Probing the red blood cell interaction in individual cell pairs by optical tweezers

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Abstract: The peculiarity of red blood cell interaction was studied by optical tweezers. The intercellular interaction force in cell pairs and the role of cell deformation and adhesion time in interaction dynamics were evaluated and discussed.

1. Introduction

Red blood cells (RBCs) tend to form tight binding structures known as rouleaux through face-to-face adhesion in aqueous protein/polymer-solutions. The rouleaux formation (i.e. aggregation) and disruption (i.e. disaggregation) that depend on both suspension and RBCs properties (e.g., RBC elasticity and zeta potential) are crucial to the blood rheology [1]. Optical tweezers (OT) is a unique laser-based Nobel Prize winning invention that allows for non-destructive manipulation of cells in their natural environment at unprecedentedly small scale hardly possible with any other technique [2]. This study focuses on investigating the mutual adhesion between human red blood cells (RBCs) in individual cell pairs with the in-house-made infrared OT, aiming at revealing the RBCs interaction mechanism and the role of intercellular adhesion time and RBC deformation in the interaction dynamics.

2. Materials and Methods

The experimental samples are low concentration (<1%) RBCs suspensions in autologous plasma donated by one single donor (female, age 27). The measurements were performed at room temperature (23 °C) within 4 hours after sample injection into the glass sample chamber consisted of a microscope slide and a cover glass. The in-house made two-channel infrared OT, consists of a laser source (ILML3IF-300 Leadlight Technology, Taiwan), two polarizing beam splitters (PBS), and a water immersion objective (LumPlanFI-100×/1.00 NA, Olympus, Japan), is the main research platform as shown in Fig.1a. The optical trapping force that is proportional to the trapping power was calibrated to carry out quantitative measurements, the calibration result is shown in Fig.1b.

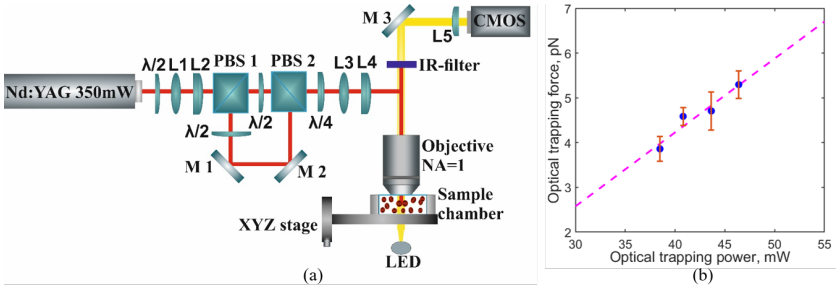

Fig. 1. (**a**) Schematic layout of the two-channel OT system; (**b**) Trapping force calibration result.

3. Results

The adhesion strength between human RBCs in individual cell pairs was evaluated by measuring the optical trapping force required to decrease the adhesion area between two cells. Two similar-sized RBCs resting on the microscope slide were captured and lifted to a height of 40 μm in the sample chamber by two trapping channels simultaneously. With the help of OT, the RBCs were put into contact and an initial overlapping area ($S_0 \approx 60\%$) was formed. After a certain contacting time, one trap was slowly moved away from the other trap and the RBCs were getting away from each other accordingly. Until the intercellular attracting force overcame the trapping force, the cells escaped from the traps and clumped together again, as illustrated by the inserted colored-micrograph in

Fig.2a. The RBC interaction energy reserved within the final adhesion area was calculated as described in our previous work [3], and was plotted against the final relative interaction area as shown in Fig.2a. A reciprocal distribution indicates that the RBC adhesion area decreased yet not proportionally with the increase of the trapping force. This peculiarity of RBCs disaggregation has been associated with the formation and migration of the macromolecule junctions between adjacent RBCs [4]. However, as RBCs underwent observable stretching in disaggregation measurement, especially with large trapping force, the role of RBC deformation in disaggregation dynamics is worth discussing. The deformation force of single RBCs was measured by directly applying the trapping force at two opposite end-points of a RBC. The deformation was denoted by the stretching ratio, which can be described as the ratio between the relative change in cell diameter and the initial diameter ($|d_1 - d_0|/d_0$), where d_0 and d_1 are cell diameters before and after stretching respectively. It can be seen from Fig.2b that the RBC was elongated proportionally with the trapping force until it reached its stretching limit. Therefore, in RBC disaggregation measurement with OT, the trapping force has to overcome both the cellular attracting force and RBC deformation force. In order to clarify the influence of mutual adhesion time on RBCs interaction, two RBCs were kept attaching to each other for different times varied from 20s to 300s before the disaggregation force was measured. The final relative adhesion areas (S_i/S_0) between two RBCs achieved by three different optical trapping forces (5.4 pN, 9.2 pN, and 12.5 pN) in the disaggregation process were recorded as shown in Fig.2c. With large trapping forces of 9.2 pN and 12.5 pN, it can be seen that the longer the RBCs contact, the harder to separate them as they remained attached to each other with larger final adhesion area. This peculiarity of RBC disaggregation is reliably detectable with large trapping force. The result indicates that the longer the RBCs contact time, the stronger the intercellular interaction.

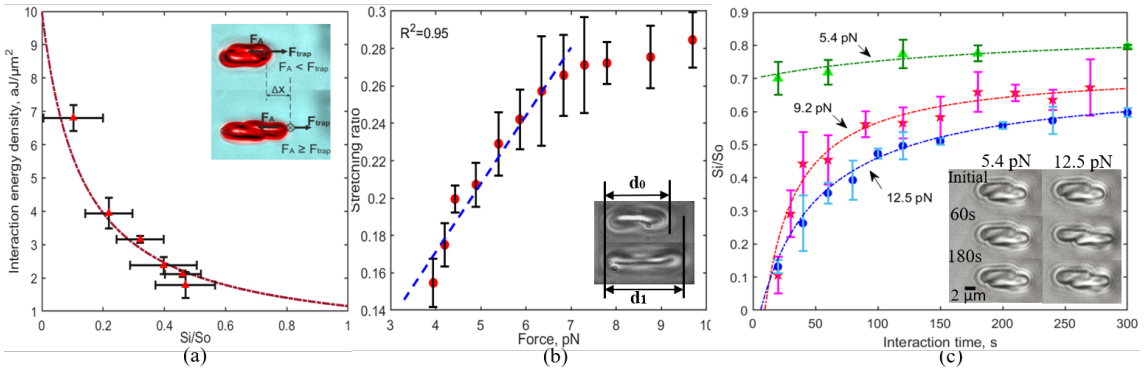

Fig. 2. (**a**) The relationship between the interaction energy density and the relative adhering area in RBC disaggregation; (**b**)The relationship between the cell stretching ratio ($|d_1 - d_0|/d_0$) and the trapping force; (**c**) The final relative interaction area (S_i/S_0) achieved by trapping forces of 5.4 pN, 9.2 pN, and 12.5 pN from an 80% relative initial overlap in RBC disaggregation process.

4. Conclusions and Acknowledgements

The interaction between human RBCs in individual cell pairs was evaluated with double-beam OT. In RBC disaggregation measurement, the trapping force has to overcome both the cellular attracting force and RBC deformation force. The intercellular interaction between RBCs increases with cells mutual contact time. Our study improves the understanding of the dynamic interaction between RBCs in autologous plasma, and clarifies possible influence factors in the measurement with OT. The China Scholarship Council (CSC No. 201706410089, R.Z.) and Tauno Tönning Foundation (grant No. 20190104, R.Z.) are acknowledged for financial support.

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