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Optical laser trapping for studying the deformability of sickle red blood cells in response to hydroxyurea

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Abstract:

BACKGROUND: Sickle cell disease (SCD) is prevalent in Basrah city and affects the red blood cell (RBC) deformability and thereby causes disease symptoms. Hydroxyurea (HU) is effective in reducing morbidity and mortality in SCD patients by different mechanisms.

OBJECTIVES: The aim of the study was to investigate the effect of HU on RBC deformability among SCD patients by direct laser optical trapping (OT) technique.

MATERIALS AND METHODS: Blood samples from SCD patients and control groups were prepared in the medical laboratory of Basrah Center for Hereditary Blood Diseases and transferred into physics laboratory wherein the laser system was presented and built-in. RBCs from each sample were exposed to three different powers of laser 5, 15, and 20 mW for 15 s and then were released and followed for 2 min. The images for each trapped RBC were obtained and at relaxation sequential times. The percentage changes in the diameters of trapped RBCs were measured for control and patient groups.

RESULTS: SCD patients were divided into two groups depending on whether they were receiving HU (39 patients) or not (43 patients). They were matched with 50 healthy individuals (control) regarding age and gender. We found that all the trapped RBCs were affected during the trapping time and then returned toward near normal with some differences between the groups and according to the power used. The deformability of HU group was better and closure to the control.

CONCLUSION: The presented laser system and OT technique with optimal power are effective to study the RBC characteristics and deformability. HU is effective in improving RBC deformability among SCD patients.

Keywords:

Hydroxyurea, laser, optical trapping, sickle cell disease

Introduction

The blood rheologic and hemodynamic properties are largely determined by red blood cells (RBCs) which is the main cellular component of the blood. The normal healthy RBCs are biconcave disc shape with approximately 7.5–8.7 μm in diameter and 1.7–2.2 in thickness, and were not subjected to an external stress. Such healthy RBCs have a flexible membrane that facilitates reversible elastic deformability during RBC passage through the microcirculation.

Some pathologic conditions affected the RBC deformability and therefore altered the circulation causing disease symptoms ranging from benign to lethal complications such as what happened in sickle cell disease (SCD).^[1] Hence, the mechanically fragile, less deformable RBCs in SCD cause hemolysis and impaired blood flow velocity contributing to other pathophysiologic aspects of disease.^[2-4] The impaired blood flow plays a major and key role in acute and chronic complications of SCD.^[5]

SCD is an inherited blood disorder with highest prevalence throughout areas of

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Africa, India, Mediterranean countries, and Middle East.^[6] Basrah city, southern Iraq, is within the regions affected by SCD in the world.^[7] The hemoglobin S (HbS) is present in large numbers in Abu-Khasib and the low numbers in Al-Shatt Al-Arab district than other districts in Basrah.^[8] In Basra, some of our patients still experienced poor quality of life despite the achievements in management of the disease and the use of hydroxyurea (HU), which is the only approved and best currently available drug used in SCD.^[9] HU is effective in reducing morbidity and mortality^[10,11] due to its well-known therapeutic benefits that related to increased production of fetal hemoglobin (HbF), improved hydration, and increased mean corpuscular volume of sickle RBCs as well as decrease in adhesiveness of sickle RBCs into internal wall of the vessels.^[12,13]

On the other hand, measuring the optical characteristics and the properties of RBC deformability has been studied as well to understand the RBC-related diseases. The technique used was based on the optical forces of the laser beam for optical trapping (OT) which was introduced in the early 1970s. Since then, advances in laser technology and progress in OT make it possible to investigate the elasticity and viscoelasticity of single cells or the microorganelle.^[14,15]

In Basrah, we have studied the clinical and hematologic effects of HU,^[16] but yet, there is no research about the RBC deformability. We hypothesized that studying RBC deformability may help to understand more aspects of HU effect on RBCs. Therefore, we have presented and built a single-laser OT technique to study the therapeutic effect of HU on deformability of a single-living RBC obtained from blood samples of SCD and healthy control participants in a comparative study.

Subjects, Materials, and Methods

Subjects and sample preparation

The study was conducted between November 2016 and August 2017 in two institutes: Basrah Center for Hereditary Blood Diseases and the Department of Physics, College of Science. The study was approved by ethical committee of college of science university of Basrah. written inform consent was obtained from each patient prior to study. The adult patients with SCD who were taking HU regularly for 3 months were included in this study to compare with SCD patients without HU. We have excluded patients with interrupted use of HU and those with severe painful crises. Blood samples from the patients and control healthy individuals were collected and prepared in the medical laboratory and then transferred within 3 h to the Department of Physics' laboratory.

For sample preparation, the blood was taken from patients and healthy controls and collected in ethylenediaminetetraacetic acid tube (4.5 mg/ml³). Then, 30 μ l of blood was washed three times by normal saline and centrifuged at 4000 rpm for a minute. The washed RBCs were then resuspended in 1 ml of normal saline. After that, two drops of bovine serum albumin is then added to prevent RBC adhesion to glass plates. On measurement, the diluted blood samples with normal saline of 1 ml were placed as sandwich between microscope slide and coverslip. Finally, piece of coverslip was attached in three edges with colorless nail polish or Dibutylphthalate polystyrene xylene (DPX).

Methods and experimental technique

Principle of optical trapping operation

The basic theory for trapping of a particle is based on the size or diameter of particle (in the order of microns) to be much larger than that the laser wavelength (λ).^[17,18] In principle, when a laser beam interacts with particle, the particle will trap due to the absorption, reflection, and refraction or scattering of light, and thus, the light induces linear momentum change that leads to generate an optical force on that interacting particle. This force attracts the particle to the region of the intensity of laser beam. Then, the trapped particle will affect two types of forces as illustrated in Figure 1a. These two forces are called the scattering force (F_{scat}) and gradient force (F_{grad}) to the direction of the propagation of the laser beam and

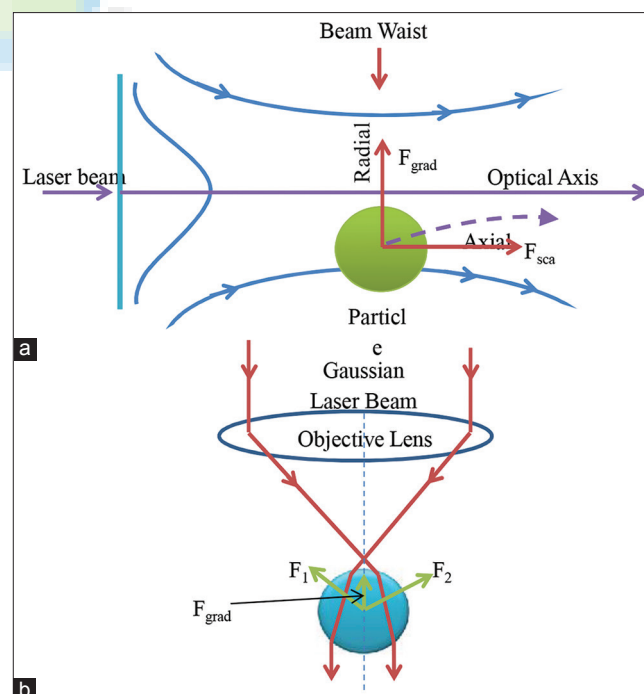


Figure 1: Principle of optical trapping. (a) Schematic illustrations of optical scattering and gradient forces on particle placed in a laser spot in the ray optics regime. (b) Schematic illustrations of optical gradient force with a single of laser beam enter the objective lens on a particle or RBC

spatial of the intensity of the beam, respectively. The scattering force is caused by reflection of the light away from the laser beam waist. The other force is caused by light intensity applying or trapping the particle at the narrowest region of the laser beam waist.^[18,19]

Thus, when a Gaussian laser beam in the TEM₀₀ mode is highly focused by an objective lens (100XOL) to a single spot in a near particle (in our case RBC) plane, the spot creates an "OT" where the particle or RBC with higher refractive index than that of the ambient medium as well as the gradient force will push toward the focus of laser beam where the intensity is highest as shown in Figure 1b.

Experimental setup

The technique is illustrated in Figure 2. A diode-pumped solid-state laser with a maximum power of 300 mW operating in a Gaussian TEM₀₀ mode at a wavelength of 532 nm is used for trapping. The output power of the laser is attenuated by a neutral density filter and the beam is passed through a dichroic mirror (DM). Then, the beam is passed through an objective lens (100XOL) and illuminated the prepared RBC sample. DM was fixed at an angle of 45° and used to guide the laser beam through the objective and allows the reflected light from the sample. The reflected laser beam aligned and passed into the back of the objective lens. The sample was placed on an adjustable three-dimensional translation stage to accommodate the microscope slide. A halogen lamp was used to illuminate the prepared RBC sample through an objective lens (10XOL).

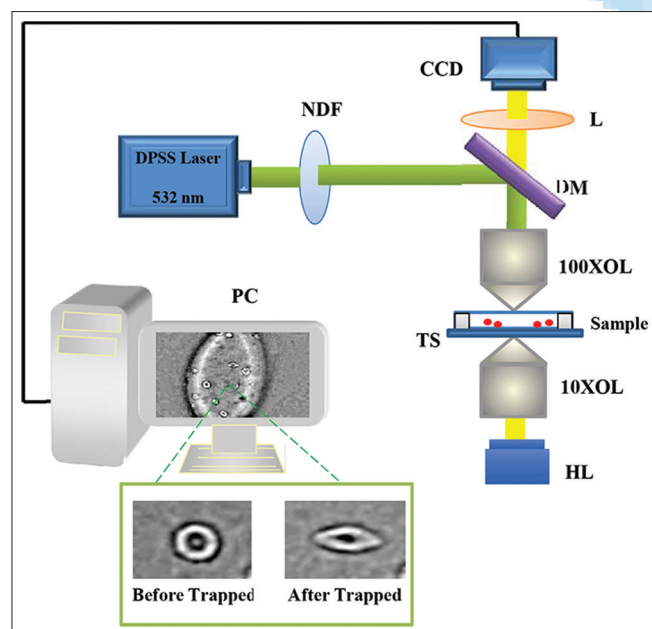


Figure 2: Schematic illustrations of the experimental technique of single-beam optical trapping. DPSS Laser: Diode pumped solid state laser; NDF: Natural density filter; DM: Dichroic mirror; 100XOL: 100X Objective lens; 10XOL: 10X Objective lens; L: Convex lens; TS: Three dimension translation stage; CCD: Charge coupled device camera; PC: Personal computer

All the samples from the controls and patients were then studied using increasing powers of laser beam during trapping starting from 5, through 15, and 20 mW. The duration of trapping was 15 s followed by relaxation periods up to 5 min. The image of the trapped cell was recorded by a charge-coupled device camera and monitored by a personal computer. Images for each trapped RBC were taken and then during the relaxation periods to compare with the images at free time.

Data analysis

After laser trapping of RBCs, a procedure of image analysis is applied to the images from RBC optical stretching and the free cell. Then, the RBC optical deformation can be described in terms of elongation along the optical axis of the laser beam (the maximum diameter) and contraction normal to the direction of the beam (minimum diameter). An ImageJ program/software (Wooster, Ohio, USA) was used to analyze and measure the sizes and the deformability of each cell type. Using these sizes or diameters, the elastic deformability of RBCs can be analyzed using the relative changes in the maximum and minimum diameters at a given incident power of laser beam as^[20]

%Change in Diameter =

$$\left[\left(\frac{\text{Free} - \text{Trapped}}{\text{Free}} \right) \right] \times 100\%$$

Results

General characteristics of the healthy controls and patients are shown in Table 1. The enrolled 82 patients were divided into two groups, the first group (G1) included 43 SCD patients without HU and the second group (G2) included 39 SCD patients treated with HU. Most of the patients, i.e. 62, have sickle thalassemia. The age range was 14–61 years with mean age of 24.8 years for G1 and 28.4 years for G2. They were matched regarding age and gender to control group of 50 healthy individuals, $P = 0.212$.

Table 1: General characteristics for healthy control and patients

Groups	Samples	Number of sample	Gender (male/female)	Age (mean)	P
G1	SCD	43	24/19	15-61 (24.8)	0.212
	SCA	12			
	S.TH	31			
G2	SCD-HU	39	19/20	15-61 (28.4)	
	SCA	8			
	S.TH	31			
	Control	50	24/26	15-43 (26.5)	

SCD=Sickle cell disease, HU=Hydroxyurea, SCA=Sickle cell anemia, G1=First group, G2=Second group

The RBCs from control and both patients' groups showed changes in size and shape to direct laser trapping by elongation (increase diameter) and then after release at relaxation by decreasing their diameter backward to near original size and shape but to a different extent according to the power of the laser beam and the type of the sample whether from control or from the patients' groups. As an example for the changes, Figure 3 shows the differences in size and shape of the RBCs from control, G1, and G2 at free time and after trapping with power of 15 mW.

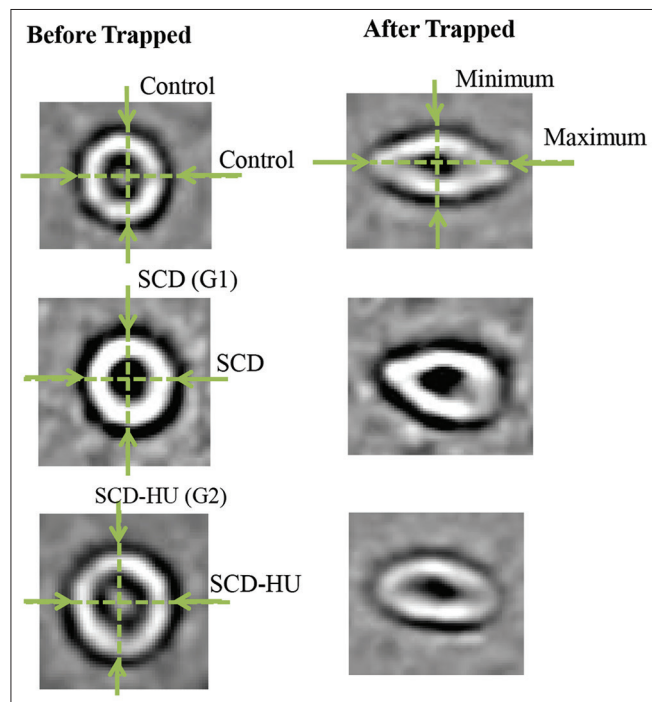


Figure 3: Optical images of RBCs before and after trapped at power of 15mW with exposure time of 15 sec for prepared samples (a) healthy control, (b) SCD, and (c) SCD-HU

The deformability of RBCs was analyzed by calculating their maximum and minimum diameters and the relative changes in these diameters as a function of trapped power were studied using the above-mentioned equation. We calculated the average values of all trapped RBCs from the studied samples at each specific setting of the power. The results of changes in diameters are illustrated in Figure 4 (a and b for maximum and minimum diameters, respectively) for the RBCs of control, G1, and G2. It is clear that the percentage changes in the diameters were proportional and dependent on the trapped power of laser beam and with reduction in maximum and increase in minimum diameters at power of 15 mW as compared to power of 5 mW. At power of 20 mW, the results were disproportionate both for maximum and for minimum diameter measurements. For maximum diameters [Figure 4a], the higher value of relative changes refers to more deformability and that is true for control group followed by G2 and lowest value for G1. The opposite applied to relative changes of minimum diameters. These results refer to better deformability of RBCs from G2 than G1 but less than that of control group.

After release, we have followed up the RBCs to study their relaxation rates by measuring the percentage changes in maximum diameters at 15-s intervals for a period of 120 s. The obtained results are given in Figure 5, and the relative average changes in the maximum diameter were plotted as a function of time for three input powers (a, b, and c for 5, 15, and 20 mW, respectively). From this figure, it is clear that the sizes and shapes of the trapped RBCs reduced back toward near (but not as exactly as) normal free cells. The pattern of return is almost the same for control and the patients' groups at powers of 5 and 15 mW with G2 closure than G1 to control. However, the rate of relaxation of G2 was not as same as the control group and the difference increased as the power of laser

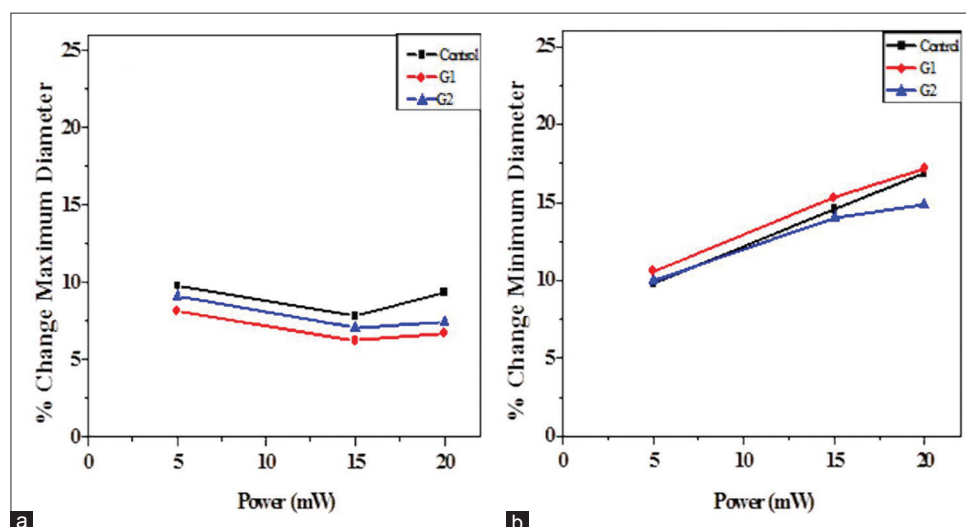


Figure 4: Percentage change of red blood cells by their (a) maximum relative elongation and (b) minimum relative contraction as a function of the trapped power for healthy controls and patients with G1 and G2

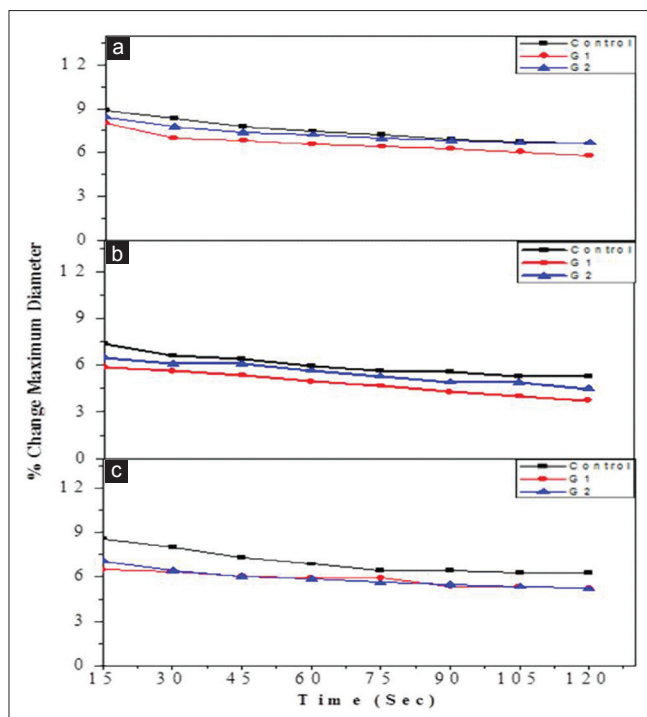


Figure 5: The percentage change in the mean maximum diameter of the healthy control, G1, and G2 as a function of the time at different powers: (a) 5 mW, (b) 15 mW, and (c) 20 mW

increased. At a power of 20 mW, the curves of changes for G1 and G2 are almost the same and obviously differ from that of the control group. We have noticed that; at the end of 120 seconds, all the trapped cells from G1, G2 and control were not fully relaxed, i.e., the relative changes did not approach to a zero value.

Discussion

SCD is highly prevalent in Basrah as compared to other parts of Iraq.^[8] HU is largely used among our patients, but some of them still experience painful attacks despite the improvement in HbF and other RBC indices. Therefore, the current work was conducted to evaluate the effect of HU on the deformability of sickle cells using an OT technique that was presented and built locally. The enrolled adult patients had age ranging between 15 and 61 years, most of them having sickle thalassemia syndrome, and they were age and gender matched with control healthy individuals. The cells from all the samples were measured at free time, and we found that the average value is interestingly higher for the patients than for controls. However, all the trapped cells were elongated, but the relative changes in the maximum diameters were different according to the power setting of laser and also the type of the sample whether control or patients' groups. The higher values of relative changes of maximum diameters mean more deformability properties of RBCs. The obtained results indicated that RBCs of control group were more deformable,

followed by HU group, and the lowest value was for sickle patients without HU. However, the changes were disproportionate at high power (20 mW) probably due to photodamage effect that disturbed the cell membrane properties leading to protein denaturation.^[21] The photodamage effect was different for control and the patient groups. Hence, in the current work, we could point out an optimum power for the used wavelength of 15 mW as a function of the relative changes in diameters for the RBCs.

When we followed RBCs after release from OT, the photos were captured for up to 5 min during relaxation time. However, the percentage changes in maximum diameters were measured at 15-s intervals for only a period of 120 s because of frequent artifacts that happened in the samples after 2 min. We found that the trapped RBCs tend to return to their near original size and shape during relaxation time, and pattern of return was almost similar for control and the patient groups at powers of 5 and 15 mW. The better response for HU group could be attributed to the effect of HU in promoting production of HbF which actively inhibits polymerization of HbS improving morphologic and physiologic properties of RBCs.^[22] This was in accord with other studies,^[23-26] although they were using different experimental setup for measuring RBC deformability.

On the other hand, the response among HU group was not as same as that of healthy control RBCs probably because some of sickle RBCs might have irreversible changes altering membrane viscosity and deformability^[3,27,28] despite the laboratory response by HU in those patients and that raises the necessity to investigate other mechanisms than polymerization of HbS causing membrane stiffening and reduced RBC.^[29] At power of 20 mW, the optical deformability for healthy control increased compared to both patient groups; this might be due to the cell photodamage affecting more sickle RBCs regardless the treatment with HU. We have noticed as well that at the end of 120 seconds the trapped cells were not fully relaxed and were not returned to their exact size and shape, i.e., the relative changes did not approach to a zero value even of control sample as expected and found in other studies^[20] because we used in our experiment different sample preparation, different system setting up and different power levels.

Conclusion and Recommendations

The results confirmed that the presented and built single-laser OT technique was effective and able to study the therapeutic effect of HU on deformability of a single-living RBC among patients with SCD. Although still worse than healthy control, but it was clear that the patients treated with HU exhibited better deformability

properties than untreated sickle patients. The obtained results could open the way for further and future prospective studies among patients with SCD or other hematologic diseases taking into consideration other factors affecting RBC and blood properties.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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