마이크로 및 마크로 수준의 적혈구 응집성 분석

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Peculiarities of red blood cells aggregation at micro- and macro- level

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Abstract : Red blood cells aggregation is one of the main processes determining the hydryodynamic resistance to blood flow in large vessels and the blood microcirculation. This process aroused basic and clinical interest over long time. However, the assessment of the cells interaction mechanism still remains a challenging problem, and many aspects of the process remains in veil. In the present study, we revisit the cells interaction issue by means of straigth-forward measurement of the cells interaction force at individual cell level using optical traps and comparing it with the results of conventional macro-scale measurements in whole blood samples using commercial devices.

1. Introduction

The red blood cells (RBC) aggregation is one of the main processes determining the blood microcirculation and is of crucial importance for homeostasis [1]. For this reason, this process was studied over several decades, for both basic and clinical interest. A number of different devices were implemented for *in vitro* measurement of RBC aggregation parameters, however the results are somewhat contradictory. [2]. While the cause of the discrepancy is poorly addressed, we speculate that the better understanding of cells interaction mechanism can bring an answer. Here, we revisit the RBCs interaction kinetics using optical tweezers (OT) - a novel technique that allows to freely manipulate and measure the interaction forces between the individual cells [3].

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2. Materials and methods

The detailed description of OT can be found elsewhere [3]. Briefly, the laser beam (1064 nm, 0-20 mW) is focused using high-numerical aperture water immersion objective (NA = 1.00, 100x) and forms an optical trap near the focal plane of the objective exerting the force proportional to the beam power. By manipulating individual RBCs in heavily diluted solutions (e.g. 0.01% RBCs in autologous plasma) we could measure the RBCs interaction forces. Details of the measurement procedure can be found in our previous paper [3].

3. Results and discussion

The results show that at the individual cells level the cells interaction forces raise with the supplementation of fibrinogen into plasma, as shown in Fig. 1 (filled circles). It is worthy mentioning that fibrinogen is generally regarded as the main protein affecting (inducing) RBC aggregation.

In the case of whole blood measurements, we borrow the data from Marton et al. [2]. As one can see in Fig 1 the dependence is changing over different measurement geometries (e.g. different commercial devices). By comparing the data with direct measurements obtained using OT, we can confirm that the results of measurements with microchannel-based aggregometers are the closest ones to the OT results. Alternative techniques have only slight or even negative dependence of RBC aggregation parameter on the concentration of fibrinogen. This result is in agreement with our previous study of temperature dependence of RBC aggregation. It revealed that both cone-plate and cup-bob systems have intrinsic artefacts [3].

4. Conclusion

Our study reveals that direct measurement of RBCs aggregation forces might serve as control measurement to solve the contradictions between the measurement techniques. Although individual cell measurements cannot be directly extrapolated to whole blood measurements, we speculate that similarity should be preserved. Moreover, considering that the most of parameters used to characterize the RBC aggregation are arbitrarily defined, it is important to connect the measurements to physical quantity - especially to avoid potential artifacts that we observed in the present work and in our previous study [3]. We empathize that among the parameters measured by commercially available measurement devices, only the critical shear stress, exhibits similar dependence, and seems to be very sensitive to changes of RBC aggregation compared to other available techniques.

5. Acknowledgements

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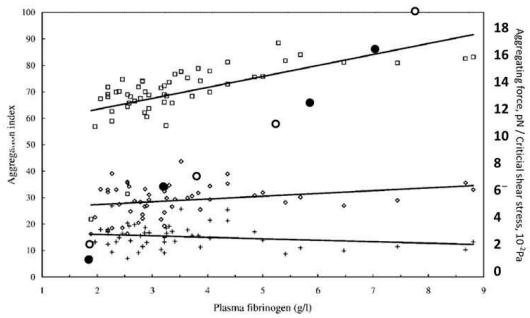

Fig. 1 The dependence of RBC aggregation parameters on concentration of fibrinogen in autologous plasma. Symbols corresponds to following measurement techniques: Circles - optical tweezers (aggregation force, pN, in-house developed) Squares - cup-bub aggregometer (aggregation index, dimensionless, LORCA), diamonds and plus signs - cone-plate aggregometer (aggregation indices M and M1, dimensionless, Myrenne), hollow circles - microchannel-based aggregometer (critical shear stress, mPa, RheoScan). For critical shear stress and aggregating force, refer axis on the right side.