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Characterization at the individual cell level and in whole blood samples of shear stress preventing red blood cells aggregation

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ABSTRACT

The aggregation of red blood cells (RBC) is an intrinsic feature of blood that has a strong impact on its microcirculation. For a number of years it has been attracting a great attention in basic research and clinical studies. Here, we study a relationship between the RBC aggregation parameters measured at the individual cell level and in a whole blood sample. The home made optical tweezers were used to measure the aggregating and disaggregating forces for a pair of interacting RBCs, at the individual cell level, in order to evaluate the corresponding shear stresses. The RheoScan aggregometer was used for the measurements of critical shear stress (CSS) in whole blood samples. The correlation between CSS and the shear stress required to stop an RBC pair from aggregating was found. The shear stress required to disaggregate a pair of RBCs using the double channel optical tweezers appeared to be about 10 times higher than CSS. The correlation between shear stresses required to prevent RBCs from aggregation at the individual cell level and in whole blood samples was estimated and assessed quantitatively. The experimental approach developed has a high potential for advancing hemorheological studies.

1. Introduction

The ability of red blood cells (RBC) to aggregate is an intrinsic feature of blood that determines its rheologic properties along with the deformability of RBCs (Kim et al., 2012; Nikitin et al., 2014; Korolevich and Meglinski, 2000). These properties are of current basic science and clinical interest (Baskurt and Meiselman, 2013a; Baskurt et al., 2012) as major determinants of the health of the microcirculation. Numerous studies of RBC aggregation were carried out during the last decades, however proper understanding of this intrinsic property of RBCs still remains an actual problem to date (Baskurt et al., 2012; Meiselman, 2009; Muravyov and Tikhomirova, 2014; Skalak et al., 1983; Zhang and Neu, 2009).

In this work, we focus on measuring the parameters that characterize the aggregation of RBCs and on assessing their interaction dynamics.

1.1. Optical tweezers – a tool for measuring the interaction forces between individual cells

The possibility to study the cell–cell interaction appeared with the introduction of optical tweezers (OT) by Ashkin (1970). OT is the tool that allows trapping and free manipulation of single microparticles, and measurement of forces in a range from sub-pN to hundreds of pN using tightly focused laser beams. These forces are in the range of cellular interactions, which makes OT a promising tool for biophysical applications. Consequently, a number of breakthrough results have been obtained using OT to study cellular and molecular interactions (Ashok and Dholakia, 2011; Bennink et al., 2001; Wang et al., 1997). Related to RBC, the OT were used to study the aggregation and deformability of single cells (Bronkhorst et al., 1997; Dao et al., 2003; Khokhlova et al., 2012; Maklygin et al., 2012; Lee et al., 2016). The quantification of

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RBC interaction forces was made on RBC doublets (Khokhlova et al., 2012; Maklygin et al., 2012; Lee et al., 2015). It was found that the disaggregating force, defined as the force required to separate two interacting RBCs, is a few dozen piconewtons. The disaggregating force showed clear dependence on RBCs interaction area and duration of the contact (Bronkhorst et al., 1997; Dao et al., 2003; Khokhlova et al., 2012). Therefore OT introduced a possibility to study the dynamics of RBC interactions in order to assess RBC interaction mechanics. In this work, we use a home-made double channel OT to characterize the RBC aggregation at the individual cell level and a commercial aggregometer RheoScan (Rheomeditech, Republic of Korea) to characterize the RBC aggregation in a large ensemble (population) of cells so as to find the correlation between the parameters measured using different techniques. These parameters are: the aggregating and disaggregating forces measured using OT and the disaggregating shear stress measured using the RheoScan aggregometer (Xue et al., 2013; Shin et al., 2009). In the limits of our knowledge, this is the first work that directly compares the results of OT measurements with the results of measurements made using a whole blood aggregometer.

1.2. Commercially available devices that measure comparable parameters (RBC critical and threshold shear stress measurements)

There are currently two devices in the world market – RheoScan (Rheomeditech, Republic of Korea) and LORCA (R&R Mechatronics, the Netherlands) that allow measurement of comparable force-related RBC aggregation parameters (Shin et al., 2009; Hardeman et al., 2011). The measured parameters are referred to as critical shear stress (CSS) and threshold shear rate (TSR). TSR can be converted to the comparable parameter-threshold shear stress (TSS) with an additional measurement of the blood viscosity η: ($[TSS]=\eta[TSR]$). For convenience in what follows, we will refer to TSS instead of TSR. The physical description of the CSS and the TSS is exactly the same – it is the minimum shear stress that prevents RBCs from aggregating. These two stresses could be considered as a minimum shear stress to disperse RBC aggregates. To measure these parameters in whole blood samples, two different approaches are used: a microfluidic approach with Pouiselle flow in the RheoScan aggregometer and a coaxial rotational cups approach with Couette flow in the LORCA aggregometer. There is approximately a two times quantitative difference between TSS and CSS, attributed to lower efficiency of shear stress action in LORCA aggregometer (Lim et al., 2011; Chien et al., 1983). More detailed descriptions of the devices and the measured parameters can be found elsewhere (Shin et al., 2009; Hardeman et al., 2011).

In our work, we used the RheoScan aggregometer and, therefore, CSS measurements will be reported here. This parameter has the advantage that there is no need to additionally make measurements of blood viscosity, which in their turn require the temperature and haematocrit of the samples to be well controlled which also makes it convenient for clinical use (Lim et al., 2011). CSS is independent of haematocrit and strongly dependent of fibrinogen and RBC deformability (Xue et al., 2013). On the other hand, this property is critical for comparison with OT measurements operating with individual cells. In the scope of comparing CSS with the results of OT measurements, the forces measured with OT can be converted to the shear stresses and quantitatively compared.

2. Materials and methods

2.1. Blood sample preparation

Aliquots of 4 ml of blood were drawn by venipuncture from 8 clinically healthy male donors of age 20–30. The experiments were undertaken with the understanding and verbal consent of each donor according the ethical policy of the Lomonosov Moscow State University. Dipotassium ethylene-diamine-tetra-acetic acid (K2-EDTA) was used as an anticoagulant at a concentration of 1.8 mg/ml. The experiments were performed within 4 h after drawing the blood. The experimental samples for the individual cells measurements consisted of highly diluted suspensions of RBCs in platelet free plasma. Platelet free plasma was obtained by the following procedure: the whole blood was centrifuged for the first time at 1800g for 10 min to separate the RBCs, and then plasma was removed and centrifuged for the second time at 12,000g for 10 min to remove any remaining platelets. The platelet free plasma was taken and the RBCs were added to achieve the final concentration of about 0.05%. Such high dilution was necessary to facilitate the individual cell measurements. The measurements using RheoScan were performed with the whole blood samples.

2.2. RheoScan: critical shear stress measurements

The CSS measurements were performed using the RheoScan aggregometer (Shin et al., 2009). A detailed description of the device can be found elsewere (Shin et al., 2009, 2007; Lim et al., 2011). Here we only briefly describe the measurement procedure. The schematic layout of the setup is as shown in Fig. 1(a). The pressure is applied using a vacuum generator and the applied shear stress is monitored through the pressure sensor. The setup uses the backscattered light (BSL) intensity as a reference of the RBC aggregation level. A portion of whole blood (0.5 ml) is put into a single-use microfluidic chamber for measurements.

The measurement sequences shown in Fig. 1(b and c) are as follows: (1) once the shear stress is applied, the blood sample enters the microchannel; (2) the shear stress decreases over time, and at first the RBC aggregates tend to break down, resulting in an increase of the backscattered light (BSL) intensity; (3) the shear stress further decreases and the dispersed RBCs start to re-aggregate resulting in a decrease of the BSL intensity. Thus, the maximum value of BSL intensity indicates the termination of RBC disaggregation and the commencement of RBC aggregation. The shear stress value corresponding to the maximum value of the BSL intensity is referred to as the CSS, which is the minimum shear stress exerted by the microchannel flow required to disaggregate the RBC aggregates. Detailed description for the principle of CSS measurement can be found elsewhere (Shin et al., 2007).

2.3. Optical tweezers: aggregating and disaggregating force measurements

Measurements of RBC aggregating/disaggregating forces in the process of a cell doublet formation/destruction were performed using our optical tweezers. A detailed description of the OT operating principles can be found elsewhere (see, e.g., the review article of Neuman and Block (2004)). Here we give only a brief description of the force measurement procedure. Generally the trapped particle can be considered as a particle held by a spring, whose stiffness k is dependent on the trapping laser power. If there is no external force applied on the trapped particle it remains in the equilibrium position. If an external force shifts the position of the particle from the trap centre, an optical trap acts with a returning force, up to a certain maximum value, depending on how much the particle is separated from the trap center.

Quantitatively, the force applied from the OT onto the trapped particle is obtained through a precise calibration procedure using one of a few different methods (Neuman and Block, 2004). In our work, a calibration method that finds the maximum returning force by matching the trapping forces with the external (viscous friction) forces was used. A more detailed description of the calibration procedure can be found in our previous work (Maklygin et al., 2012).

The schematic layout of the OT setup used in this work is shown in Fig. 2. The two traps are formed by the orthogonally polarized continuous wave laser beams from two single mode diode pumped Nd:YAG lasers with wavelength of 1064 nm and output power up to 250 mW. Large numerical aperture (NA 1.00) 100 × water immersion objective (Olympus, ∞-corrected LumPlanFi) was used to focus the laser beams and form the optical traps. The RBC trapping force could be varied in the range from 1 to 20 pN. The position of one of the traps inside the sample was controlled in the focal plane of the focusing objective by a conjugated beam shifting mirror. Visual control of the trapped objects was implemented in the transmission configuration using the CMOS camera (Thorlabs, DCC1545M). Heating of the trapped cell by the laser beam could be neglected as the laser wavelength (1064 nm) is in the transparency window of the blood absorption spectrum. The minor heating of the trapped cell is estimated to be ~1 °C per 10 mW of the laser power (Khokhlova et al., 2012; Maklygin et al., 2012; Neuman and Block, 2004; Liu et al., 1995). In our work, the laser power after the objective did not exceed 20 mW.