Stretching Red Blood Cells with Optical Tweezers

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Abstract: In this work we report on experiments to measure the deformability of red blood cells (RBCs) when subject to stretching with optical tweezers. Individual red blood cells are trapped directly in a dual optical tweezers, and subject to a stretching protocol that is the same for all cells under test. Differences in the resulting extension are therefore indicative of differences in cell deformability. The targets for investigation are RBCs taken from patients with type 2 diabetes mellitus (T2DM) who exhibit diabetic retinopathy (DR), and from patients with birdshot chorioretinopathy (BCR). We find a statistically significant change in deformability for RBCs from DR patients compared to a control group, but no significant change for BCR patients. These results offer support to the importance of the role of RBC biomechanical properties in the progress of these conditions.

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1. Introduction and motivation

Blood is a complex two-phase fluid, made up of plasma and formed elements including red blood cells (RBCs or erythrocytes), white blood cells (WBCs or leukocytes) and platelets. Of these, RBCs are predominant, forming ≈ 99 % of the cellular component. The primary role of RBCs is the transportation and delivery of oxygen to the peripheral tissues, however, their mechanical and flow properties are responsible for the complex fluid dynamics which occur in microvessels [1]. As such, altered biomechanical properties of RBCs can result in impaired oxygen and nutrient supply to peripheral tissues, as well as altered haemodynamics. In the microvasculature, where the delivery of oxygen to the cells takes place, the diameter of capillaries is comparable to or even less than the diameter of RBCs (typically $\approx 8\ \mu$m). Here it is suggested that alteration of biomechanical properties of RBCs can severely affect the microcirculation, resulting in ischaemia and necrosis [2].

Diabetes is known to impair the deformability or RBCs [3], and this has previousy been linked to microvascular complications in the kidney [4]. It remains unclear, however, whether reduced deformability of RBCs correlates with the presence of microvascular complications in the retina. Diabetic retinopathy (DR) is a disease of the eye that is caused by hyperglycaemic damage to the microvascular system of the retina. In the early stages, abnormalities due to hyperglycaemia can cause microaneurysms to develop in the walls of the microvasculature which leak red blood cells (RBCs) and serous fluid into the retina. In the later stages, the blood vessels can become occluded leading to retinal ischaemia. In response to this ischaemia, new blood vessels grow rapidly out of the retinal surface and into the vitreous humor where bleeding can obscure vision. Over time, scar tissue forms which may lead to retinal detachment and, ultimately, blindness. In many countries, DR is the most frequent cause of preventable blindness in the working age population [5]. We have performed experiments using optical tweezers [6] to measure the deformability of RBCs from DR patients to assess the correlation between RBC deformabiility and complications arising from diabetes.

2. Experiment

We use a dual optical tweezers constructed around an inverted microscope equipped with a $\times 100$, NA = 1.3 oil immersion objective lens. The trapping beam is derived from a Nd:YAG laser, wavelength $\lambda = 1064$ nm, maximum power $P = 3$ W. The beam is split into two, and re-combined using polarization optics to produce two nearly co-propagating beams with orthogonal circular polarizations to avoid interference effects [7]. In the section of the optical

path where the two beams are separated, one of them is reflected from two orthogonally-mounted galvanometer mirrors (GMs), enabling it to be steered automatically in two dimensions [8]. The dual optical traps are used to capture RBCs directly. As shown in figure 1(a) & (c) the two beams are initially positioned over a cell lying on a substrate, with a beam separation less than the cell diameter. As the cell is lifted away from the substrate it rotates to adopt the 'side-on' orientation shown in figure 1(b) & (d). RBCs are subject to optical stretching by slowly increasing the separation between the laser foci along the direction parallel to the line joining the foci using the GMs to steer one beam. The separation of the foci is increased from $d_0 = 5.06\ \mu$m to $d_1 = 6.47\ \mu$m in a time of 6 s to stretch the cell. The stretched cell is then released by quickly setting the separation to a large value for 2 s during which time the cell relaxes to its unstretched length. The cell is then recaptured by returning the foci separation to d_0 and the stretching cycle repeated.

Samples of RBCs were acquired from seven diabetic patients and eight age and gender-matched healthy control patients presenting at Moorfields Eye Hospital (London). The samples were maintained at 4°C during transport, and experiments were performed at room temperature. Each RBC sample was diluted with PBS (1 μl of RBC suspension with 1 ml of PBS) and with 1% bovine serum albumin (BSA) to prevent RBC aggregation and adhesion to the slide. 100 μl of the diluted RBC suspension was pipetted into the well of a microscope slide, and sealed beneath a cover slip. Following this preparation procedure cells were visually inspected under the microscope. The overall appearance was healthy when RBCs were in optimal concentration with fewer than 5% echinocytes per slide [7]. Data from the stretching experiments was acquired by means of digital video microscopy and subsequent image analysis using custom-written Matlab software to extract the length of the cell in each frame of the video recording, and thus the inital cell length, l_1 and fully stretched cell length l_2 in each stretching cycle [9]. Up to 10 cells in dilute suspension were tested for each patient, and each cell tested was subjected to up to 3×10 stretching cycles.

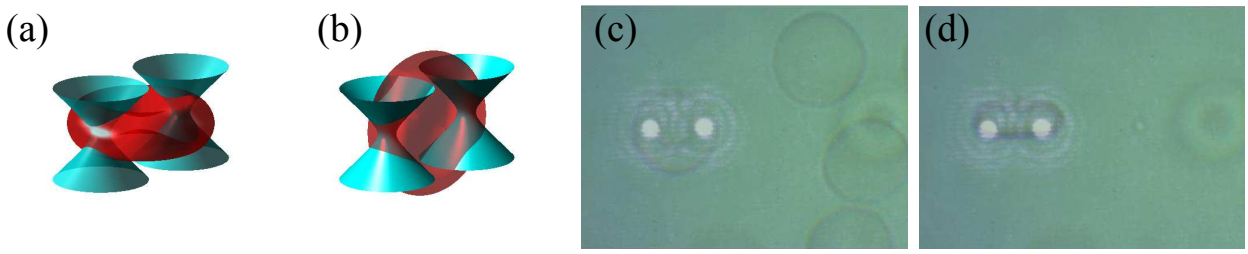

Fig. 1. Trapping red blood cells with dual optical tweezers. (a) two focused beams (shown in blue) are positioned over a red blood cell initially lying flat on a substrate; (b) when lifted away from the substrate the cell rotates to adopt the orientation shown; (c) micrograph of untrapped cell lying on coverslip with optical trapping beams superimposed; (d) cell held in the optical tweezers with trapping beams superimposed.

3. Results

In total over 900 stretching events resulting in measurements of (l_1,l_2) were recorded for cells from each of the DR patients group and the control group. A kernel density estimation (KDE) of the the frequency of (l_1,l_2) pair recordings is shown in figure 2(a) for the control cells, and 2(b) for the DR patients' cells. In both cases there is a linear relationship between stretched and initial lengths for cells subject to this stretching protocol. To quantify cell deformability we define the fractional deformation of the cells subject to this stretching protocol as:

$$d = \frac{l_2 - l_1}{l_1}. \tag{1}$$

A quantitative comparison of the fractional deformations from these samples shows that $d = 0.0703 \pm 0.0008$ for the control, and $d = 0.0645 \pm 0.0001$ for the diabetic retinopathy patients ($p < 0.001$, Wilcoxon signed-rank test). These results are suggestive of a statistically significant decrease in deformability (increase in stiffness) of RBCs from DR patients. We have performed similar experiments on RBCs from patients with the condition birdshot chorioretinopathy (BCR) [10]. BCR is an uncommon, chronic, recurrent ocular vasculitis with rare occurrence of retinal vascular occlusion. Here we found no statistically significant difference in this measure of deformability between RBCs from BCR and healthy controls (however it should be noted that the BCR sample size was, in practice, limited by the small

number of patients presenting with the condition at Moorfields over the period in which these experiments were carried out).

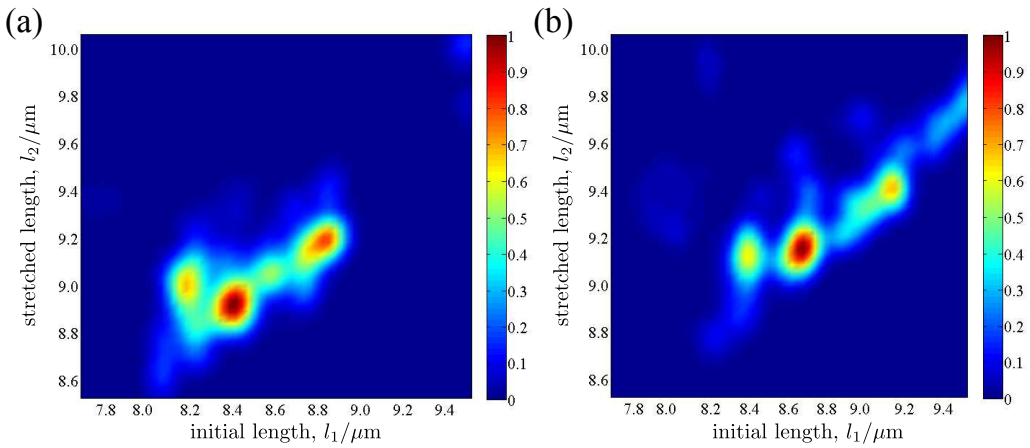

Fig. 2. Kernel Density Estimations (KDEs) for stretched cell length, l_2, and initial unstretched cell length, l_1 for (a) control cells; (b) cells from diabetic retinopathy patients. The color scale indicates the nomalized frequency of occurrence.

4. Conclusion

We have used an optical tweezers stretching method to quantify the deformability of RBCs from patients with eye conditions diabetic retinopathy and birdshot chorioretinopathy. We find a decrease in deformability for patients with DR that is associated with vascular occlusion, but no statistically significant change for RBCs from patients with BCR. Although caution must be exercised in interpreting the data due to the limited sample sizes in this pilot study, taken together, these data are suggestive of a link between the occurrence of retinal vascular occlusion and a change in deformability (stiffening) of red blood cells.

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