretching The Cells

Fig 1B. Geometry of The Liquid Gap which Contains the Liquid Medium and The Cells

Fig 1C. RBCs Imaged Before, During and After The Cavitation Event The last frame shows the deformed cells at 18.5 ms after the bubble has collapsed.

Fig 1D. Shape Recovery of an Individual Cell. The first frame is taken at 222 ms after the bubble has collapsed and the successive frames are taken at intervals of 1ms. L is the length of the major axis that is used to define the strain.

RESULTS AND DISCUSSION

RBCs images were also inspected to come across if any deformation in RBCs shape exists (when no laser trapping guide present) as shown in figure 2. Cells were made distinctive on the basis of morphology like might be they are slightly disturbed in shape (for example membrane zeiosis or blebbing which is actually an irregular bulge in plasma membrane and cell getting flat), one more evidence in shape change is intermediately disturbed cells (membrane blebbing is very extensive with a little quantity of cell contraction and emergence of spiny form) or extremely agitated (wide cell reduction and spiny form).

Different collections of RBCs were sighted and examined to recognize diverse groups of perturbed cells. To account for average perturbation of cells, least count of approximately 100 cells (and in general ≥ 200 cells) were examined for all data sets.

Fig 2. Schematic Plan of RBC Folding in an Optical Trap

A biconcave RBC (right) near the surface of a flow chamber (rectangular box), moves (horizontal arrow) towards the trap focus and folds into a rod like shape (inset of actual image of an RBC folded in the trap). Vertical arrow: direction of laser propagation.

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