Hypoxic Storage of Red Blood Cells: Assessment of Adhesion Properties Using a Standardized Endothelium-on-a-chip Microfluidic Platform

#1072

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INTRODUCTION

- Blood transfusions are routine medical procedures in which stored blood or blood products (i.e., red blood cells, RBCs) are given to the patient to prevent adverse health outcomes due to acute or chronic anemia.
- The current regulations require RBCs to be stored at 4 ºC not more than 42 days before a transfusion. However, RBCs undergo extensive rheological changes during storage and may contribute to complications associated with transfusion.
- Hemanext has recently introduced an innovative storage system to ameliorate such storage lesions, in which RBCs are stored in a hypoxic environment, and thus they are exposed to much lower oxidative stress during storage.
- To this end, we report the changes in adhesion properties of stored RBCs to human endothelial cells following a 42-day storage in normoxia vs hypoxia, using a standardized endothelialized microfluidic platform: **Endothelium-on-a-chip** (**Fig. 1**) [1].

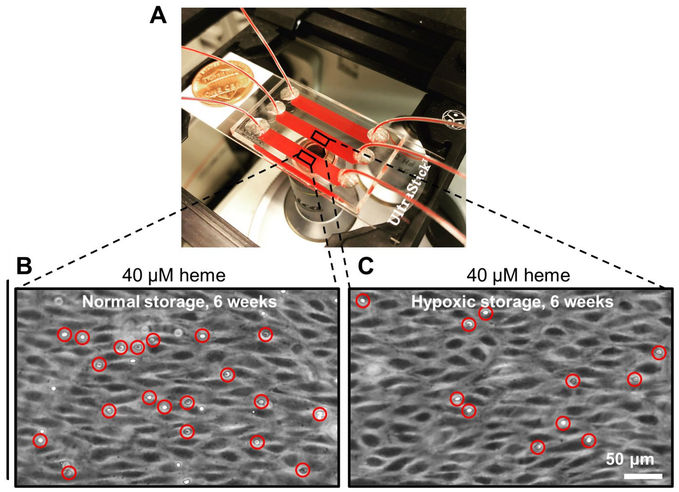

Figure 1. Adhesion assessment of stored erythrocytes to heme-activated HUVECs under physiologic flow. (**A)** A representative image of the Endothelium-on-a-chip microfluidic platform. **(B)** Representative images of adherent erythrocytes that were stored for 6 weeks either in conventional normoxia **(A)** or hypoxia **(B)** to 40 µM heme-activated HUVECs. Each circle indicates an adherent erythrocyte.

METHODS

- Microfluidic channels consisted of a bottom glass slide, a polymethyl-metacrylate (PMMA) top cover, and a laser-micromachined double sided adhesive (DSA) that was sandwiched between the top and bottom layers to create the flow domain **(Fig. 1A).** The dimensions of the microfluidic channels were (width x height x length): 4 mm x 0.05 mm x 24 mm.
- Microfluidic channels were first functionalized with fibronectin and then seeded with human umbilical vein endothelial cells (HUVECs) followed by a static-culture for 2 hours at 37ºC and 5% CO_2. The seeding density were aimed to be around 8x10^6 cells/mL for each endothelialization process.
- The endothelialized microchannels were then cultured under flow (~15 dyne/cm^2) for 48-hours at 37ºC and 5% CO_2 prior to adhesion experiments.
- **Storage of RBCs:** Two units of 1-day old blood type O positive leukocyte reduced RBCs in additive solution were pooled and divided into equal aliquots (300mL each) A and B. Unit A was stored under conventional normoxic storage condition at 4°C for 42 days. Unit B was deoxygenated so that the percent oxygen saturation of the hemoglobin was less than 20%. The deoxygenated RBCs were stored in an oxygen impermeable storage bag for 42 days at 4°C. Adhesion levels were tested at the beginning (baseline, Week 1) and the end of the storage period (Week 6).
- **Activation of endothelial cells:** Following a 48-hours culture under flow, cells were removed from the flow line and treated with 40 µM of heme for 4 hours. The heme solution was prepared in FBS-free basal culture medium (EBM-2, Lonza) to eliminate the effect of heme-deactivating molecules in serum. The microfluidic channels were rinsed with heme-free EBM-2 prior to the adhesion experiments.
- **Adhesion experiments:** RBCs were removed from the storage bags and centrifuged at 500xg for 5 minutes to remove the additive solution. Isolated RBCs were then rinsed with PBS twice and resuspended in basal culture medium (EBM-2) supplemented with 10 mM of HEPES at a hematocrit of 40%. A 15 µl of RBC solution was then injected over heme-activated HUVECs at a shear stress of 1 dyne/cm2 followed by a 10-minute rinse with fresh basal culture medium to remove non-adherent RBCs. At the end of the experiment, adherent erythrocytes were manually quantified. Control experiments were conducted with non-activated HUVECs.

RESULTS AND DISCUSSION

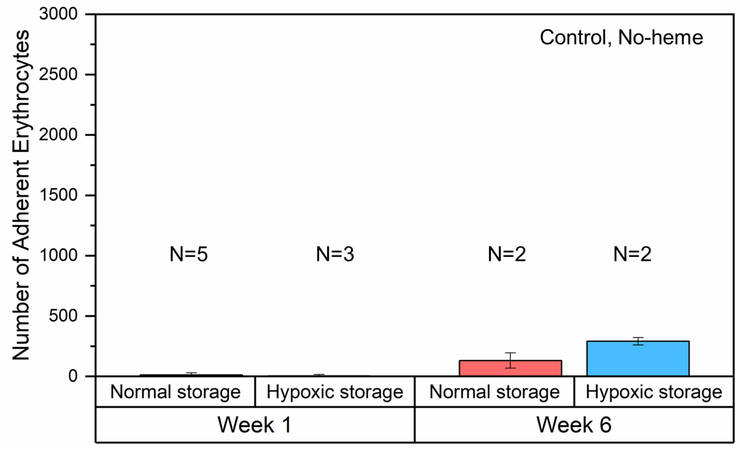

Figure 2. Stored erythrocyte adhesion to quiescent (non-activated) HUVECs. Erythrocyte adhesion increases by storage time at week 6 while adhesion levels at week 1 are negligible. No difference was observed in terms of RBC storage conditions. Error bars=standard error of the mean.

- The baseline adhesion levels (Week 1) to non-activated HUVECs (control) were negligible (**Fig. 2**, normoxia: 13±6; hypoxia: 18±10, p>0.05) while both cell populations had higher adhesion levels to non-activated HUVECs at Week 6 (**Fig. 2**, normoxia: 132±64; hypoxia: 292±31). While the p-values were greater than 0.05 when the Week 1 and Week 6 results were compared for both normal and hypoxic storage conditions, we expect to see a statistically significant difference as the sample size increases.
- Following a 6-week storage, adhesion of erythrocytes stored in normoxia to heme-activated HUVECs was higher compared to those stored in hypoxic conditions while the p-value was borderline **(Fig. 3**, 1333±407 vs 544±149, p=0.09).
- Overall, erythrocyte adhesion to heme-activated HUVECs was significantly greater compared to non-activated HUVECs, combining the Week 1 and Week 6 data (heme: 681±150 vs non-activated:81±33).
- Stored erythrocyte adhesion to heme-activated HUVECs at Week 6 vary from sample to sample while 4 out of 5 samples that were tested displayed lower adhesion when stored in hypoxia **(Fig. 4).**
- These results collectively suggest that storage-mediated erythrocyte adhesion to heme-activated HUVECs may be ameliorated by the novel hypoxic-storage condition.
- This study showed a decrease in the adhesion of hypoxic erythrocytes to heme-activated HUVECs when compared to conventionally stored erythrocytes for transfusion. Based on our findings, we postulate that hypoxic erythrocytes may reduce the risk of developing vaso-occlusion (VOC) after transfusion in patients such as in sickle cell disease where adhesion to heme-activated HUVECs has been implicated in the pathogenesis of VOC [1].

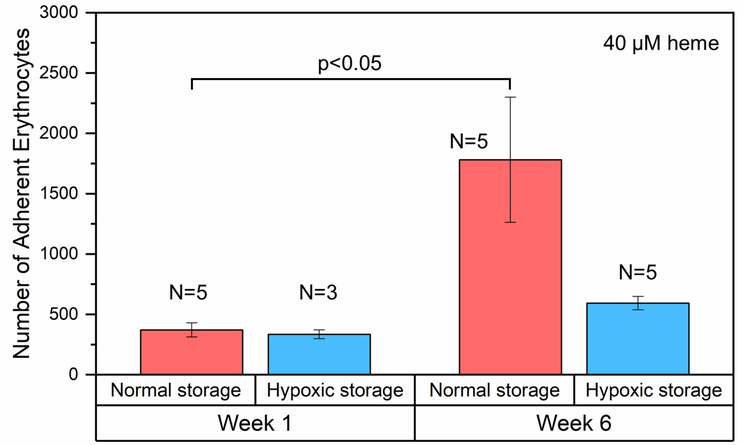

Figure 3. Stored erythrocyte adhesion to 40 µM heme-activated HUVECs. Erythrocytes stored in normoxia had greater adhesion to heme-activated HUVECs in comparison with those stored in hypoxia. P values were based on non-parametric Mann-Whitney test. Error bars=standard error of the mean.

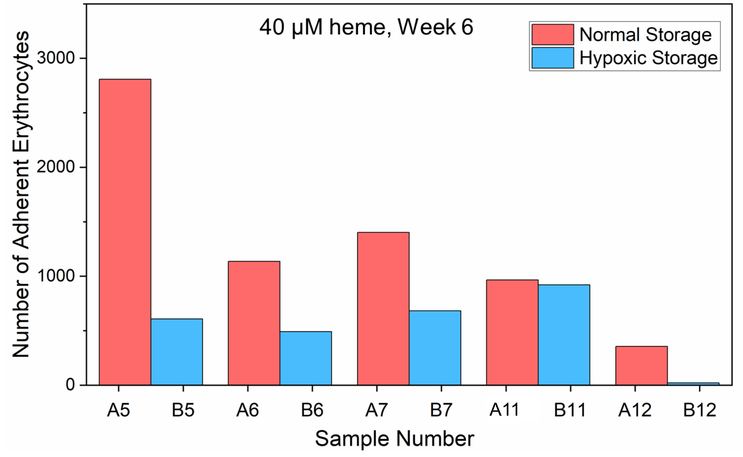

Figure 4. Stored erythrocyte adhesion to 40 µM heme-activated HUVECs at Week 6. Total adhesion levels vary by different samples. Letters A and B denote normal and hypoxic storage conditions, respectively. Each number represents a unique sample donor.

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