INTRODUCTION

The Red Blood Cell (RBC) is of biconcave shape in the static state, but easily deforms and elongates under flow due to some physiological reasons, those are to facilitate oxygen transfer and to reduce flow resistance. This characteristic of RBC, called deformability, has been exploited as one of the clinical diagnosis methods for specific diseases such as cancer, malaria and so on [1].

When RBC flows in vivo, it experiences stress both from shear and extensional flows. The flow in the blood vessel is not slow except in some capillaries, and the RBC deforms to some extent. However, when RBC flows in vitro as in the bypass to the extracorporeal cardiovascular devices, it experiences significant deformation, which sometimes leads to a mechanical hemolysis that is not observed in vivo. This may be related with the rigidity of blood vessel, and it may cause a serious trouble in a human body. So it is important to understand the mechanism of mechanical hemolysis and to reduce such an undesirable phenomenon, which is especially true when the extracorporeal cardiovascular devices such as artificial heart are designed and exploited. In this regard, for practical use of artificial organs, the quantitative correlation of shear stress and mechanical hemolysis has been studied intensively [2,3].

In this study, we control the shear flow and investigate the deformation of RBC cells, and the correlation between the applied shear stress, RBC damage and mechanical hemolysis. The effect of pre-conditioning on RBC deformability has also been investigated.

METHODS

A commercial instrument (LORCA™) which is based on the back light scattering technique and an optical microscope (Olympus BX51) with a shearing system (Linkam CSS 450) have been used to measure the RBC deformability. The deformed RBC shapes were investigated in diffraction patterns and with real images under the well-defined flow conditions.

To prevent coagulation, the RBC was put into the K3EDTA tube. The RBC was dispersed to the solution made by phosphate buffered saline (PBS) and Poly (vinypyrrolidone) to increase the viscosity of the medium, which transfers more stress to the cell and induce more cell deformation. The sequence of experiment is as following: (a) measure the deformability of normal RBC (b) impose controlled shear stress to the RBC dispersed in the polymer solutions during the designated time (c ) measure the deformability of pre-conditioned RBC.

RESULTS AND DISCUSSION

Elongational Index (EI) is a dimensionless number to quantity the RBC elongation. EI is defined as the ratio of length difference between long and short axis to the sum of axis lengths. EI increases fast at low shear stress and saturates to a limiting value at high shear stress region (Figure.1). This phenomenon comes from the elasticity of RBC membrane and the RBC’s character that tends to sustain the cell volume. After imposing the mechanical shear stress (step b), the deformability of damaged cells decreases according with the imposed stress intensity. The difference of EI between normal RBC and the

![Figure 1. RBC Deformability change decrease by shear stress (56.4Pa) during the designated time](image-url)
pre-conditioned cell reflects the shear stress effect as well as the degree of RBC damage and hemolysis. As stress magnitude increases, the shear stress effect increases too. As the imposed time increases, similar behavior is observed (Figure 1). And the suspended polymer viscosity also affects on the RBC damage. It was found that the relative viscosity difference between the internal cell medium and the suspended polymer solution is an important factor to cell damage.

The effect of shear and the threshold of cell membrane damage is shown in (Figure 2.). In the low shear stress region (below 30Pa of pre-conditioned stress), the shear effect is not significant. But in the higher stress than 30Pa, the degree of RBC damage increases rapidly. This behavior is similar to the mechanical hemolysis. It is reported that the mechanical hemolysis has the shear stress threshold at 150Pa-400Pa [2,3,4].

The stress threshold of 30Pa is quite small compared to the previous results [2,3,4]. It may be that the RBC membrane which sustains the elongated shape is partially damaged. It allows the mass transfer across the membrane and increases the intra cell viscosity, which leads to the decrease of cell deformability. The result that high shear stress with long imposed-time makes RBC less deformable, could support this explanation. Also a few less deformable RBC could lysis at lower stress level below 150Pa. The diffraction pattern intensity decreases after imposing the pre-condition, and the mixed pattern was observed at 30Pa (experiment sequence c). When we investigated the RBC shape by optical microscope, some tiny fragmented cells were observed below 150Pa.

CONCLUSIONS

In this study, we have investigated the cell damage and hemolysis by measuring the RBC deformability on controlling the shear stress. Using the diffraction technique as well as direct microscopy, the deformability was found to decrease with the degree of RBC damage. And the RBC membrane damage threshold was suggested by illustrating pre-conditioned shear stresses. Also we found that the less deformable RBC could be damaged and hemolyzed at shear stress level much lower than that reported as a hemolysis threshold. This result is meaningful in that it suggests the governing factors – shear imposed time, magnitude, relative viscosity and RBC damage threshold - for designing and controlling the artificial organs.

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