

S12: Macro and micro hemorheology in vitro and in vivo

S12-1 The “tipping point” of mechanical stress on erythrocyte biology

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Mechanical stresses are important stimuli for many biological processes. Such stresses especially impact blood: its classic shear-thinning viscosity profile results, in part, from disaggregation of red blood cells (RBC), and cellular deformation, that are each induced by mechanical forces. While it is thus well-known that shear is important for governing blood rheological processes, it is becoming increasingly recognised that other active processes – e.g., ion flux, free radical production – are sensitive to shear. Nitric oxide, for example, is produced in RBC in response to shear, with resultant augmentation of cell deformability over that induced by external forces.

Increased attention is now being directed to exploring the interactions between the level and duration of shear exposure on RBC physiology. In general, it appears that the physiological shear stress range (0~10 Pa) induces beneficial responses in RBC. Once shear exposure is suprphysiological there appears to be three primary domains that characterise blood responses: i. suprphysiological, but below the “sub-haemolytic threshold” tends to have no obvious permanent and detrimental impact on RBC function; ii. above the sub-haemolytic threshold, but less than the “haemolytic threshold” tends to result in impaired RBC deformability, and alterations to cell biochemistry, and; iii. above the haemolytic threshold, which induces RBC rupture. Accumulating evidence indicates that shear exposure within the second domain of suprphysiological shear exposure induces RBC dysfunction that likely contributes to widespread impairments in the microcirculation. Understanding these effects are increasingly important given increased dependence on mechanical circulatory support that operate above the sub-haemolytic threshold.

S12-2 Testing the sensitivity of red cell fragmentation and deformability measurements for shear-mediated mechanical damage

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Under physiological conditions red blood cells (RBC) can withstand to shear stresses (SS) up to 150 Pa, without being hemolysis, and have the ability to fully recover back to its original shape. However, alterations in membrane stability may significantly reduce this hemolytic threshold and decreased stability can lead to cell fragmentation. In this study; we focused on: a) evaluating the distribution and the magnitude of the force applied in the ektacytometer via computational fluid dynamics (CFD), b) how suprphysiological SS affects RBC fragmentation and deformability parameters in two damage models (oxidative damage and

metabolic depletion). Subsequently, various levels of shear stress (0-100 Pa) were applied to the RBC in a Couette-type shearing system for 300 seconds. RBC deformability was measured immediately following shear stress exposure via ektacytometer. RBC fragmentation was determined using a cell counter system (Beckman Coulter, USA). In each sample, the number of cells in the range of 20-60 fL was divided by the total number of cells and multiplied by 100 to determine the percentage of fragmented cell. The CFD simulations showed that 29% volume percent of the suspension undergoes non-uniform shear stress at the bottom part of the ektacytometer. While the percentage of fragmented cells increased at all shear stresses applied in the group of oxidative damage, it was elevated at 80-100 Pa shear stress in the metabolic depletion group. RBC deformability changes were somewhat similar to the fragmentation rates in these groups. In summary, the subhemolytic threshold and fragmentation measurements are useful tools for close monitoring of shear-mediated mechanical damage and sufficiently sensitive to the damage models employed in the present study.

S12-3 Discussion about high shear stress induced erythrocyte's damage and lysis - Interpretation of hemolysis in cardiovascular devices based on our visualized erythrocytes' behaviors -

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It is well known that high shear stress would be the main cause of hemolysis in cardiovascular device, however, exact erythrocyte's damage process leading to its lysis is still in big discussion. In order to elucidate such high shear stress induced hemolysis, we have successfully prototyped the microscopic observation test setup which incorporating the counter-rotational flow field mechanism between top cone and bottom plate through referring previous great test setup by Dr. Fischer at al. Our originality is its availability to generate extremely high shear stress over 200Pa. Erythrocytes had been diluted in the high viscous polyvinylpyrrolidone phosphate buffered saline solution. And then such suspension fluid was exposed to extremely high shear stress using our shear device. What we saw through such flow visualization tests are followings; even though all the intact erythrocytes showed excellent ellipsoidal shapes at the beginning, as time prolonged a part of them started to cyclically waving their shape and further transformed into abnormal dissymmetric shape. Furthermore, they tumbled and fragmented. Additionally, the cell destruction through the cell-cell collision was also observed. Taking these aspects into our consideration, we speculate that high shear stress had induced erythrocytes their localized membrane damage and less deformability, then it has resulted in abnormal asymmetric their shape and occurrence of tumbling behavior. Under such abnormal cellular behavior, the Couette flow regulation became into quite difficult then it induced disturbance in extra-cellular flow field. Under such condition, cell-cell collision maybe more easily happens and then hemolysis can be further accelerated. Similar phenomenon discussed here would happen within clinical cardiovascular devices.

S12-4 Mechanical sensitivity of blood in sickle patients on chronic blood transfusion – understanding erythrocyte exposure to chronic physiologic shear vs. chronic supra-physiologic but sub-hemolytic shear stress

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Background: Sickle cell disease (SCD) is a hemoglobinopathy where deoxygenated hemoglobin S polymerizes, leading to rigid red blood cells (RBC), and thus increased sensitivity to mechanical stress. Survival is improving in SCD but chronic cardiovascular disease is increasing, potentially necessitating procedures requiring cardiopulmonary bypass. Transfusion therapy acts as primary and secondary prevention of ischemic stroke in SCD. Whether blood transfusion alters the mechanical sensitivity of RBC to sub-hemolytic shears is unknown.

Aims: We hypothesized individuals with SCD undergoing chronic blood transfusion would have improved sensitivity to shear, compared with patients not on transfusion therapy.

Methods: RBC from individuals with SCD not receiving and receiving chronic simple-transfusion were conditioned to shear for various duration. Deformability of RBC was immediately measured after each. Comparisons were made with healthy controls. A surface-mesh was interpolated to determine the effect of blood transfusion on RBC mechanical sensitivity.

Results: Impaired mechanical sensitivity to prolonged supra-physiologic shear was observed in SCD RBC compared to control. Longer shear exposure over the supra-physiologic shear range was required to impair RBC in the transfused group compared with non-transfused. When exposed to prolonged shear stress in the physiologic range, mechanical sensitivity improved in the transfused group. There was no improvement in the non-transfused group.

Conclusion: Simple transfusion may be an effective method to improve the mechanical sensitivity of RBC in SCD. Given SCD is listed on the current exclusion criteria for popular ventricular assist devices, these findings may affect strategies for cardiovascular therapies; surgical and otherwise.

S12-5 Drag-reducing polymer effects on macro- and microcirculation

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Drag-reducing polymers (DRPs) are long-chain soluble polymers with fairly linear molecular structure have been discovered to significantly reduce flow resistance in a developed turbulent flow in pipes, thus increasing flow rate at a constant pressure gradient or decreasing pressure at a constant flow rate when added to the flowing fluid at minute concentrations (Toms effect). The flow conditions associated with the Toms effect do not occur in blood circulation through vascular system, however, many studies have shown that IV or IP administration of DRPs to experimental animals produce significant beneficial effects on blood circulation increasing tissue perfusion, tissue oxygenation and other hemodynamic phenomena. This paper will review major results obtained over the last decade in vivo in various animal models and in vitro experiments which employed DRPs as blood additives. Furthermore, the potential mechanisms behind the DRP phenomena in blood circulation including their effects on the traffic of RBCs and other blood cells will be discussed.