Measuring micro-interactions between coagulating red blood cells using optical tweezers

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Abstract: Agents that alter the dynamics of hemostasis form an important part in management of conditions such as atherosclerosis, cerebrovascular disease, and bleeding diatheses. In this study, we explored the effects of heparin and tranexamic acid on the efficiency of blood coagulation. Using optical tweezers, we evaluated the pN-range micro-interaction between coagulating red blood cells (RBCs) by measuring the minimum power required to trap them. By observing the mobility of RBCs and the intensity of cellular interactions, we found that the coagulation process can be separated into three phases. The effects of heparin and tranexamic acid were examined by observing variations in cellular interaction during the coagulation phases. Heparin attenuated the interaction between RBCs and prolonged the first phase whereas the samples containing tranexamic acid bypassed the first two phases and immediately proceeded to the final one.

OCIS codes: (170.0170) Medical optics and biotechnology; (170.1470) Blood or tissue constituent monitoring; (170.4520) Optical confinement and manipulation; (350.4855) Optical tweezers or optical manipulation.

References and links

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1. Introduction

One of the functions of blood is the transportation of metabolic substrates in the human body. Hemostasis is critical to survival particularly to control hemorrhage and ensures that circulating blood is maintained within the intravascular space [1]. Optical tweezers are powerful tools used for the micro-manipulation of biological molecules and are created using laser beam technology [2]. In this study, the interaction between coagulating red blood cells (RBCs) was determined by measuring the optical power required to trap them. By observing the degree of interaction and mobility of coagulating RBCs, we were also able to demonstrate that the coagulation

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process could be discriminated into three distinct phases. Antithrombotic drugs include agents that can inhibit the activity of platelets or the coagulation process, whereas antihemorrhagic drugs include agents that cause platelet activation or inhibit fibrinolysis and clot dissolution [3]. We found that with the addition of an antithrombotic reagent, blood samples exhibited a delayed first phase and a prolonged coagulation phase. The addition of an antifibrinolytic reagent caused samples to proceed through the first two phases immediately to the third phase and complete the whole coagulation process.

2. Materials and methods

2.1 Estimation of optical trapping force

To estimate the trapping force of optical tweezers, a laser spot of power P was applied to a sample particle, as shown in Fig. 1(a). The sample stage was dragged to the right where a viscous drag force F_D was applied to the particle, as shown in Fig. 1(b). At the same time, a transverse trapping force F_L was applied to the particle by a fixed laser spot (shown by a circle with a cross) in the opposite direction [4].

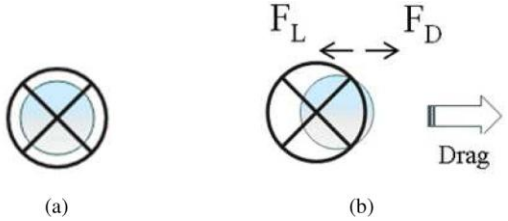

Fig. 1. Estimation of trapping force on a particle by dragging the sample stage.

When the particle is displaced from the equilibrium, the trapping force increases to a power-dependent maximum value called escape force, and then begins to decrease to zero [5]. When the viscous drag force F_D was smaller than the escape force, the particle was confined by optical tweezers; when F_D was equal to or slightly larger than the escape force, the particle began to be dragged out of the spot and escape the trap. The viscous drag force F_D applied to the dragged particle was determined by Stokes' Law [6]:

$$F_D = 6\pi\eta R v, \tag{1}$$

where η denotes the viscosity coefficient of fluid, R denotes the radius of particle, and v the dragging speed of the stage. As the trapping force F_L was balanced with the viscous drag force F_D at equilibrium, Eq. (1) was used to evaluate the trapping force F_L of the optical tweezers.

According to the theory of optical tweezers [7]:

$$F_L = nP / c, \tag{2}$$

where n denotes the index of incident media, P is the power of source, and c the vacuum speed of light. Varying the optical power P applied to the particle, we measured the trapping force F_L and derived the curve of $F_L(P)$ versus P. By using the relation curve, we determined the microscopic trapping force F_L directly for a given laser power P.

2.2 The interaction between red blood cells in coagulation

During the process of coagulation, an RBC is attracted by a coagulative interaction F_B from the right coagulated group, as shown in Fig. 2. In order to stop the cell from moving to this group, sufficient power was applied to a spot to make the trapping force F_L greater than the coagulative interaction F_B. With the power reduced to a critical value P, the cell began to escape the trap and was attracted to the right, as illustrated in Fig. 2. At this point, F_B was balanced with F_L. Using

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the relation curve of F_L versus P, the coagulative force F_B was derived by the minimum power P required to trap the coagulating RBC.

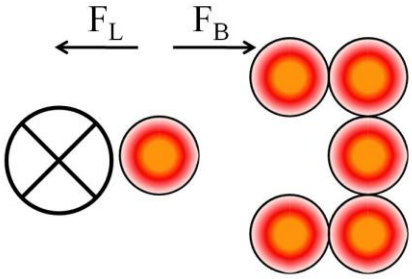

Fig. 2. Evaluation of the interaction between RBCs by the trapping force of optical tweezers.

2.3 Apparatus and materials

A 1064 nm IR laser, an optical microscope and a 100 × oil lens were utilized to establish the optical tweezers system, as depicted in Fig. 3. A motorized X-Y stage was equipped to the system to induce viscous drag force, by which the trapping forces of the optical tweezers were determined. In the below coagulation experiments, samples were prepared by diluting 20 μl of blood in 1 ml of normal saline. Control samples were prepared without the addition of drug substances. In the other samples, 20 μl of 100 IU heparin and 20 μl of 3% tranexamic acid were added.

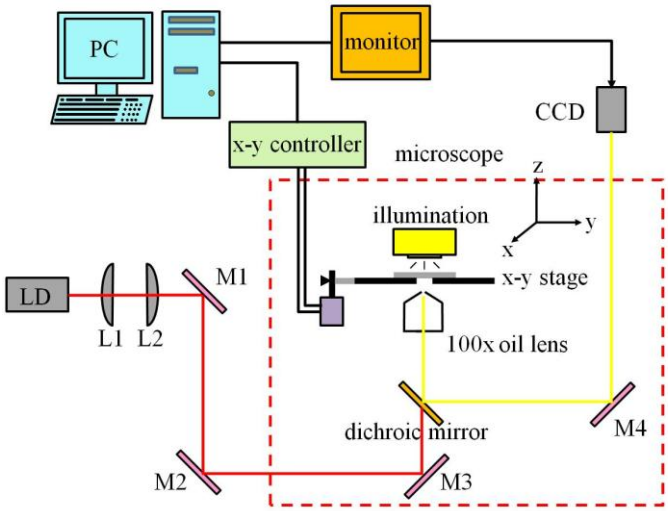

Fig. 3. A schematic diagram of the optical tweezers where the red dashed rectangle illustrates the microscope.

3. Results and discussion

3.1 The evaluation of coagulating interaction

As stated previously, dragging experiments were performed to determine the trapping force F_L of the optical tweezers. The dependence of trapping force F_L on varying laser power P is summarized in Table 1 and Fig. 4, which are used accordingly to derive the interaction between coagulating RBCs.

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Table 1. Trapping force induced by varying laser power, where "Power" denotes the laser power *P*, "Speed" indicates the dragging speed *v*, and "Force" the trapping force of the optical tweezers. The trapping force is derived from Eq. (1) with the viscosity coefficient η of 0.0055 N-sec/m^2 [8] and the cell radius *R* of ~4.5 μm

Power (mW)	Speed (μm/s)	Force (pN)
30	0.69	0.32
60	1.05	0.47
90	3.06	1.43
120	3.31	1.55
150	3.88	1.81
180	4.38	2.04
210	5.00	2.33
240	6.25	2.92
270	7.38	3.44
300	7.50	3.50
330	8.13	3.79
360	8.25	3.85
380	8.38	3.91

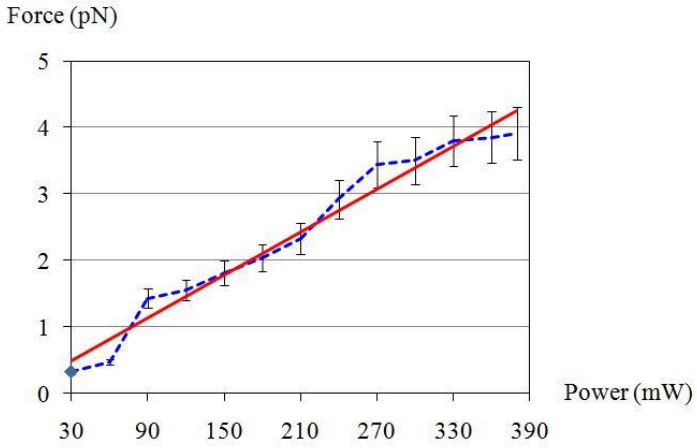

Fig. 4. Trapping force as a function of optical power, where "Power" denotes the laser power and "Force" the induced trapping force. Based on the data listed in Table 1, the blue dashed curve with 10% error bars is plotted here along with its red linear trend line.

The process used to evaluate coagulating interactions is illustrated in Fig. 5. In Fig. 5(a), the coagulating RBCs 1 and 3 were attracted to the blood group on the left. By applying higher laser power, RBC 1 was trapped and stopped moving to the left, as shown in Fig. 5(b). However, when the power was reduced to ~130 mW, cell 1 was released and was attracted to the blood group again, as shown in Fig. 5(c) and Fig. 5(d). According to the data shown in Table 1, the coagulating force F_B on RBC 1 was estimated to be ~1.64 pN.

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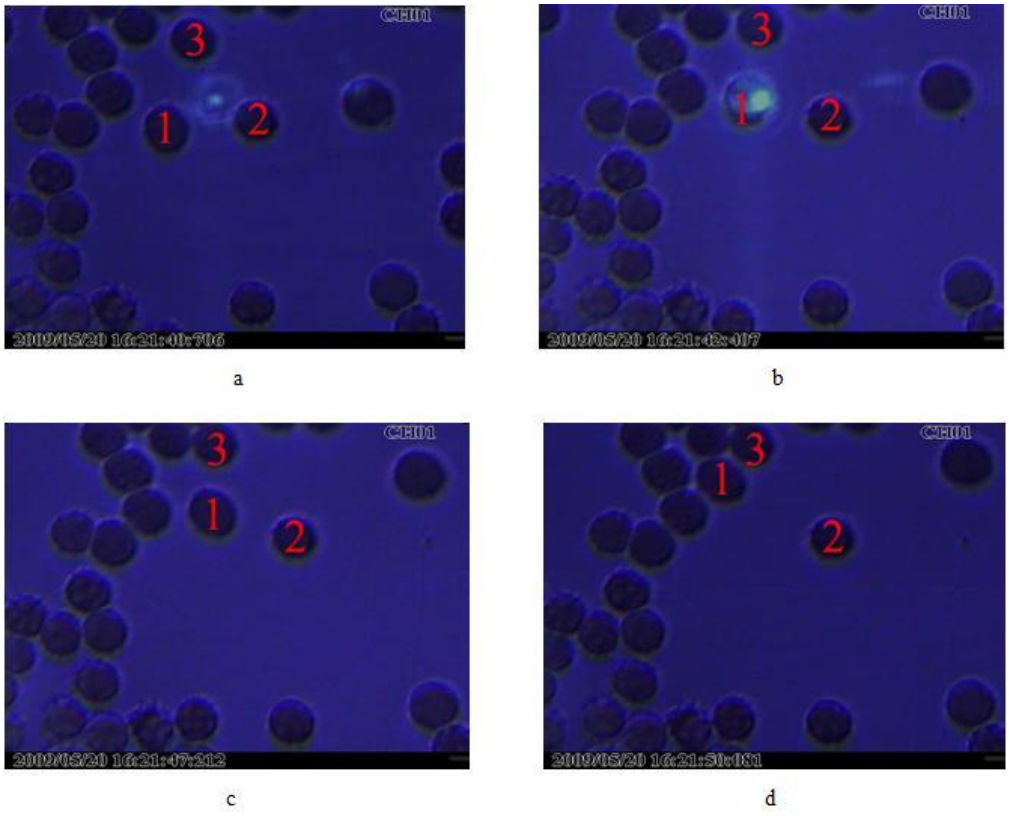

Fig. 5. (Media 1) The process to evaluate the interaction between coagulating RBCs.

3.2 The phases of blood coagulation

The blood samples were prepared on microscope slides and situated on the stage of optical tweezers for coagulation experiments, as shown in Fig. 6. Coagulation was initiated in blood solution located outside the edge of the coverslip, which exerted outward attractive forces on the remainder of the sample. As the coverslip and slide were bonded by a two-sided adhesive tape which served as a spacer as thick as 50~100 μm, the attracted RBCs could move outwards free of effects from the substrates. Based on the mobility of the RBCs and the strength of intercellular interactions, we observed three distinct coagulation phases. In the first phase, the RBCs vibrated around their equilibrium positions. In the second phase, individual RBCs began to shift outwards to the coagulated region, while the final phase featured intense group migration until the entire process was complete.

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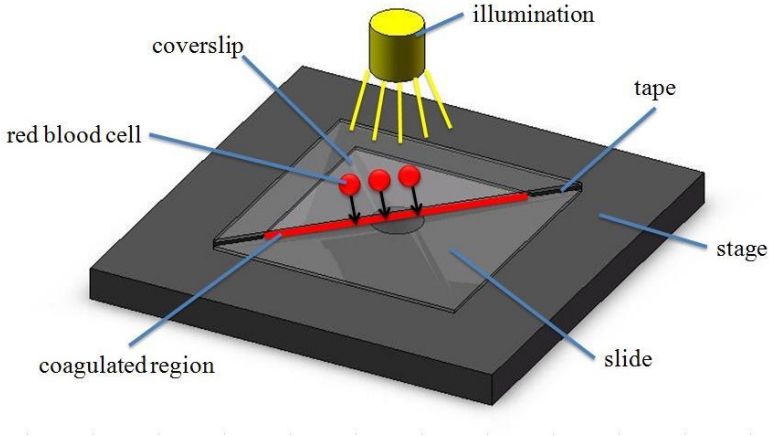

Fig. 6. The preparation of blood samples for coagulation experiments.

For blood samples with no additives, RBCs were randomly distributed inside or outside the coverslip at the beginning. After the coagulated region was formed, RBCs located on the inside exhibited attraction and began to shift individually and slowly outwards. After the process proceeded for roughly five minutes, extensive cell migration commenced, marking the third phase. Cellular interaction versus coagulation time is summarized in Fig. 7(a). In the second phase, interaction ranged from ~1.0 pN at beginning and increased to ~3.9 pN at around five minutes. In the third phase, the interaction was so intense that the 380 mW source power was insufficient to trap the rapidly-moving RBCs.

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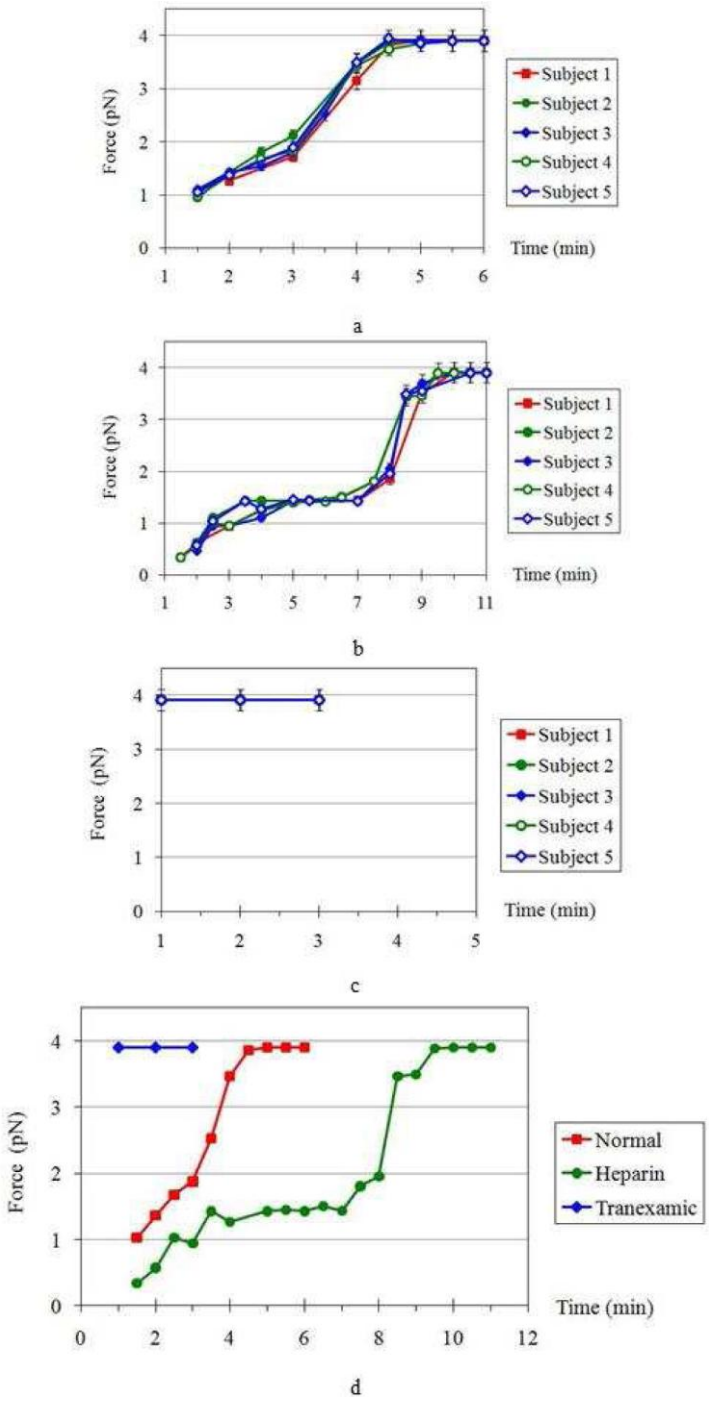

Fig. 7. (Media 2) (a) The variation of interaction between coagulating cells in normal blood samples. Note that subject 1 is a male of age 15, subject 2 is a male of age 22, subject 3 is a female of age 23, subject 4 is a female of age 33, and subject 5 is a male of age 39. (b) Variations in cellular interactions in blood samples treated with heparin [9]. (c) Variations in cellular interactions in blood samples treated with tranexamic acid [10]. (d) Variations in averaged cell interactions in three types of blood samples. The control samples are denoted as "Normal"; the ones treated with heparin or tranexamic acid are denoted as "Heparin" or "Tranexamic", respectively.

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The blood sample containing heparin entered its first phase at the beginning. Heparin attenuated the inter-cellular interaction and prolonged the coagulation time. Figure 7(b) illustrates the time variation of cellular interaction in the blood samples. The addition of heparin extended the first phase by keeping the interaction at ~1.5 pN for four minutes. At around seven minutes, the samples proceeded to the second phase with a corresponding increase in force. The interaction increased to 3.9 pN at ten minutes at which point the third phase commenced. The addition of heparin postponed coagulation for about five minutes.

The sample containing tranexamic acid immediately entered the third phase at the beginning. The fast and intense group migration allowed the overall coagulation process to be completed within two minutes. Figure 7(c) shows the variation in cellular interactions of the samples. As illustrated, we found that the interaction increased towards and stabilized at 3.9 pN till the end of the process.

Time variations in the micro-interaction of the three samples are summarized in Fig. 7(d). The control sample entered the second phase at the beginning and the intensity of cellular interactions increased with time until the third phase began at five minutes. Heparin containing samples exhibited a prolonged first phase without group migration or net coagulation. Contrastingly, samples containing tranexamic acid proceeded immediately to the third phase and completed the coagulation within two minutes.

4. Conclusion

In this study, we used optical tweezers to evaluate the microscopic interactions between RBCs during coagulation. The pN-scale interaction was derived by the optical power required to trap the cells. Variations in the intensity of cellular interactions over time allowed us to describe the coagulation process as three distinct phases. We also confirmed the effects of heparin and tranexamic acid on coagulation by examining the cellular interactions throughout each phase. The results of this study provide an objective assessment of the effects of medications on the efficiency of coagulation.

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