Rapid Altitude Acclimatization Through Hemoglobin Loading into Red Blood Cells

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Introduction

The current standard for improving high altitude warfighter performance relies on time-consuming acclimatization steps that leverage physiological responses triggered by HIF-1 in low-oxygen environments or on treatment with erythropoiesis-stimulating agents. The acclimatization response includes increased hematocrit and 2,3-bisphosphoglycerate levels, hereby increasing oxygen delivery to tissues. Yet reliance on this method to improve altitude performance requires substantial prior knowledge of mission environmental conditions to support extended preconditioning. Autologous blood donation and erythropoiesis-stimulating agents can rapidly increase hematocrit but carry safety risks, including increased blood clotting due to increased blood viscosity. DesiCorp, Inc proposes an alternative strategy to increase oxygen-delivery capacity by transiently raising the hemoglobin concentration within RBCs.

Materials and Methods

Packed red blood cells from human donors were obtained from a commercial vendor (Innovative Research, Novi, MI, USA). Human hemoglobin was purified from lysed RBCs and labeled with ATTO-655 maleimide (AAT Bioquest, Pleasanton, CA, USA). RBCs were loaded with ATTO 655-labeled hemoglobin (ATTO-Hgb) using ultrasound in a 3D-printed ultrasound-integrated, concentric spiral flow chamber using 10% phospholipid-coated perfluorobutane microbubbles at a flow rate of 90 mL/h (Fig. 1). RBCs suspended in PBS containing 150 µg/mL of ATTO 655 hemoglobin were exposed to ultrasound pulses at 3.3 MHz input with a 0.1% duty cycle and 250 mVpp amplitude to induce microbubble cavitation. After loading, RBCs were transferred to CPD-AS3 storage solution and monitored over 40 days. Molecular delivery and hemolysis were measured via spectrophotometric assays, and RBC morphology and size distribution were evaluated via complete blood counts on a hematology analyzer (Advia 2120i, Siemens, Munich, DEU) at 0, 7, 21, and 40 days post-acoustofluidic processing.

Objective

1. Discuss the method of increasing intracellular hemoglobin concentration as a means of mimicking altitude acclimatization.
2. Analyze methods for determining changes in intracellular hemoglobin concentration.
3. Discuss the potential safety considerations of increasing hemoglobin concentration in high-altitude environments.

Results

Acoustofluidic loading of RBCs yielded a loading efficiency (the ratio of the intracellular cargo concentration to the cargo concentration in the loading buffer) of 21.11% ± 4.86%, which is significantly higher than that achieved by incubating the ATTO-Hgb with RBCs in the loading buffer (5.82%) (Fig. 2). ATTO-Hgb retention was monitored over 40 days and remained detectable through Day 7, at 63.34% ± 9.04% of the initial intracellular mass, approximately 1.7 times greater than the retention of hemoglobin observed in the cargo incubation control (Fig. 3). Hemolysis increased over time in all groups and was elevated in RBCs with acoustofluidic processing at all timepoints compared with the other groups, peaking at 23.40% ± 1.55% on Day 40 (Fig. 4). Although some significant differences were observed, hematology analyzer results indicate similar morphology and size distribution trends across groups and, as previously observed, across storage in CPD-AS3 (Fig. 5,6).

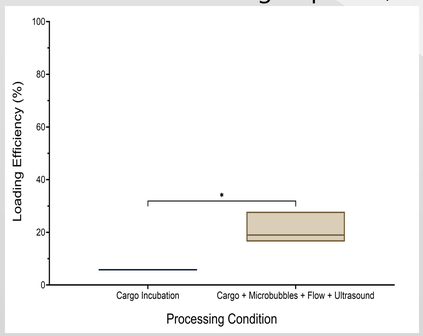

Fig. 2: RBCs processed with acoustofluidics showed significantly higher loading efficiency than the cargo incubation control (n = 1-3; α = 0.05).

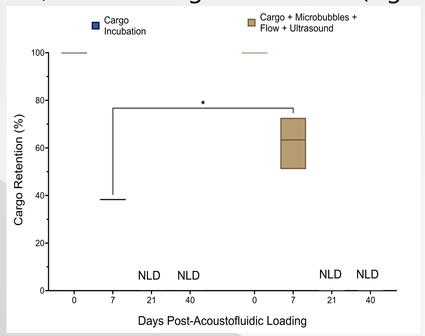

Fig. 3: Cargo retention was significantly higher at 7 days post-processing than at the same timepoint in the cargo incubation control (n=1-3; α = 0.05).

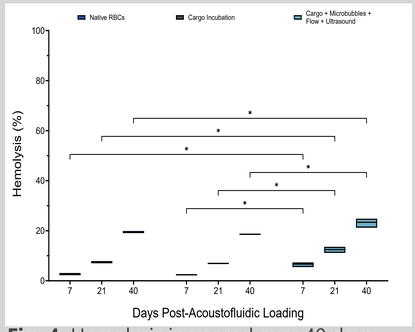

Fig. 4: Hemolysis increased over 40 days across all samples and was significantly higher in samples with acoustofluidic processing (n=1-3; α = 0.05)

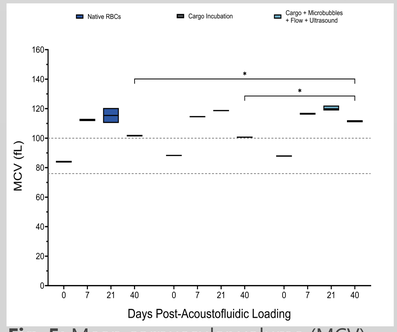

Fig. 5: Mean corpuscular volume (MCV) was comparable across all sample groups over time. MCV was significantly higher on Day 40 in the processed group (n=1-3; α = 0.05) but remained similar to previously observed values during storage in CPD-AS3. Dotted lines indicate the physiological ranges for whole blood

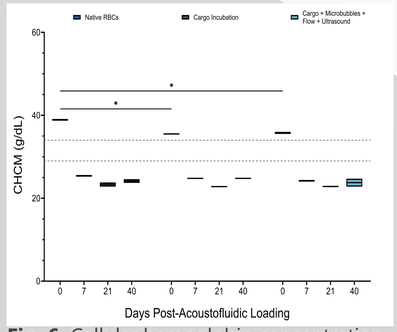

Fig. 6: Cellular hemoglobin concentration mean (CHCM) was comparable across all sample groups over time. CHCM was significantly higher initially in the native RBCs group (n=1-3; α = 0.05), but it was similar to previously observed values during storage in CPD-AS3. Dotted lines indicate physiological ranges for whole blood

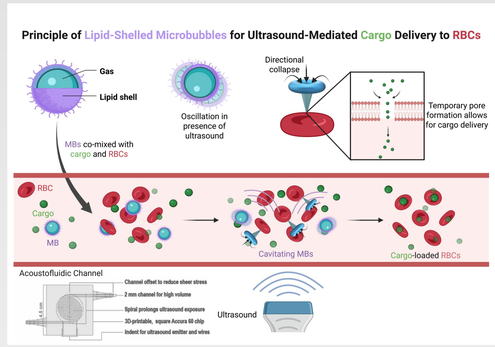

Fig. 1: Principle of acoustofluidic loading.

Conclusions

Our results demonstrate that the intracellular hemoglobin concentrations can be increased, and future experiments will aim to raise them to levels comparable to those observed during hematocrit increases with altitude acclimatization. Further studies are needed to validate RBC safety *in vitro* and *in vivo*, to optimize loading efficiency, and to establish hemoglobin loading as a suitable means of highly accelerated altitude acclimatization. However, applications of this approach with significantly increased intracellular hemoglobin concentrations would enable the preparation and storage of autologous blood for intracellular loading and infusion immediately before altitude exposure, thereby enhancing warfighter performance and readiness.

Disclaimer

Brett Janis is a founder, board member, and shareholder of DesiCorp, Inc.

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