Functional Manufactured Red Blood Cells from Refrigerated or Glycerol-Free Cryopreserved Formulations

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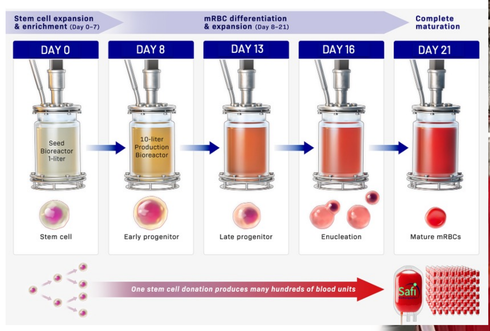
Manufactured red blood cells (mRBCs) have the potential to improve civilian and military blood logistics by enabling on-demand manufacture and longer-term storage options

Introduction & Methods

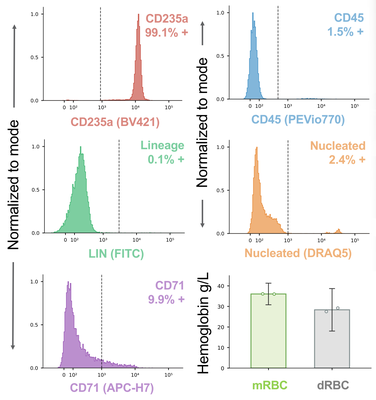
- Blood availability is a readiness constraint — cold-chain dependence and short shelf-life limit reach in austere settings.
- A scalable, storage-stable, universal-donor red cell would enable pre-positioning and rapid response.
- One leukopak → thousands of uniform, "younger" mRBC units — free of immune cells & pathogens.

Schematic of Safi's 10L mRBC production



Safi's 10L mRBC production bioreactor; insert shows harvested product

Product – representative flow plots and hemoglobin content at harvest

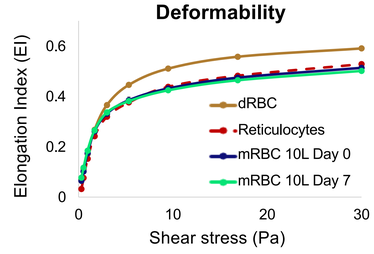


- Reproducible 10 L bioreactor production yields 1 unit of transfusable mRBCs.
- Transferred to US GMP-ready CDMO — ARMI | BioFabUSA.
- Product profile at harvest: >97% CD235a positive; <2% CD45; non-erythroid lineage negative; >90% enucleated (DRAQ5 histogram); Hemoglobin content higher and more consistent than donor (dRBCs).

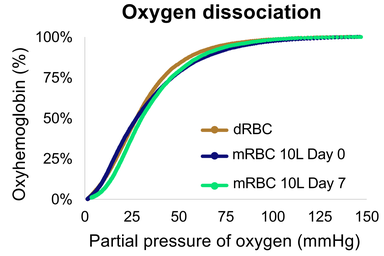
Results - Harvest & Refrigerated Storage

- mRBC Deformability: Provides evidence of capacity to pass microcapillaries (Target >0.4 EI at 30 Pa).
- mRBC O_2 dissociation curves during storage: Normal Hb oxygen affinity demonstrated via p50 saturation point.

Deformability

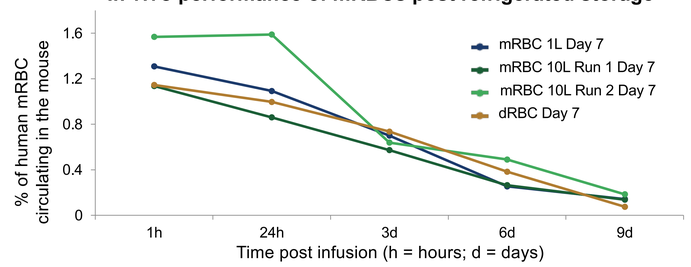


Oxygen dissociation

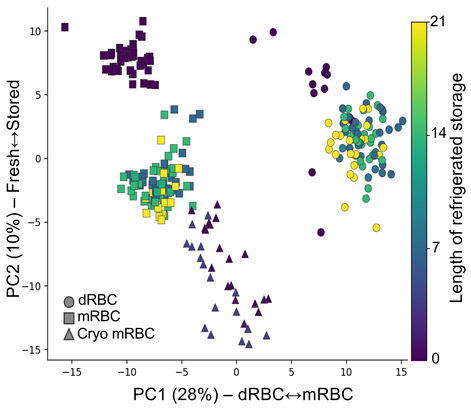


- *In vivo* circulation models show equivalent circulatory half-life for dRBC vs 1 L and 10 L scale manufacture mRBC after 7 days of storage.

***In vivo* performance of mRBCs post refrigerated storage**



Storage cell metabolome: mRBC vs dRBC vs Cryo mRBC

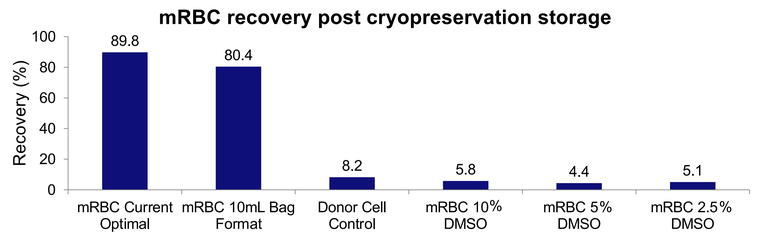


- Metabolomic profiling identified structured differences between mRBC and dRBC, reflecting mRBCs' lack of plasma conditioning and their retained young-red-cell metabolism (predominantly PC1).
- Both mRBC and dRBC undergo the same storage changes over time eg 2,3-DPG and ATP reduction (predominantly PC2).

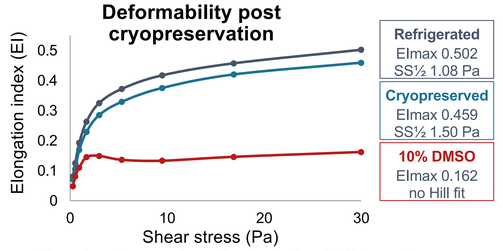
Results - Cryopreservation Storage

- mRBCs' robustness and homogeneity support glycerol-free cryopreservation.
- mRBCs recover ~90% with preserved deformability versus <10% for donor cells under equivalent storage media conditions or mRBCs in standard DMSO cryoprotectants.
- mRBCs' higher glutathione/ATP content relative to donor cells provides a mechanistic basis for this advantage.

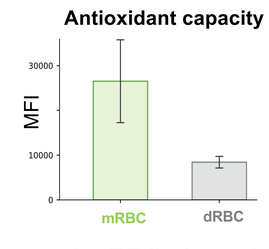
mRBC recovery post cryopreservation storage



Deformability post cryopreservation

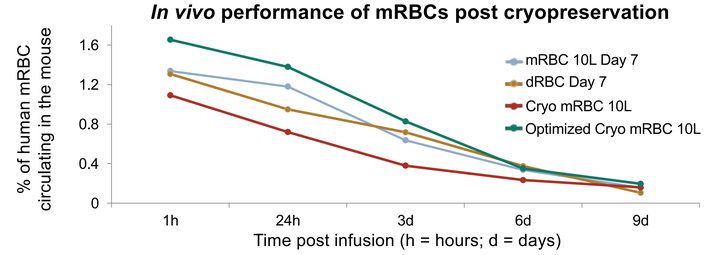


Antioxidant capacity



- Equivalent circulatory half-life of cryopreserved mRBCs in an *in vivo* model

***In vivo* performance of mRBCs post cryopreservation**



Conclusion

- mRBCs retain key functional attributes across manufacturing scale and storage conditions relevant to clinical supply.
- mRBCs will tolerate more flexible formulation than donor cells, creating potential for simpler long-term storage approaches, including glycerol-free cryopreservation for defense and civilian applications.

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