전단유동하에서의 적혈구 변형에 관한 연구

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Red blood cell deformation under shear flow

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Introduction

Normal human red blood cells (RBCs) in quiescent plasma assume a biconcave disk shape with an average diameter of 8㎛, thickness of 2㎛. RBCs classify into the category of suspended particles known as capsules: capsules are particles with elastic membranes that enclose a fluid, as opposed to droplets and bubbles whose interfacial forces arise from surface tension. The membrane of the RBC is easily deformed by shearing forces while maintaining its local area nearly unchanged[1,2,3]. The average deformation of a suspension of red blood cells was most recently studied[4,5]. The RBC membrane can be ruptured by the extremely high shear rate-they cannot sustain their shape at very high deformation. The extreme shear-induced hemolysis is referred as the crucial defect of artificial heart. In this study, we investigated the history of RBC deformation and confirmed the shear-induced hemolysis in polymer suspended medium by scattering patterns, thereby related RBC deformability and shear-induce hemolysis.

Experiment

Sample preparation Human blood was anti coagulated with K3-EDTA. For measurement of RBC deformability, either 10, 20, 100㎕ of blood was diluted in 5㎖ of a solution of 0.14mM poly(vinyprrolidone) (PVP, Mw=360,000, Sigma-Aldrich) in phospate buffered saline (PBS, pH 7.4 made by Sigma Digonostics Kits) or a solution of 35% (weigh%) Dextran 40 (Mw-40,210, Sigma-Aldrich) in PBS.

Instrument Ektacytometry is a technique that gives a quantitative measure for the average RBC deformability. In ektacytometry a laser beam traverses a suspension of blood cells between two concentric cylinders and generates a diffraction pattern on a small projection screen. In this study, *LORCA*™ (ektacytometry, made by Mechatronics) was used to measure the RBC deformability. The RBC shapes are observed by Optical Microscope (BX 51, Olympus) and the shear is controlled by

CSS 450 which is mounted on the Microscope. The images are captured by high shutter speed video camera (TK-C1380, JVC). The rheological properties of Dextran and PVP are measured by rheometer (C-VOR, Bohlin Instrument). Each medium polymer solution shows Newtonian behavior in the shear rate range investigated in this study.

Results and Discussion

Microscope Observation Normal human red blood cell formed aggregates that are called rouleaux[6]. To avoid the aggregation phenomena, we suspended the red blood cells to Dextran 40 and PVP 360 polymer solution which is soluted by PBS. As the viscosity of polymer medium is higher than RBC intrinsic viscosity, the RBC membranes can be deformed in shear flow.

The behavior of red blood cells in shear flow was observed from low shear rate to high shear rate. At low shear rates the cells tumble, but the red blood cells reach at equilibrium state as the shear rate increases. Fig 1.(a) is the image of RBC before imposing the shear flow. It shows the normal RBCs biconcave shapes and non-aggregate shapes in Dextran solution medium. On imposing the high shear, the cells form elongated shape to the high shear flow. Fig 1.(b) shows the elongated RBC. As increasing the shear rate, the degree of elongation increases and saturates at the critical shear rate.

After stoping the shear flow, we could observe relatively tiny cells (Fig 1.(c)) They are assumed to be ghost cells whose internal fluid is the same as the suspending fluid plasma[7]. The ruptured RBCs release hemoglobin and internal substances. And they are filled with polymer medium, resealed instantly and finally make the ghost cells. The ghost cells are less deformable due to small viscosity difference to medium. They are evidences of shear-induced hemolysis.

Ektacytometry- Back light scattering method When deformed under shear, RBCs change gradually from the biconcave towards a prolate ellipsoid morphology and orient themselves along the flow direction in the gap. This is accompanied by transition from a circular into an elliptic diffraction pattern which is oriented perpendicular to the orientation of the elongated cells. The Elongation Index (EI) captured in scattering, is used as a measure of RBC deformability. It is defined as (A-B)/(A+B), where A and B are the vertical and horizontal axes of ellipse, respectively. Normally, the elongation index is linear to elongation index[8]. Firstly, we measure the normal RBC deformability(EI), and impose the extremely high shear rate (10, 15, 20 rps) to RBC suspended in PVP 360 to make the shear-induced hemolysis. At the end of experiment, we measure the hemolyzed RBCs deformability.

Increasing either shear rate or imposed time, the value of saturated EI decrease. (Fig. 2 (a),(b)). It mean the cells temporary align to the flow obstructive direction due to cell rigidity. The scattering intensities of hemolysis cells are lighter than normal ones in that experiments. Also high medium viscosity is effective to induce hemolysis. (Fig. 2(c))

Conclusions

From microscope, we observed RBCs tumbling and elongation. Also, we confirm that RBC hemolysis is the function of shear rate, medium viscosity and imposed-time in polymer suspended medium. The scattering intensities and patterns changes reflect the ghost cells made by shear-induced hemolysis

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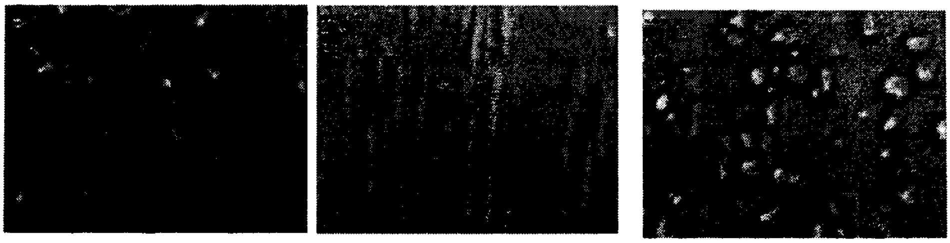
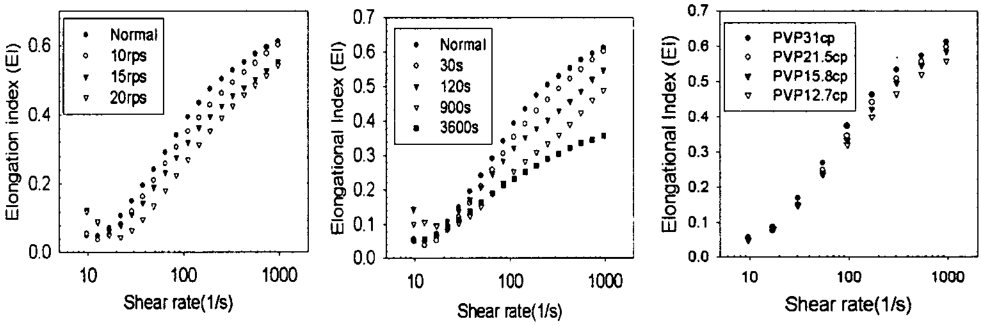


(a) (b) (c)

Fig 1. (a) RBC in Dextran 40 medium before imposing the shear flow
(b) RBC in Dextran 40 medium in the high shear rate flow
(c) RBC in Dextran 40 medium after imposing the shear flow



(a) (b) (c)

Fig 2. (a) Imposed-shear dependence of hemolysis(shear-imposed time: 30sec)
(b) Imposed-time dependence of hemolysis
(c) Medium viscosity dependence of hemolysis