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# In vitro influence of 520 nm diode laser irradiation on red blood cell spontaneous aggregation studied by optical tweezers and light microscopy

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## ABSTRACT

The laser has biomodulation effects on blood properties thus could regulate the microcirculation. The laser-RBC interaction mechanism is unclear, whereas most studies provide statistical results and lack detailed observations of laser irradiation effects on blood rheology. This study is designated to probe the in vitro effects of 520 nm diode laser irradiation on red blood cells (RBCs) mutual interaction properties in spontaneous aggregation process in autologous plasma by optical tweezers (OTs), with an attempt to reveal the laser-RBC interaction outcomes at a single-cell level. The results preliminarily show that though the laser irradiation statistically inhibited the increase of the size of RBC aggregates compared with the non-irradiating group, the aggregation force between single RBCs increased slightly with the time of irradiation.

**Keywords:** Red blood cells (RBCs), RBC aggregation, laser irradiation, optical tweezers, light microscopy.

## 1. INTRODUCTION

Red blood cells (RBCs) are hemoglobin-filled bi-concave disk-shaped cells that are developed in the bone marrow, circulated in the body for an average of $120 \pm 20$ days, and get recycled by the reticuloendothelial cell. The flexible membrane and the large surface-to-volume ratio of RBCs, as well as the lack of cell organelles including nucleus and mitochondria, empower them to carry and transport oxygen from lungs throughout all parts of the body with maximum efficacy.[1] In static and low-shear conditions, RBCs suspended in plasma or plasma mimicking protein solutions will spontaneously aggregate into face-to-face attaching structures, which could grow in size through face-to-edge and edge-to-edge connections. This intrinsic property of RBCs is critical in hemostasis and weak aggregation is closely related to hemophilia, whereas strong aggregation will cause thrombotic and ischemia.[2] The monitoring and intervention of RBCs aggregation behavior have been attracting scientific and clinical attention as the degree and state of the reversible dynamic aggregation is a fundamental hemorheological determinant that sensitively affects the microcirculation.[3] Compared with the statistical analysis of the degree and speed of RBC aggregation, the in-depth comprehensive understanding of the cell-to-cell interaction mechanism at the single-cell level is necessary to explore methods of regulating RBC aggregation.[4–6] During the last few decades, a novel laser-based non-invasive technique, optical tweezers (OTs), has been developed into a popular method in living cell analytical studies, which enables precise manipulation and detection of bio-forces simultaneously.[7–9]

The interactions of light with biological tissue through different mechanisms, including photo-thermal actions, photochemical reactions, and photobiomodulation, can elicit stimulatory and inhibitory effects on tissue conditions, depending on the preset light parameters, thus have been drastically investigated.[10] Tremendous valuable results have been obtained, especially after the invention and development of laser technology.[11] Low-level light therapy (LLLT) has become a matured field of utilizing different light sources, including lasers, lamps, LED, etc., for diagnostic and especially for therapeutic purposes.[12] Various methods for LLLT, including extracorporeal

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and intravenous blood irradiation, have been developed, and LLLT has received considerable clinical outcomes.[13] However, the interaction mechanism of laser with blood cells and the effects of major laser characteristics, including wavelength, power density, and radiation dose, on the rheological properties of microcirculation remain in discussion, and in-depth investigation is still necessary to reveal the laser biomodulation effects on the human circulation system. It is widely believed that in blood irradiation, the absorption of irradiation energy by hemoglobin is the main reason for the improvement of rheological performance. As listed in Table 1, some of the studies investigating the regulatory effects of laser irradiation on blood properties showed that the stronger effect was obtained with laser wavelength closer to the absorption peak of hemoglobin, especially after long time irradiation (up to 130 min) before thermal denaturation occurs.[14,15] In this paper, an attempt was made to test the effect of 520nm laser irradiation for up to 120 min on RBC spontaneous aggregation in autologous plasma by light microscopic observation, and the RBC mutual interaction in individual cells pairs was evaluated with single-cell analytical method OTs. The OTs observations showed that the laser irradiation slightly enhanced the RBC aggregation force in the single-cell level, and promotes the formation of small RBC aggregates. However, the light microscopic observation statistically showed that the laser irradiation prohibited the formation of large aggregating structures, as the sizes of RBC aggregates after irradiation were generally smaller than without irradiation. The study demonstrates OTs as a useful tool in studying the laser-RBC interaction mechanism and provides us a new insight into the understanding of the laser irradiation effects on blood cell properties.

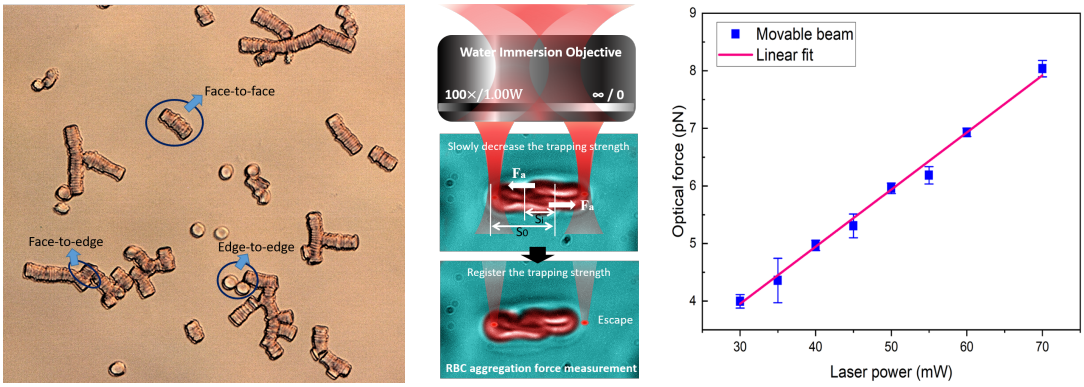
Table 1. Results of low-Level laser irradiation on some rheological factors in human RBCs in vitro.

| Irradiation laser source and method | Results | Study |
|---|---|---|
| He-Ne laser source (wavelength 632.8 nm, power output 8.0 mW). Direct irradiation at a distance of 20 cm by the focused laser beam (circular spot, 0.8 cm in diameter) for 5 min. | The deformability of RBCs was decreased by 30% after 5-min laser irradiation. Laser irradiation and epinephrine act antagonistically on RBC deformability. | 16 |
| He-Ne laser (632.8 nm, 1 mW and 6 mW) and laser diodes (630 – 990 nm). Direct continuous irradiation by beam with spot of 0.2 cm in diameter. | Laser radiation has a revitalizing effect on the RBCs in preserved blood in reducing the erythrocyte sedimentation rate (ESR). | 12, 14 |
| 632.8 nm He–Ne laser and 532 nm YAG solid laser. The power of each laser on the samples was 30 mW with an irradiation spot of the 5 mm diameter. | ESR were lowered by laser irradiation of both wavelengths. The RBC viscosities of hyperviscosity blood samples were reduced and the RBCs with poor deformability was improved after irradiation. | 15 |
| 405, 589 and 780 nm laser are used for irradiation with output power 10 mW and fixed power density 30 mW/cm$^2$ | The ESR was decreased after irradiation with the maximum effect observed with 405 nm laser at 72 J/cm$^2$ radiation dose. ESR of non-irradiated RBCs in irradiated plasma is significantly lower than in non-irradiated plasma. | 17 |
| Continuous and pulsed low-level He–Ne laser (633 nm, 4 mW, beam diameter 0.48 mm) short time (up to 300 s) irradiation. (Power density: 2.21 W/cm$^2$. | Up to 300 s continuous He–Ne laser irradiation, showed no influence on RBC aggregation, whereas the RBC aggregation force decreased after 120 s irradiation by the same laser with pulse modulation of 225 Hz. | 18 |

## 2. MATERIALS AND METHODS

The blood samples are diluted RBCs suspension (0.6 - 1 µl) in autologous platelet-free plasma (100 – 150 µl). The low concentration of RBCs ensured that the RBCs were irradiated individually and the changes of the cell-cell interaction force between single irradiated RBCs were measured by OTs. The blood sample was donated by a healthy female donor via venipuncture at a Nordlab clinic (Oulu, Finland), and the plasma was separated

from the whole blood by double-centrifugation at 6500 RPM (4732 g) for 10 min. RBCs were obtained freshly each time before experiments by fingertip prick and were washed in phosphate buffer saline. The blood samples were injected into the sample chamber made of a microscope slide and cover glass. The sample chamber was irradiated directly by the laser beam from the diode laser source (520 nm, 50 mW, beam diameter 1.1 cm) at a distance of 15 - 20 cm. The power density of the irradiation is calculated to be about 52.6 mW/cm$^2$, and for irradiation time of 30, 60, 120 min, the radiant doses are calculated to be about 94.7, 189.5, and 378.9 J/cm$^2$. The irradiation was carried out at room temperature (23 $\pm$ 1 °C). To estimate the change of RBC aggregation degree with time and after laser irradiation from a statistical point of view, the light microscopic images were taken using an Eclipse LV100DA-U microscope (Nikon, Japan), as shown in Figure1(a). The total areas of RBC face-to-face aggregates (including large aggregates formed by face-to-face aggregates connected through face-to-edge interactions) were calculated and the frequency distribution curves were used to show the trends of RBC aggregation. The OTs setup used for RBC aggregation force measurement is the same as we used in our previous studies and the aggregation force measurement procedure, as demonstrated in Figure 1(b), is the same as we did before.[19,20] Basically speaking, two orthogonally polarized laser beams separated from one infrared trapping Nd:YAG laser source (1064 nm, 350 mW, ILML3IF-300 Leadlight Technology, Taiwan) were focused by a high numerical aperture water immersion objective (lLUMPlanFl 100×/1.00 W, Olympus, Tokyo, Japan) to form to independent optical traps with the position of the non-center beam controllable. The relationship between the laser power and the optical trapping force is shown in Figure 1(c).




a b c

Figure 1. (a) Illustration of microscopic image of RBCs aggregation structures formed by face-to-face, face-to-edge, and edge-to-edge interactions. (b) Illustration of RBC aggregation force measurement procedure by OTs. (c) The calibration relationship between optical trapping power and the trapping force.

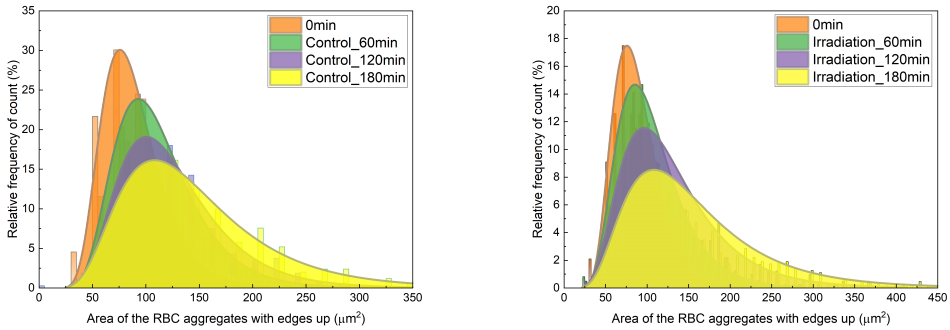
## 3. RESULTS

### 3.1 Light microscope studies

One group of the RBCs within the microscope slide sample chamber were let stand for up to 180 min, and were observed and recorded using a Nikon Eclipse LV100DA-U microscope every 30 min. The other group of RBC samples were irradiated for the same time before the microscopic images were taken. Around 5000 objects (a single RBC and an aggregate formed by several RBCs were both counted as 1 object) were registered for area size distribution calculation. The observations demonstrate that for both groups, the RBCs within the sample chamber spontaneously attached to each other and the size of the aggregates grows with time, as shown in Figure 2. However, the comparisons between the control and irradiation group at each time interval illustrate that the formed RBC aggregates were more distributed in the small size area for the irradiation group, and more larger size aggregates appears in the control group as shown in Figure 3.

### 3.2 Optical tweezers studies

The RBC aggregation force was measured as the minimum force applied to stop the attached RBCs from forming larger attaching area and clumping tightly together. As shown in Figure 1(b), two single RBCs were




**a** b

Figure 2. Demonstration of changes in the size distribution of RBC aggregates with edges up according to the conventional light microscopic images under (a) control condition and (b) after continuous direct laser irradiation for up to 180 min with time interval of 30 min. Only results of 0, 60, 120, and 180 min were shown, and all results follow the same trend that the size of aggregates grows with time.

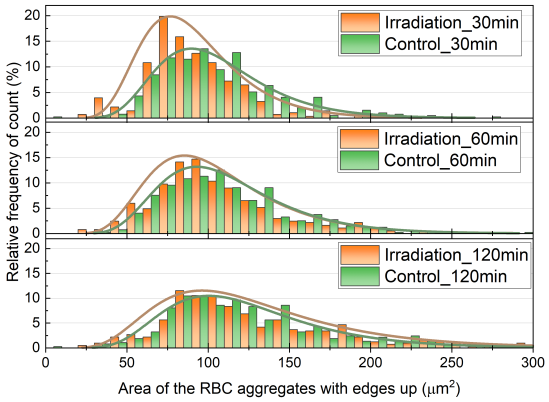



Figure 3. The comparison of size distribution of RBC aggregates between the control and irradiation group after 30, 60, and 120 min.

independently trapped by the two beams with a sufficient trapping strength. After a stable interaction area was formed, the trapping strength was slowly decreased by decreasing the trapping power, under the action of RBC intercellular mutual attracting force, the RBCs escaped from the trap and moved closer until totally clumped. At the point of cell escaping, the trapping force is considered to match with the cell interaction force, which is called the aggregation force. The measurement was repeated with different initially formed RBC interaction area and after different irradiation time. As we have presented before,[18,19] the relationships between the RBC aggregation force and the initially formed interaction area in Figure 4 follow the linear manner. After the laser irradiation, the aggregation force was slightly increased and slowly restored to normal. Moreover, no hemolysis or morphological changes were observed for irradiated samples, the differences between the control and irradiated RBCs were not observable from microscopic images by bare eyes. However, it is obvious in the OTs measurements, that the RBCs fall into small aggregates after irradiation, especially after 120 min irradiation, whereas the non-irradiated RBCs were more in single forms at the same concentration.

## 4. CONCLUSIONS

The evaluation of the RBC interaction force by OTs showed that the RBC aggregation force was enhanced slightly by laser irradiation, and the effect was more obvious within 60 min of irradiation and became weaker after 120 min

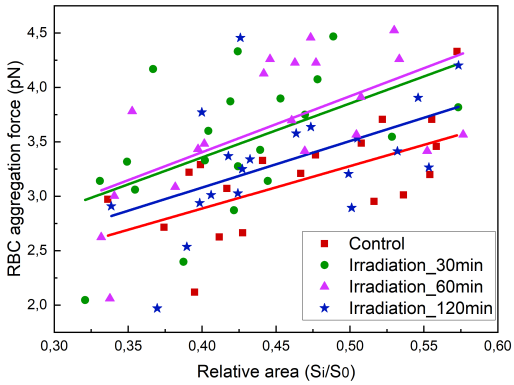



Figure 4. The relationships between the RBC mutual intercellular aggregation force and the relative interaction area (the ratio between the initially formed RBC interaction area $S_i$ and the up surface area of a single RBC $S_0$) measured after different irradiation times.

irradiation. The enhanced aggregation force was also reflected in the fact that more small RBC aggregates were observed after 120 min irradiation, while the number of aggregates among non-irradiated RBCs was relatively fewer. Moreover, the light microscopic observation statistically showed that the laser irradiation prohibited the formation of large aggregating structures, as the sizes of RBC aggregates after irradiation were generally smaller than without irradiation. The study provides a new type of method of evaluating the laser irradiation effects on blood rheological properties, especially on RBCs, and provides new insights into the understanding of the laser-cell interaction. More investigations will be continued to reveal deeper laser-cell interaction mechanisms and possible biphasic dose response as indicated by other studies.[17]

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## REFERENCES

[1] Zhu, R., Avsievich, T., Popov, A., Bykov, A., and Meglinski, I., "In vivo nano-biosensing element of red blood cell-mediated delivery," *Biosensors and Bioelectronics* , 112845 (2020).

[2] Chien, S. and Sung, L. A., "Physicochemical basis and clinical implications of red cell aggregation," *Clinical Hemorheology and Microcirculation* **7**(1), 71–91 (1987).

[3] Baskurt, O. K. and Meiselman, H. J., "Erythrocyte aggregation: basic aspects and clinical importance," *Clinical Hemorheology and Microcirculation* **53**(1-2), 23–37 (2013).

[4] Lee, K., Danilina, A. V., Kinnunen, M., Priezzhev, A. V., and Meglinski, I., "Probing the red blood cells aggregating force with optical tweezers," *IEEE Journal of Selected Topics in Quantum Electronics* **22**(3), 365–370 (2015).

[5] Avsievich, T., Popov, A., Bykov, A., and Meglinski, I., "Mutual interaction of red blood cells influenced by nanoparticles," *Scientific Reports* **9**(1), 1–6 (2019).

[6] Lee, K., Priezzhev, A., Shin, S., Yaya, F., and Meglinski, I., "Characterization of shear stress preventing red blood cells aggregation at the individual cell level: The temperature dependence," *Clinical Hemorheology and Microcirculation* **64**(4), 853–857 (2016).

[7] Zhu, R., Avsievich, T., Popov, A., and Meglinski, I., "Optical tweezers in studies of red blood cells," *Cells* **9**(3), 545 (2020).

[8] Avsievich, T., Zhu, R., Popov, A., Bykov, A., and Meglinski, I., "The advancement of blood cell research by optical tweezers," *Reviews in Physics* **5**, 100043 (2020).

[9] Avsievich, T., Tarakanchikova, Y., Zhu, R., Popov, A., Bykov, A., Skovorodkin, I., Vainio, S., and Meglinski, I., "Impact of nanocapsules on red blood cells interplay jointly assessed by optical tweezers and microscopy," *Micromachines* **11**(1), 19 (2020).

[10] Harris, D. T., "The action of light on blood," *Biochemical Journal* **20**(2), 271–279 (1926).

[11] Anders, J. J., Lanzafame, R. J., and Arany, P. R., "Low-level light/laser therapy versus photobiomodulation therapy," *Photomedicine and Laser Surgery* **33**(4), 183–184 (2015).

[12] Siposan, D. G. and Lukacs, A., "Relative variation to received dose of some erythrocytic and leukocytic indices of human blood as a result of low-level laser radiation: an in vitro study," *Journal of Clinical Laser Medicine & Surgery* **19**(2), 89–103 (2001).

[13] Siposan, D. G. and Bobe, S., "Effects of hene laser on stored blood," *Laser Therapy* **19**(4), 245–255 (2010).

[14] Siposan, D. G. and Lukacs, A., "Effect of low-level laser radiation on some rheological factors in human blood: an in vitro study," *Journal of Clinical Laser Medicine & Surgery* **18**(4), 185–195 (2000).

[15] Mi, X., Chen, J., Cen, Y., Liang, Z., and Zhou, L., "A comparative study of 632.8 and 532 nm laser irradiation on some rheological factors in human blood in vitro," *Journal of Photochemistry and Photobiology B: Biology* **74**(1), 7–12 (2004).

[16] Yova, D., Haritou, M., and Koutsouris, D., "Antagonistic effects of epinephrine and helium-neon (he-ne) laser irradiation on red blood cells deformability," *Clinical Hemorheology and Microcirculation* **14**(3), 369–378 (1994).

[17] Al Musawi, M. S., Jaafar, M., Al-Gailani, B., Ahmed, N. M., Suhaimi, F. M., and Bakhsh, M., "Erythrocyte sedimentation rate of human blood exposed to low-level laser," *Lasers in Medical Science* **31**(6), 1195–1201 (2016).

[18] Zhu, R., Avsievich, T., Bykov, A., Popov, A., and Meglinski, I., "Influence of pulsed he–ne laser irradiation on the red blood cell interaction studied by optical tweezers," *Micromachines* **10**(12), 853 (2019).

[19] Zhu, R., Avsievich, T., Popov, A., and Meglinski, I., "Influence of interaction time on the red blood cell (dis) aggregation dynamics in vitro studied by optical tweezers," in [*European Conference on Biomedical Optics*], 11075_12, Optical Society of America (2019).

[20] Zhu, R., Popov, A., and Meglinski, I., "Probing the red blood cell interaction in individual cell pairs by optical tweezers," in [*CLEO: Applications and Technology*], AW3I–4, Optical Society of America (2020).