SIMULTANEOUS PROBING OF SINGLE ERYTHROCYTE BIOCHEMCAL AND MECHANICAL PROPERTIES FOR EFFICIENT BLOOD TRANSFUSION

Sha Huang[1], Han Wei Hou[1], Jongyoon Han[1,2]

[1]Department of Electrical Engineering and Computer Science, [2]Department of Biological Engineering
Massachusetts Institute of Technology, Cambridge, MA, USA

ABSTRACT

The age of stored blood plays a critical risk factor in blood transfusion [1, 2]. The strong connection between RBC clearance and the age of stored blood leads us to believe that age-dependent biochemical and mechanical changes play a key role in RBC clearance after transfusion. In this project, we are interested in finding out if these changes are more specific to a subpopulation of stored blood. Furthermore, a label-free RBC deformability based sorting [3] is performed to pre-filter less deformable RBCs. It is hoped that this pre-filtration prior to blood transfusion can potentially reduce *in vivo* RBC clearance of transfused blood, and other side effects of blood transfusion.

KEYWORDS: Red Blood Cells, Deformability, Blood Storage

INTRODUCTION

The maximal shelf life for stored red blood cells (RBCs) is 42 days and the age of RBC storage indeed plays a critical risk factor in blood transfusion [1, 2]. Recipients of 30-42 day-old stored RBCs exposed to 5% excess mortality compared to those receive 10-19 day-old RBCs [4]. Within the first hour after blood transfusion is when most RBCs get cleared, releasing toxic iron and inducing inflammation [5]. Rapid clearance of transfused RBC is believed to account for the added risk of blood transfusion.

Storage age dependent changes on RBC biochemical and mechanical properties are believed to play a key role in RBC clearance after transfusion. Studies show that RBC deformability decreases with blood storage time, accompanied with a rise in the intracellular calcium level [6]. We are therefore interested if these changes are more specific to a subpopulation of stored blood or are simply general shifts within a uniform population. Furthermore, we are interested to see if a label-free RBC deformability based sorting [3] can be performed prior to blood transfusion to reduce *in vivo* RBC clearance.

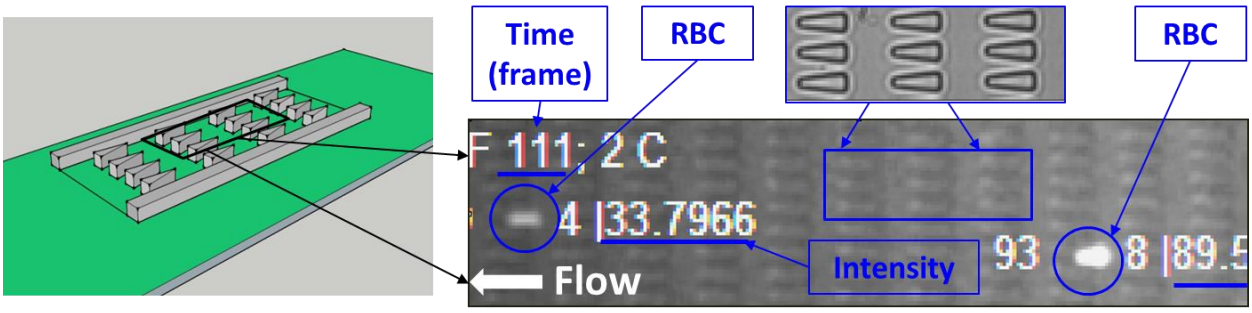

Figure 1: Device schematics (left). The velocity and intracellular calcium intensity are captured simultaneously when single red blood cells deform and transit through the narrow pillar array structures.

THEORY

In this work, single RBC deformability and intracellular calcium concentration ($[Ca^{++}]_i$) were simultaneously measured using a microfluidic device (Figure 1). The deformability of RBCs is quantified by measuring their transit velocity through narrow gaps, that higher velocity corresponds to high deformability [7]; and $[Ca^{++}]_i$ is measured based on the fluorescence intensity, that higher $[Ca^{++}]_i$ is reflected as higher intensity read out. A margination device was then used to sort the stored RBCs based on deformability [3] (Figure 5, left). Less deformable cells are marginated to the side outlet, enriching a more deformable population in the center outlet.

EXPERIMENTAL

Fluo-4 Calcium assay kit was used to measure RBC $[Ca^{++}]_i$. To test the performance of our microfluidic device, RBCs were first treated with Calcium ionorphore A23187 (company name), and then exposed to PBS buffer solutions containing different Calcium concentrations (Figure 2, left). The sensitivity of our microfluidic-based fluorescence probe was compared and calibrated with standard fluorescence activated cell sorting (FACS) machine with good agreement (Figure 2, left). Simultaneous measurement on $[Ca^{++}]_i$ and RBC deformability was performed, and the scatter plot was presented in Figure 2 (right). In general, increased $[Ca^{++}]_i$ correlates with reduced cell deformability.

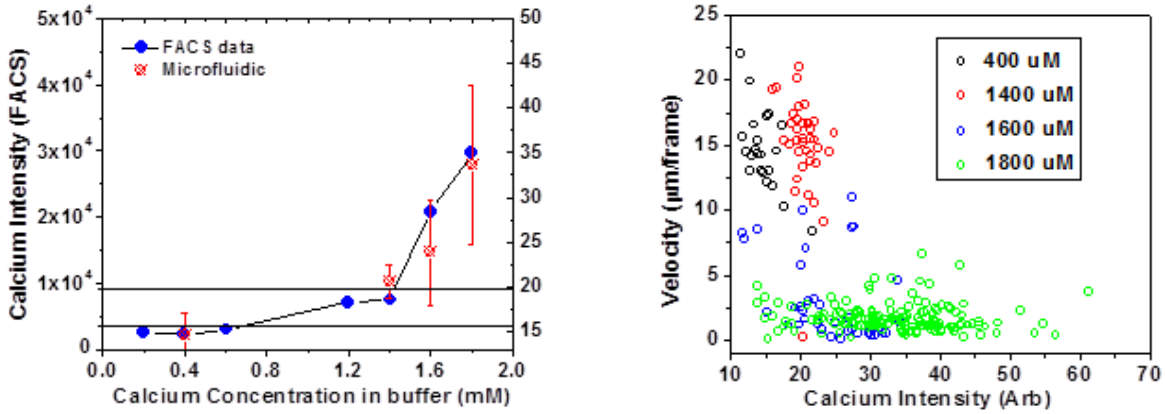

Figure 2: Microfluidic based calcium fluorescence measurements are calibrated against standard FACS result (left). Intracellular calcium level is regulated by A23187 ionorphore treatment. The velocity (i.e. deformability) of single RBCs is correlated with their intracellular calcium level (right).

RESULTS AND DISCUSSION

Changes in RBC deformability and $[Ca^{++}]_i$ were simultaneously traced over a period of 22 days. An overall increase in $[Ca^{++}]_i$ and drop in deformability was observed over storage time. Interestingly, a bimodal distribution in both $[Ca^{++}]_i$ (Figure 3, left) and deformability (Figure 4) became evident from day 13. These biochemical and mechanical changes were accompanied with substantial changes in RBC morphology, that more echinocyte and spherocyte formation was observed in aged blood (Figure 3, right). This observation is consistent with previous study showing that both echinocytes and spherocytes are significantly stiffer as compared to normal discocytes [8].

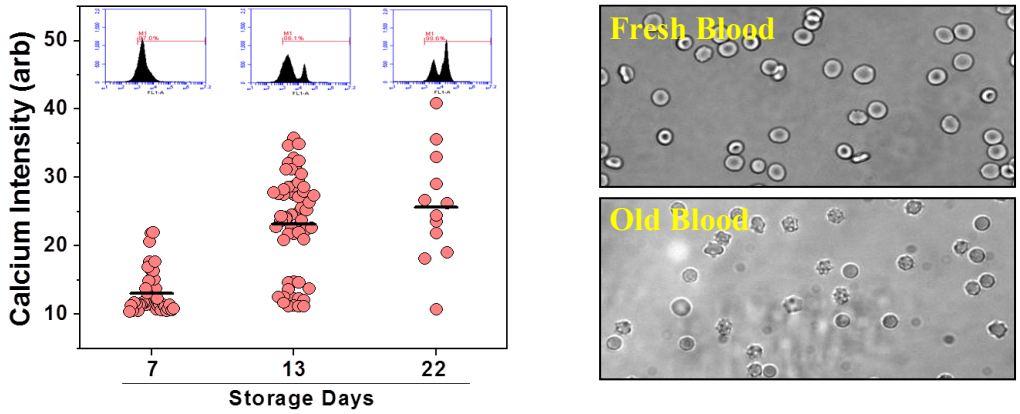

Figure 3: Changes in intracellular Calcium levels are monitored for up to 22 days with 4°C stored RBC concentrates. (Left). More echinocytes and spherocytes are observed in old blood (30-40 storage days) in comparison to fresh blood (less than 1 week storage).

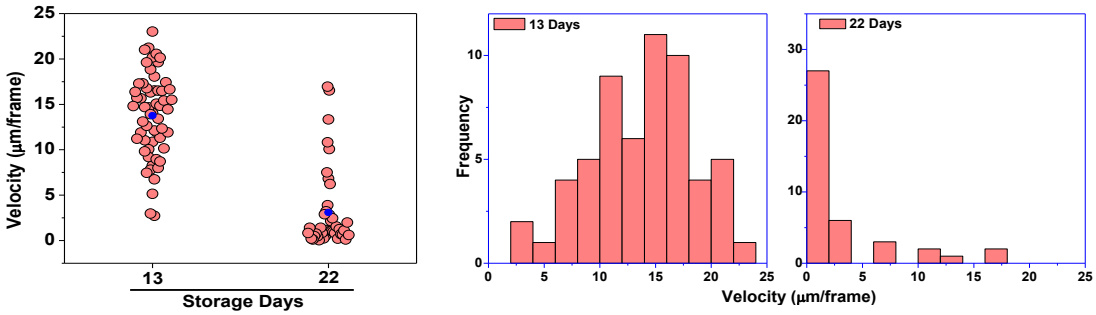

Figure 4: Changes in RBC deformability are monitored for up to 22 days with 4°C stored RBC concentrates. Significant decrease in RBC microcirculatory velocity is observed.

Human spleen, known a mechanical filter, removes less deformable RBCs [9]. We therefore speculate that the significant drop in RBC deformability could be an important biomechanical attribute accounting for the increased RBC splenic clearance, as evidenced by the increase spleen in weight shortly after blood transfusion [1]. We hypothesized that

a pre-filtration that removes "less deformable" RBCs prior to blood transfusion could significantly reduce blood age related risk in transfusion.

To enrich the subpopulation of "deformable RBCs", a margination based sorting device was used to remove less deformable RBCs to the side outlet. Enriched deformable RBCs can then be collected in the center outlet (Figure 5). Downstream deformability measurement confirmed that RBCs collected from side outlets are significantly stiffer.

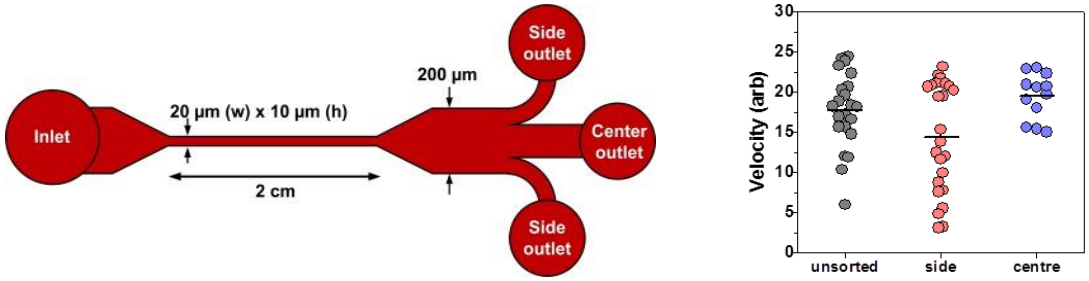

Figure 5: RBCs are passed through a margination based sorting device [3] that less deformable RBCs go to "Side" outlet (left). Sorted RBCs from center outlet are more deformable (right).

CONCLUSION

In this project we found that during blood storage, instead of a population-wide uniform shift in RBC biochemical and mechanical properties, subpopulations of RBCs with elevated $[Ca^{++}]_i$ and/or reduced cell deformability emerge. This could be related to RBC morphological transition from discocytes to echinocytes and spherocytes during natural ageing process. We have demonstrated that enrichment of the more deformable RBC subpopulations can be achieved using a microfluidic based sorting device, which is capable of process large amount of blood at high flow rate [3]. It is hoped that RBC deformability based pre-filtration could reduce blood age related transfusion risks and lead to a better clinical outcome.

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CONTACT

*J.Han, tel: +1-617-253-2290; jyhan@mit.edu