# Exploring Osmotic Characteristics and Survival of Red Blood Cell Subpopulation Extremes Post-Deglycerolization: A Biotinylation Approach

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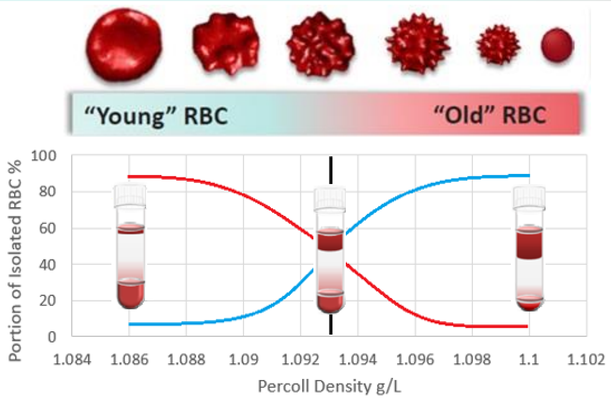
## BACKGROUND AND OBJECTIVES

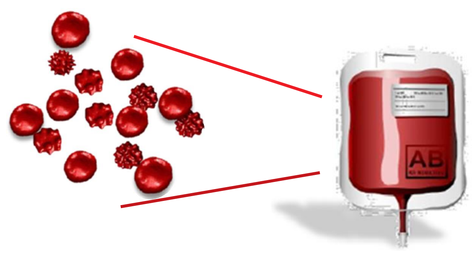
Cryopreservation subjects RBCs to osmotic stresses during the addition/removal of cryoprotectants and the freezing / thawing steps. Assessing the RBC osmotic properties within diverse biologically aged subpopulations is essential in optimizing cryopreservation protocols.

**Objective:** We aimed to analyze the osmotic characteristics of RBC subpopulation extremes and assess the survival of young (**Y-RBCs**) and Old red blood cells (**O-RBCs**) following cryopreservation through an innovative biotinylation approach.

## METHODS

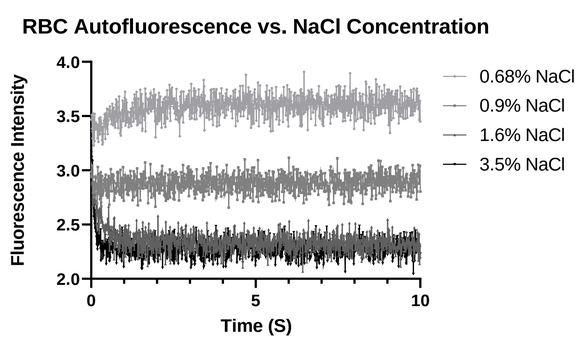
Red cell concentrates (**RCCs**) underwent age profiling; old (10.02%) and young (13.18%) RBCs from the two ends of the density distribution spectrum were isolated using the Percoll separation method.

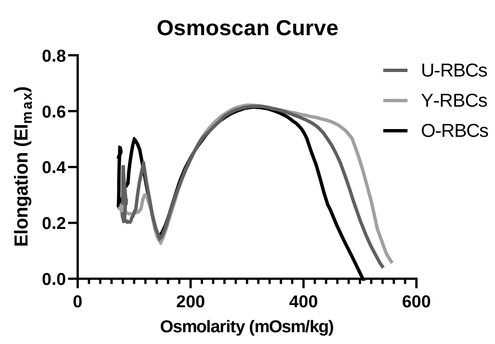



Heterogeneous population of RBCs

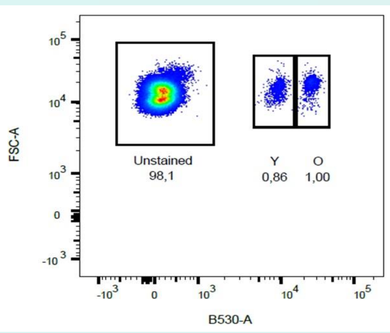
The hydraulic conductivity (**Lp**) and deformability of unseparated (**U-RBC**), Y-RBCs, and O-RBCs were assessed using stopped-flow spectroscopy and a laser ektacytometer, respectively.

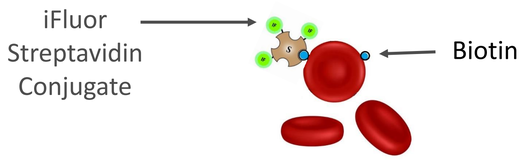



Young and old RBCs were labeled using 15 µg/mL and 48 µg/mL biotin solution, respectively, and spiked back into the RCC units.


The number of BioRBCs was evaluated following spiking, using Streptavidin antibody conjugated with fluorescence probes.



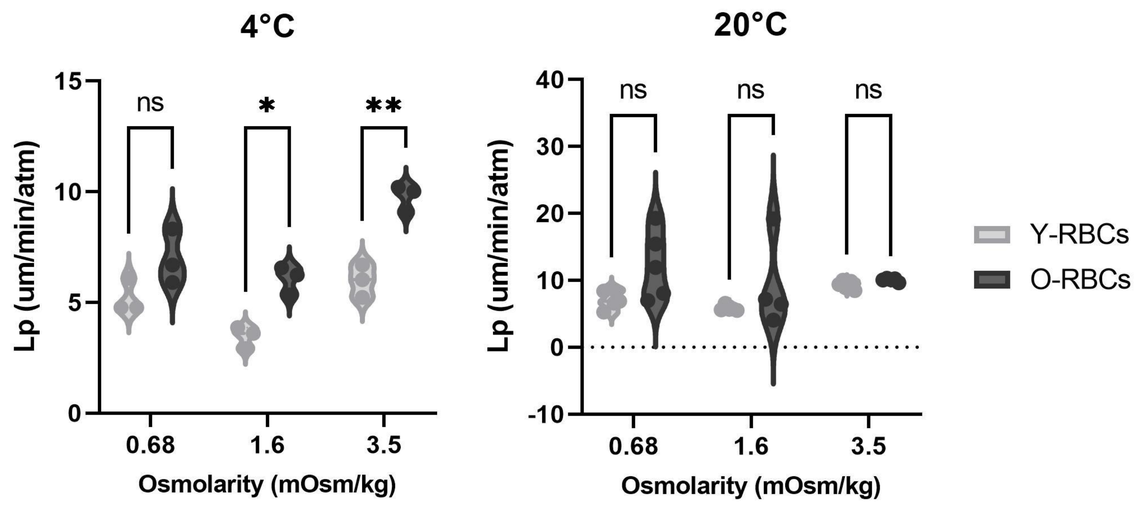



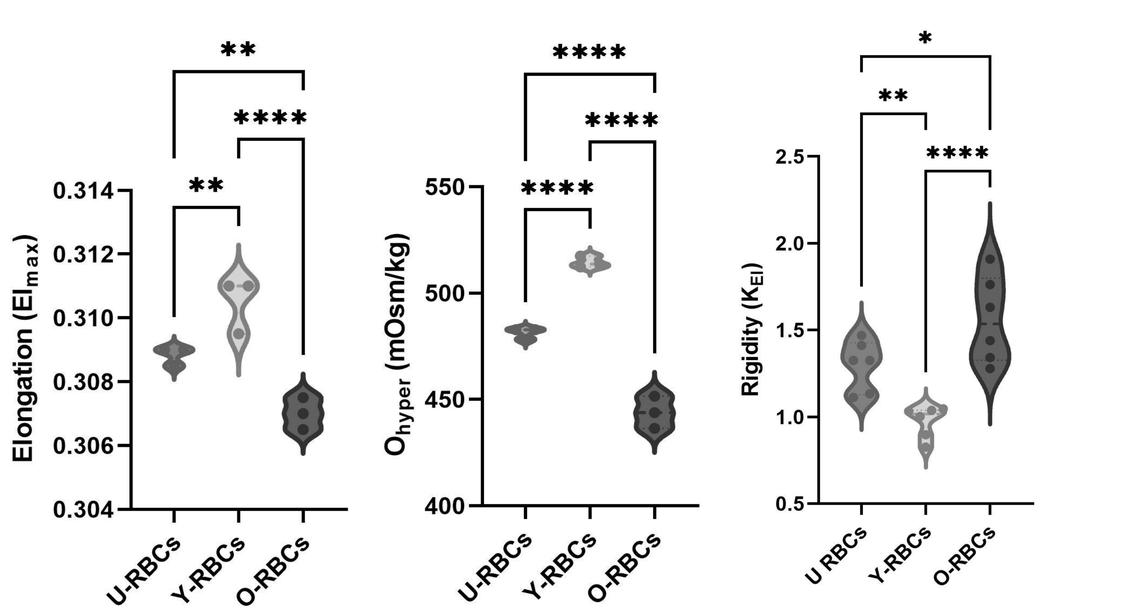

Units were subjected to cryopreservation using the High Glycerol Method (HGM) with 35-40% w/v glycerol and frozen for one month.


Units were thawed, and Flow cytometry was employed to measure the number of BioRBCs at three time points (1, 7, and 14 days) post-thaw.

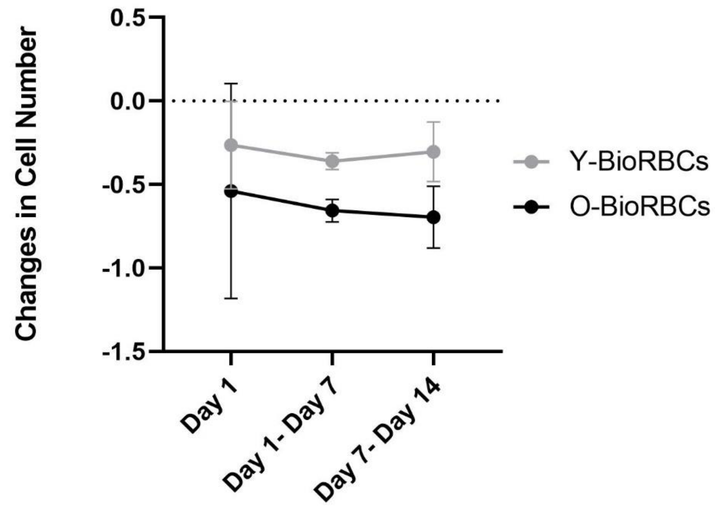
## RESULTS

- At 4 °C, O-RBCs had higher hydraulic conductivity (**Lp**) than Y-RBCs ($p = 0.0043$), while no significant differences were seen at 20°C.
- O-RBCs respond faster to hypertonic solutions than Y-RBCs.
- Y-RBCs exhibited superior elongation (**EImax**) and O-hyper than U-RBCs and O-RBCs while O-RBCs can tolerate a limited osmolality range. ($p < 0.0001$)
- O-RBCs were the most rigid among the subpopulations. ($p < 0.0001$)
- The number of O-BioRBCs demonstrates a steeper drop (slope ≈ -0.07683) than Y-BioRBCs (slope ≈ -0.01663) at three different time points post-deglycerolization. (1, 7, and 14) However, these trends lack statistical significance.




*BioRBC Dynamics Post-Thaw: Changes in Cell Count during Hypothermic Storage*




## CONCLUSIONS

Despite Y-RBCs showing superior osmotic characteristics compared to O-RBCs, we did not observe a significant post-deglycerolization survival advantage in Y-BioRBCs, which may be attributed to biolabeling-induced membrane damage.

The future research direction involves optimizing cryopreservation protocols based on observed osmotic differences between young (Y-RBCs) and old (O-RBCs) red blood cells, as well as exploring strategies to mitigate biolabeling-induced membrane damage.

## Acknowledgment

We are grateful to Canadian Blood Services' donors who made this research possible.