Figure 1. The dependence of the aggregation force on the density fraction for RBC (a) in autologous plasma and (b) dextran 70 kDa solution (50 mg/ml). The higher the density fraction number the older the cells. Each point corresponds to one measurement on a pair of RBCs. Errors here are standard errors.

CONCLUSIONS

Thus, we can conclude that the RBC aggregation process alters for RBC of different ages. The older the RBC, the higher the aggregation force between them and the more they aggregate. These results will help us to better understand the age changes of RBC and the changes of their aggregation properties.

The work was supported by Russian Foundation for Basic Research (grant No. 19-52-51015).

REFERENCES

1. Baskurt O.K., Neu B., Meiselman H. Red Blood Cell Aggregation. Boca Raton: CRC Press, 2012. 288 p.

2. Ermolinskiy P. et al. Effect of red blood cell aging in vivo on their aggregation properties in vitro: measurements with laser tweezers // Appl. Sci. 2020. Vol. 10, P. 7581-1-7581-10.

3. Lee K. et al. Optical tweezers study of red blood cell aggregation and disaggregation in plasma and protein solutions // J. Biomed. Opt. 2016. Vol. 21, № 3. P. 035001-1-035001–035010.

УДК 535.8; 577.352.336; 577.325.3

А.А. Капков, А.Н. Семенов, П. Б. Ермолинский, А.Е. Луговцов, А.В. Приезжев

ПРИМЕНЕНИЕ ЛАЗЕРНО-ОПТИЧЕСКИХ И МИКРОФЛЮИДНЫХ МЕТОДОВ ДЛЯ ИССЛЕДОВАНИЯ ВЗАИМОДЕЙСТВИЯ КЛЕТОК КРОВИ В СРЕДЕ С ЭНДОТЕЛИАЛЬНЫМИ КЛЕТКАМИ

Аннотация: Взаимодействие клеток эндотелия и эритроцитов имеет важное значение при различных заболеваниях человека, например, сахарном диабете. Одно из главных таких взаи-

модействий — адгезия эритроцитов к эндотелиальному слою. Адгезия может препятствовать движению эритроцитов в потоке по сосудам в организме человека. В данной работе мы представляем результаты нашего исследования о влиянии эндотелиальных клеток на гидродинамическую прочность агрегатов клеток крови, а также деформируемость эритроцитов in vitro, выполненного с помощью лазерных методов.

Ключевые слова: эритроциты, эндотелиальные клетки, сахарный диабет, адгезия, лазерные методы

A.A. Kapkov, A. N. Semenov, P.B. Ermolinskiy, A.E. Lugovtsov, A.V. Priezzhev

APPLICATION OF LASER-OPTIC AND MICROFLUIDIC TECHNIQUES FOR THE STUDY OF BLOOD CELLS INTERACTATION IN THE MEDIA CONTAINING ENDOTHELIAL CELLS

Annotation. The interaction between endothelium cells and red blood cells (RBC) plays a crucial role in different human diseases, e.g., diabetes mellitus. The main part of this interaction is the erythrocyte adhesion to endothelium layer of the vessel. The adhesion can interfere RBC movements in the flow in human vessel. In this work, we present the results of our study of the effect of the endothelial cells on the hydrodynamic strength of RBC aggregates and deformability of the RBC *in vitro*, performed by laser techniques.

Keywords: red blood cells, endothelial cells, diabetes mellitus, adhesion, laser techniques

INTRODUCTION

Vascular endothelial cells (EC) constitute the inner surface of arteries, veins and capillaries and therefore directly interact with different components of blood. The interaction between EC and red blood cells (RBC) is important because many hemorheological disorders are accompanied with the elevated adhesion of RBC onto vessel endothelium. The mechanisms of this pathology are not completely studied yet [1-3]. Laser techniques allow for a non-invasive study of the features of the interaction between EC and RBC [4]. This work was aimed at implementation of optical techniques to

investigate *in vitro* the effects of EC on the RBC microrheologic properties, in particular, on their spontaneous aggregation and shearing deformation.

MATERIALS AND METHODS

We performed our experiments with fresh blood samples drawn from a cubital vein of a healthy volunteer. The human umbilical vein endothelial cell (HUVEC) were cultured and grown in Koltzov Institute of Developmental Biology of Russian Academy of Sciences. Then the cells were placed inside the microfluidic vessels, that are used in the optic techniques. Subsequently, to activate EC, Tumor necrosis factor alpha (TNF) and Adenosine diphosphate (ADF) activators were used as in refs [4-7]. In our experiments, we used light scattering techniques to conduct the measurements. We used laser aggregometry based on diffuse light scattering from whole blood samples which allows to obtain the critical shear stress (CSS) parameter, which characterizes the hydrodynamic strength of RBC aggregates [8]. By analyzing the dependency of laser light, scattered by RBC suspension under different shear-stress, we measured CSS of RBC aggregates in the microchannel covered with endothelial cells layer. To assess the effects of endothelium on RBC deformability we used laser ektacytometry technique, which allows for measuring the RBC elongation index in shear stress flow. All measurements were conducted with blood samples from 10 donors. All experiments were performed at 37 .

RESULTS

The experiments carried out using laser aggregometry showed a decrease in the parameter CSS in the case of EC covered channel wall (about 40 12 % lower than in the control). As for the control sample we used similar microvessels without the administration of the EC. We performed measurements with control vessels, then with the vessels covered with the EC before and after activation. The results are presented in Fig.1:

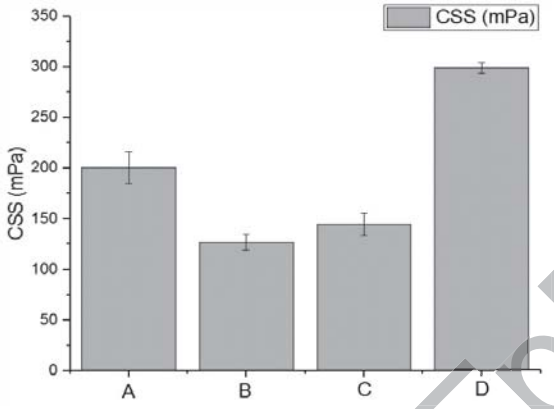

Figure 1.Left to right: parameter CSS, measured in control vessels (A) , and in vessels covered with the endothelial cells without activation (B), activated with TNF (C), activated with ADF endothelium.(D)

In this histogram, it can be seen that the administration of the endothelial cells into the vessels without activation leads a decrease in the parameter CSS. Activation of EC with TNF results in small increase in CSS (16 4% higher than in the control) while activation of EC with ADF results in crucial growth of CSS (50 7% higher than in the control).

The second histogram shows changes in deformability of RBC depending on the presence of EC. We can see that the ability of erythrocyte to deform under shear stress slightly decreases under the presence of EC (13 5% lower than in the control).

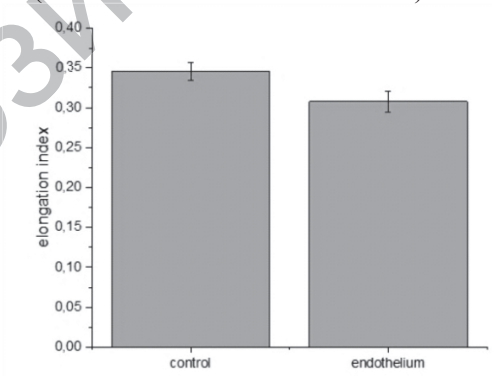

Figure 2. Alterations in RBC elongation index measured with and without endothelium cells

CONCLUSIONS

The parameter CSS was obtained and compared with control value. It was found that there is a decrease in CSS if RBC move along the vessels with endothelium layer covered walls. However, if EC were previously activated, CSS showed an increase by about 16 4% and 50 7% as compared to non-activated cells. RBC deformability under shear-stress was also studied and showed a decrease in RBC elongation index in the case of presence of EC (13 5% lower than in the control). We believe this process is due to RBC adhesion to endothelial layer. Our future work will be aimed at more detailed study of the interaction between activated EC and different blood components.

The work was supported by Russian Foundation for Basic Research (grant Nº 19-52-51015).

REFERENCES

1. Wautier J. L., Wautier M. P., Molecular basis of erythrocyte adhesion to endothelial cells in diseases // Clin. Hemorheol. Microcirc. 2013. Vol. 53. №. 1–2. PP. 11–21.

2. Yedgar S., Kaul D. K., Barshtein G. RBC adhesion to vascular endothelial cells: More potent than RBC aggregation in inducing circulatory disorders // Microcirculation. 2008. Vol. 15. №. 7. PP. 581–583.

3. Kucukal E., Quinn E. Red blood cell adhesion to ICAM-1 is mediated by fibrinogen and is associated with right-to-left shunts in sickle cell disease // Blood Adv. 2020. Vol. 4. №. 15. PP. 3688–3698.

4. Hocheng H., Tseng C. Mechanical and optical design for assembly of vascular endothelial cells using laser guidance and tweezers // *Opt. Commun.* 2008. Vol. 281. №. 17. PP. 4435–4441.

5. Wagner M. C., Eckman J. R., Wick T. M. Sickle cell adhesion depends on hemodynamics and endothelial activation // J. Lab. Clin. Med. 2004. Vol. 144. №. 5. PP. 260–267.

6. Brown M. D., Wick T. M., Eckman J. R. Activation of vascular endothelial cell adhesion molecule expression by sickle blood cells // *Pediatr. Pathol. Mol. Med.* 2001. Vol. 20. №. 1. PP. 47–72.

7. Hess C. N., Kou R., Johnson R. P., Li G. K., Michel T. ADP signaling in vascular endothelial cells: ADP-dependent activation of the endothelial isoform of nitric-oxide synthase requires

the expression but not the kinase activity of AMP-activated protein kinase // *J. Biol. Chem.* 2009. Vol. 284. №. 47. PP. 32209–32224.

8. Lee K.,Kinnunen M., Danilina A.V., Ustinov V.D., Shin S., Meglinski I., Priezzhev A.V. Characterization at the individual cell level and in whole blood samples of shear stress preventing red blood cells aggregation // *J. Biomech.* 2016. V. 49, № 7. PP. 1021–1026.

УДК 535.8; 577.352.336; 577.325.3

Приезжев А.В., Виндбергер У.,
Луговцов А.Е., Гурфинкель Ю.И., Никитин С.Ю., Шин С.

ОПТИЧЕСКИЕ МЕТОДЫ ИЗУЧЕНИЯ МИКРОРЕОЛОГИЧЕСКИХ И БИОМЕХАНИЧЕСКИХ СВОЙСТВ КРОВИ В НОРМЕ И ПАТОЛОГИИ

Аннотация: Захват микрочастиц с помощью лазерных пинцетов, рассеяние света, лазерная дифрактометрия и оптическая капилляроскопия открывают новые горизонты для широкого спектра фундаментальных и прикладных исследований в гемогеологии, поскольку дают новые возможности для изучения изменения и взаимодействия на уровне отдельных клеток *in vitro* and *in vivo* в норме и при патологиях.

Ключевые слова: лазерный пинцет, агрегометрия, дифрактометрия, оптическая капилляроскопия, гемореология.

A.V. Priezzhev, U. Windberger,
A.E. Lugovtsov, Yu.I. Gurfinkel, S.Yu. Nikitin, S. Shin

OPTICAL TECHNIQUES FOR STUDYING MICRORHEOLOGICAL AND BIOMECHANICAL PROPERTIES OF BLOOD IN NORM AND PATHOLOGY

Annotation. Trapping of microparticles with laser tweezers, light scattering, laser diffractometry and optical capillaroscopy open up new horizons for a wide range of fundamental and applied research in hemogeology, as they provide new opportunities for