S6: Advances in Hemorheological Measurements-2

S6-1 Optical study of red blood cells interactions in vitro mediated by different plasma components

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Red blood cell interaction resulting in their reversible spontaneous aggregation and shear induced disaggregation is one of the major processes that affect hemorheology and blood microcirculation. It commonly known to be dependent on the concentration of plasma proteins. The exact role of each plasma protein in RBC aggregation is not well understood so far. Sometimes the experiments conducted using different techniques with whole blood samples or RBC suspensions containing mixtures of different plasma proteins yield somewhat controversial results. We assumed that there might be an unaccounted synergetic effect of proteins (e.g. interference between proteins) on RBC aggregation. The aim of this work was to assess the kinetics of RBC interaction in-vitro in samples with varying plasma proteins and their concentrations by direct measurement of cells interaction forces using optical tweezers. We found that albumin in plasma changes its role from agonist to inhibitor of RBC aggregation with increasing the concentration of fibrinogen. When the concentration of fibrinogen is relatively high, an increase in albumin concentration does not increase the aggregation force but weakens the binding force between the RBCs. Furthermore, a model solution including five major aggregation-inducing proteins yields a weak aggregation force that can hardly be measured. These results indicate that there is an apparent interference among various plasma proteins involved in RBC aggregation and that the synergetic effect of plasma proteins determines the degree of RBC aggregation as well as the aggregation and disaggregation forces.

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S6-2 Effect of integrin glycoproteins inhibition on specific adsorption of cells adhesion macromolecules on red blood cell membrane: a microrheologic study

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Fibrinogen-induced red blood cells (RBC) aggregation was assumed to be caused by nonspecific binding of fibrinogen molecules to cell membranes and further leading to molecular bridging between interacting cells. In contrast, platelets are known to have membrane integrin IIb/IIIa glycoproteins highly specific to fibrinogen. In this work, we present the results of microrheologic study conducted

by means of laser aggregometer RheoScan AnD-300 (RheoMedTech, Korea) of the effect of integrin IIb/IIIa glycoproteins inhibition on fibrinogen adsorption on RBC membrane. We measured the hydrodynamic strength of RBC aggregates in the flow of whole blood suspension samples in terms of critical shear stress (CSS). CSS represents the minimal shear stress required to disperse RBCs aggregates in blood sample flow. We studied the effect of several commonly used inhibitors: RGDS, eptifibatide, tirofiban. After incubating RBC in buffer solution, containing fibrinogen (3 mg/ml) and any of these inhibitors, after resuspension in platelet poor plasma at 40% HCT, the CSS significantly decreased by 23±4% for RGDS in concentration 2.6 mg/ml, by 24±3% for eptifibatide (3.3 μg/ml), by 30±5% for tirofiban (4.8 μg/ml) in comparison with control. Similar results were obtained after resuspension in serum. All experiments were performed on the blood of healthy male donor using EDTA as anti-coagulant. We conclude that there is an inhibition effect which may serve as an evidence of the existence of fibrinogen specific binding sites with IIb/IIIa glycoprotein related structure on RBC membrane. The observed effect of CSS decrease was not strongly dose-dependent which points on more complicated molecular structure of such binding sites.

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S6-3 Electrochemical impedance spectroscopy of blood for blood aggregation, sedimentation, and hematocrit

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The study of blood hematocrit, erythrocyte aggregation and erythrocyte sedimentation rate (ESR) is very important both for basic research and medical applications. Electrochemical impedance spectroscopy (EIS) is a highly promising tool for the analysis of blood. The electrical properties of plasma and blood cells provide fundamental insights into the health status of patients.

A small chamber with two planar electrodes placed at the bottom was designed for sensitive detection of blood aggregation, sedimentation, hematocrit, and dielectric properties of plasma and erythrocytes. A method for correcting the polarization effect was proposed.. The changes in the blood impedance spectrum is measured at frequencies between 40 Hz and 110 MHz. A digital camera was used to verify the blood sedimentation curve and to determine the hematocrit profile.

Analytical and numerical models were developed for calculating the effective permittivity and conductivity of whole blood in the case of randomly distributed and aligned erythrocytes. An algorithm was proposed to extract the electrical properties of erythrocyte cytoplasm and membranes from the impedance spectrum. Various models of erythrocyte shapes such as spherical, disk-shaped, spheroidal, and biconcave shapes were investigated.

The EIS of blood samples reveals β- and δ-dispersions. It was found that the electrical properties of membranes have a significant influence on the blood impedance at frequencies between 100 kHz and 10 MHz (β-dispersion), while the cytoplasm has an effect at frequencies between 10 MHz and 1 GHz (δ-dispersion). Based on the proposed model, the ESR, blood sedimentation curve and hematocrit profiles can be numerically restored using only the first 400 seconds of the recorded changes in blood conductivity.

S6-4 Comparison of critical shear stress in RheoScan and adhesion force between RBCs measured in optical tweezer

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The aggregation of red blood cells (RBC) is a reversible dynamic phenomenon that has a strong effect on blood microcirculation. For past decades, RBC aggregation had been measured with various devices and methods but the results were quantified in a relative values such as AI and M, which are relative or arbitrary units. Our previous study introduced a critical shear stress measured by RheoScan, which is defined as a minimum stress to disaggregate RBCs and is known as hematocrit-independent index. Another research with optical tweezers confirmed that the CSS is the minimum shear stress to aggregate between RBCs. Therefore, the CSS was proved to be an index to represent RBC aggregation having an absolute dimensional unit such as stress. According to clinical data, CSS for healthy people yield 150 ~ 300 mPa and that for cardiovascular patients yield higher than 350 mPa. Therefore, it is strongly required to accumulate clinical data for further application of CSS as a diagnostic index of hemorheology.