Temperature Dependencies of the Aggregation Properties of RBC in Dextran Solutions *In Vitro*

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Abstract. The process of reversible spontaneous aggregation of red blood cells (RBC), as well as the process of their shear stress induced disaggregation, both affect hemorheology and blood microcirculation in the human body. The aim of this work is to estimate the influence of temperature on the aggregation properties of RBC suspended in PBS dextran solutions *in vitro*. Laser method based on diffuse light scattering was used to estimate the RBC aggregation properties. The results demonstrate a clear dependence of the critical shear stress aggregation parameter of RBC in PBS dextran solutions on temperature. These results would help to better understand the process of RBC aggregation. © 2020 Journal of Biomedical Photonics & Engineering.

Keywords: RBC aggregation; temperature; erythrocytes; diffuse light scattering; dextran; critical shear stress.

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1 Introduction

Processes of red blood cells (RBC) aggregation and disaggregation strongly influence the viscosity of blood in vessels and have a general impact human health [1, 2]. Aggregation properties of blood may be changed due many factors: alteration of blood plasma composition, change in blood temperature, injection of different synthetic macromolecules, RBC aging, pathological alterations, and others [2, 3]. The mechanisms of RBC aggregation, as well as the influence of these factors on it, are not fully understood so far. At the present, there exist two main hypotheses explaining the RBC aggregation process: the “Bridging” model and the “Depletion” model [2, 4, 5]. There is still no evidence that would fully confirm or completely refute any one of these models. There exist some suggestions that the RBC aggregation process can be described as a combination of both models [6]. The development of the RBC aggregation models is vital for clearer predicting the changes of the microrheologic properties of blood due to the alterations of concentration of plasma proteins, temperature, etc. The aggregation properties of blood change due to many socially significant diseases [2]. That is why the new

knowledge about RBC aggregation process can be used for the development of future clinical applications.

Dextran is synthetic macromolecules with molecular weights from 3 to 2000 kDa [7]. Dextran solutions are widely used both in clinical practice to adjust viscosity of blood, in the synthesis of drug-delivering nanoparticles [8], and in experimental studies of RBC aggregation [2, 9]. Thermodynamic parameters of polymer solutions (including dextran solutions) are strongly dependent on temperature [10]. Therefore, the temperature should inevitably influence the aggregation properties of RBC in these solutions. If we consider *in vivo* conditions, the temperature of the patient’s body can alter due to pathologies, and dextran injections can lead to slightly different effects under different temperatures accordingly. The influence of temperature on the processes of RBC aggregation and disaggregation in different solutions, e.g. in dextran solutions, has not been studied in detail so far. However, it is known that *in vitro* aggregation properties of RBC in autologous plasma are dependent on the temperature [3, 11].

The aim of this work is to estimate the influence of temperature on the RBC aggregation properties in

solutions of dextran of different molecular weights and concentrations *in vitro*.

2 Materials and Methods

The laser aggregometry technique was used to estimate the aggregation properties of RBC in dextran solutions at different temperatures [12, 13]. It is based on measuring the intensity of diffuse light scattering from a blood sample and is implemented in the slit-flow type system RheoScan (Rheomeditech, Seoul, Korea) [14]. In more detail, this technique and measured parameters are described in Refs. [3, 13, 15]. Briefly, RheoScan allows to measure kinetics of scattered light and to relate them to the RBC aggregation and disaggregation parameters. One of these parameters is critical shear stress (CSS) that characterizes the shear stress required to balance the process of aggregates formation and aggregates destruction, and therefore CSS is related to the hydrodynamic strength of the RBC aggregates. In this work, only the CSS parameter was measured.

Blood from one healthy individual was drawn using venipuncture technique and was stabilized by EDTA anticoagulant. RBCs were separated from the plasma using standard protocols, then they were suspended and washed in PBS 3 times at 600g for 3 min. Washed RBC were suspended in dextran solutions (PBS + dextran) at $hct = 0.4$. In this work, dextran macromolecules with different molecular weights (70, 150, 500 kDa) were used and suspended in PBS at various concentrations (10 mg/ml to 100 mg/ml). Prepared samples were incubated for 40 min at room temperature (22 °C). After that, the blood samples were placed for 5 min into the RheoScan measurement chamber that was preheated up to 30, 37, or 40 °C and then measurements were conducted. The temperature of the RheoScan measurement chamber was maintained. Each sample corresponds to one temperature. All experiments were carried out during 5 h after blood drawing. This time period on its own is not sufficient to cause any significant CSS changes.

3 Results and Discussion

Fig. 1 shows the CSS values measured for 3 different temperatures (30, 37, 40 °C) and for the dextrans of 3 different molecular weights (70, 150 and 500 kDa) and 4 concentrations (10, 30, 60 and 100 mg/ml). The bell shaped dependencies of CSS on dextran concentration are observed in all cases. The graphs shift horizontally at different temperatures. In particular, for dextran 150 kDa (Fig. 1b) the increase in temperature corresponds to shifting to the left, so that the maximum CSS is reached at lower concentration. In the case of dextran 70 kDa and dextran 500 kDa (Fig. 1a and Fig. 1c), the trends are different. The maximum CSS values for 37 and 40 °C are almost the same and differ from the one at 30 °C. Thus, the molecular weights influence the way how CSS changes under different temperatures. These results mean that there is a nonlinear dependence of CSS parameter on dextran

molecular weight, its concentration and the temperatures of the sample. Also, it means that there may be complex influence of combination of these 3 factors (dextran molecular weight, dextran concentration, and temperature) on the RBC aggregation properties.

Fig. 1 CSS at different temperatures and concentrations of dextran (a) 70, (b) 150 and (c) 500 kDa. Bars represent standard deviations. Each point is an average of at least 5 measurements. Dotted lines are parabolic approximations of experimental data. Colored arrows on horizontal scale correspond to the maximum CSS values.

Dextran 70 kDa is widely used in clinical practice, namely 6–7.5% dextran 70 kDa solutions are commonly used for injections to control blood viscosity [16]. Therefore, a more accurate observation of CSS changes was made for dextran 70 kDa. Results in Fig. 2 show CSS as a function of temperature for 50 mg/ml concentration of dextran 70 kDa. Interestingly, the minimum CSS values are observed at 30 °C. This also indicates that there is a complex dependence of CSS on temperature.

Fig. 2 CSS at different temperatures of dextran 70 kDa solution at concentration of 50 mg/ml. Bars represent standard deviations. Each point corresponds to the one measurement.

In future, it is planned to relate the obtained results with one of the aggregation models. The temperature dependencies of these models are not presented explicitly and need to be developed. The thermodynamic parameters of dextrans depend on

temperature, therefore the temperature influences the adsorption of dextran macromolecules on RBC membrane in the case of the “Bridging” model. Also, temperature influences the osmotic pressure in the case of the “Depletion” model. That is why these kinds of results could verify one of the RBC aggregation models in the future.

4 Conclusion

In this work, the aggregation of RBC was studied by assessing the critical shear stress (CSS) that characterizes the hydrodynamic strength of the aggregates. The results show a dependence of CSS in dextran solutions on the temperature changes *in vitro*. In particular, the bell shape dependence of CSS on dextran concentration changes under different temperatures and different molecular weights of dextrans. For example, the concentration of dextran 150 kDa that corresponds to the maximum CSS value is lower for 40 °C than for 30 °C. These observations mean that there is a non-linear effect of dextran concentration, dextran molecular weights and temperature on RBC aggregation properties. In future, the fundamental knowledge about RBC aggregation can be used in the development of new methods of patient treatment in order to control the viscosity of blood and rheological parameters.

Disclosures

All authors declare that there is no conflict of interests in this paper.

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