IN VITRO VASCULAR ENDOTHELIAL RESPONSE TO NOVEL WHOLE BLOOD ANALOGUE FOR RESUSCITATION

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- There are no conflicts of interest to declare.
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NMR&D

- Naval Medical Research Unit (NAMRU) San Antonio plays a vital part within Navy Medicine Research & Development (NMR&D), supporting medical readiness to ensure warfighter lethality.

- In addition to Navy platforms, NMR&D is heavily engaged in research addressing health and readiness threats in a range of operational environments, including the undersea domain, remote areas, and environments where extreme cold, heat, and altitude are factors.

- Understanding and addressing the evolving health concerns in these environments is critical to service member readiness, fitness, and lethality.

- NMR&D, with its insight into challenges faced by Sailors and Marines, is uniquely qualified to lead U.S. Navy research efforts to ensure healthy, fit, and lethal service members.

DARPA'S FSHARP PROGRAM

FIELDABLE SOLUTIONS FOR HEMORRHAGE WITH BIO-ARTIFICIAL RESUSCITATION PRODUCTS

"Develop a ***deployable, shelf-stable, universal whole blood analogue (WBA)*** as a hemorrhage countermeasure to sustain injured warfighters in austere, pre-hospital settings."

Hemoglobin-Based Oxygen Carriers (HBOCs)

ErythroMer (EM)

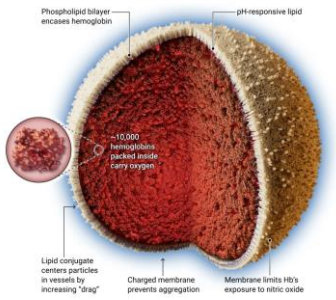
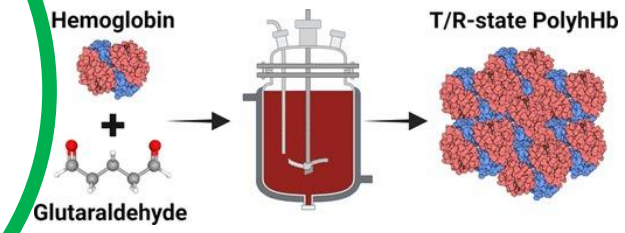


Image credit: Matt Roth for Science Magazine

Polymerized Hemoglobin (PolyHb)



Khan MA, …A, Palmer AF on behalf of the CONCERT research program. Biomacromolecules. 2024 Nov 11;25(11):7334-7348.

SynthoPlate (SP)

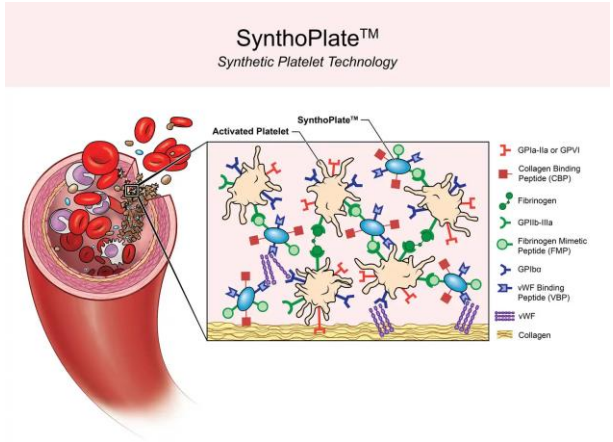


Image credit: Haima Therapeutics, LLC

Freeze-Dried Plasma (FDP)

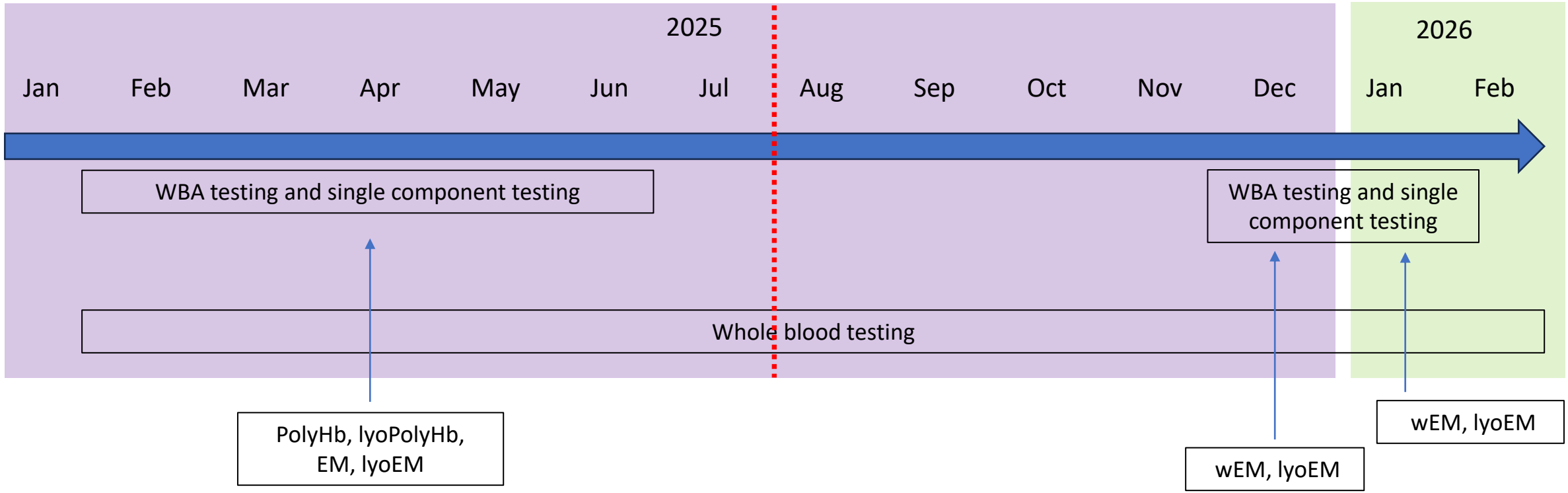
Image credit: Teleflex, Inc. via Military Times

IV&V PARTNER

Independent Validation & Verification (IV&V) Tasks

- Validate progress throughout the program
- Evaluate performer products against increasingly stringent metrics
- Work collaboratively with performers and provide feedback
- Identify and apply most appropriate experimental models and assays for evaluations

Y3 Activities: In Vitro Testing

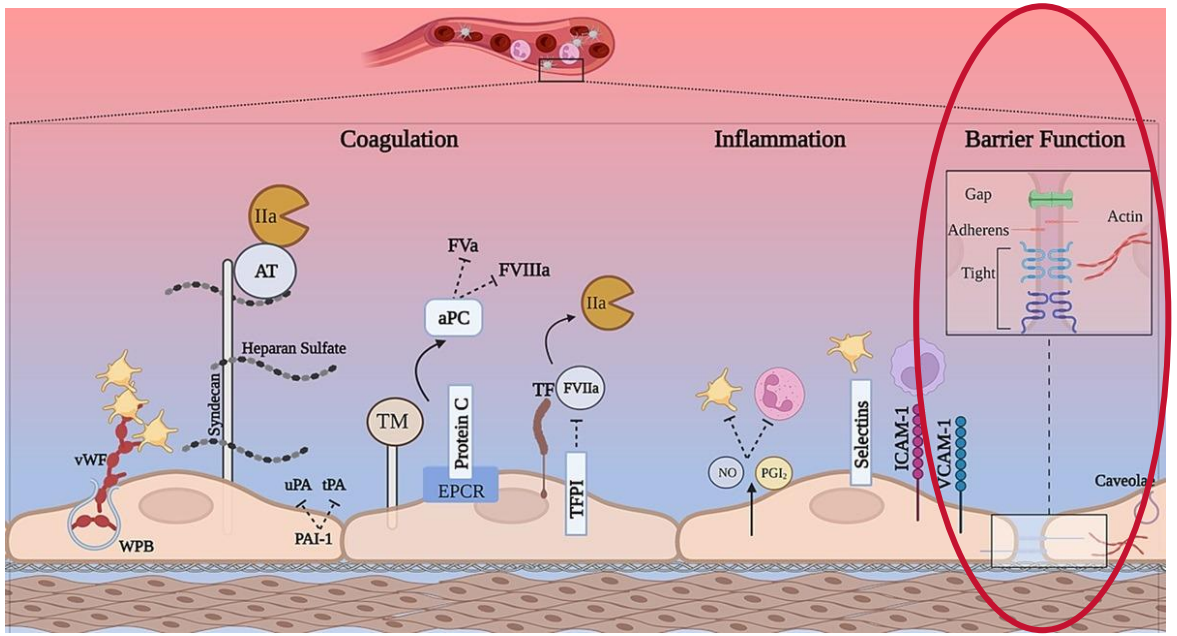


Abbreviations: EM = Erythromer; lyo= Lyophilized; PolyHb = Polymerized Hemoglobin; w = Wet; WBA = Whole Blood Analogue

STUDY OBJECTIVE

Evaluate the effects of whole blood analogue (WBA) on vascular endothelial permeability using a cell-based model of the vascular endothelium.

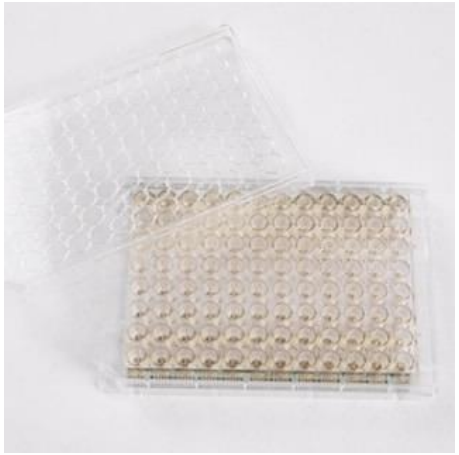
In Vivo Endothelial Function



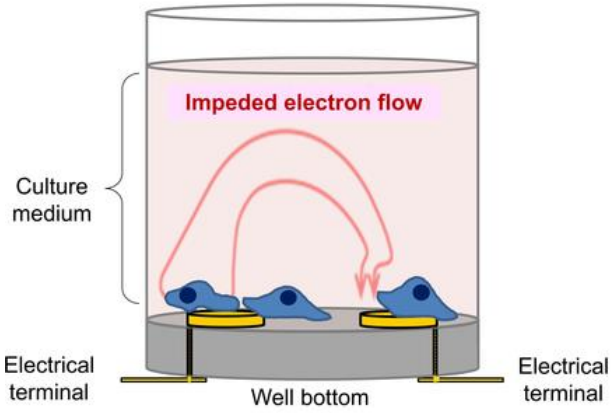
Cardenas, Jessica C. PhD; Dong, Jing Fei MD, PhD; Kozar, Rosemary Ann MD, PhD. Injury-induced endotheliopathy: What you need to know. Journal of Trauma and Acute Care Surgery 95(4):p 454-463, October 2023. | DOI: 10.1097/TA.0000000000004082

In Vitro Model of Endothelial Function

96-well Titer Plate Format



Transendothelial Electrical Resistance (TEER)



Luong A, Cerignoli F, Abassi Y, Heisterkamp N, Abdel-Azim H. Analysis of acute lymphoblastic leukemia drug sensitivity by changes in impedance via stromal cell adherence. PLoS One. 2021 Sep 30;16(9):e0258140. doi: 10.1371/journal.pone.0258140.

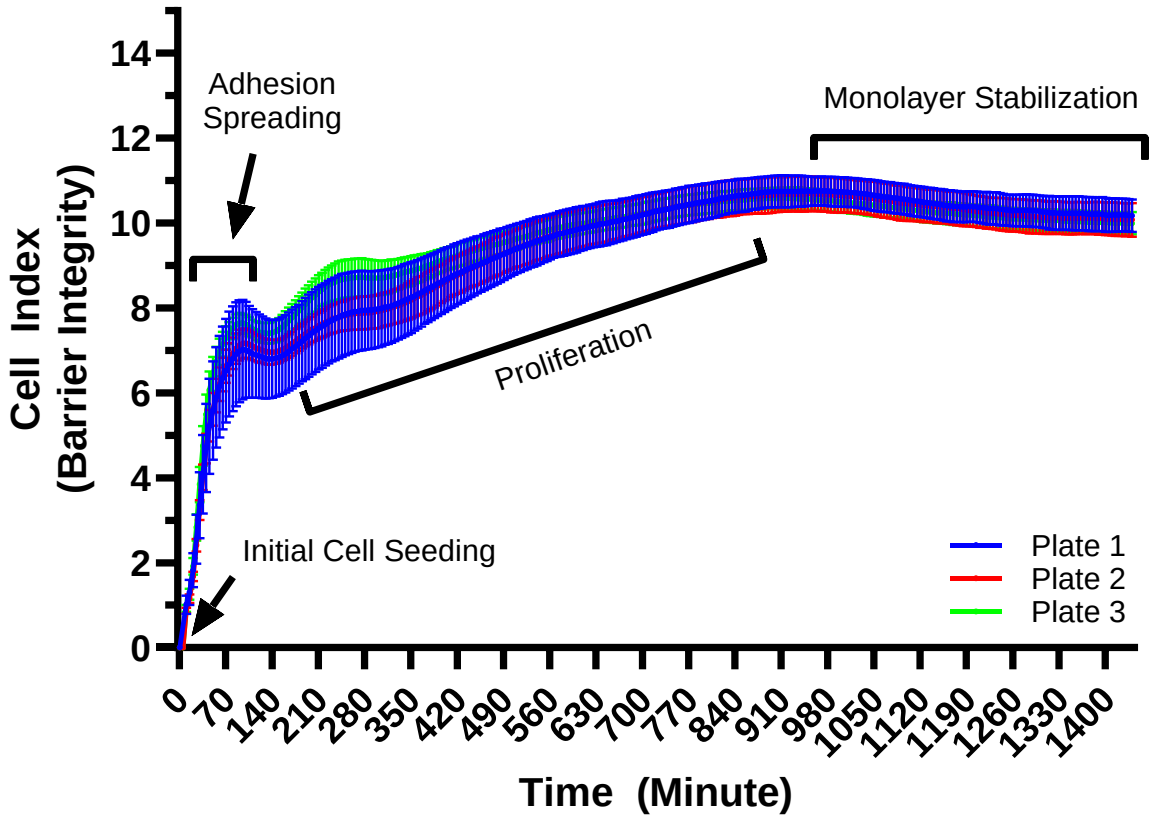
EXPERIMENTAL MODEL

In Vitro, Cell-based Model to Assess Endothelial Permeability

Cell Impedance System

Photo credit: Burrell Parmer, NAMRU-SA

Cell Index = (impedance at time point n – impedance in the absence of cells)/nominal impedance value



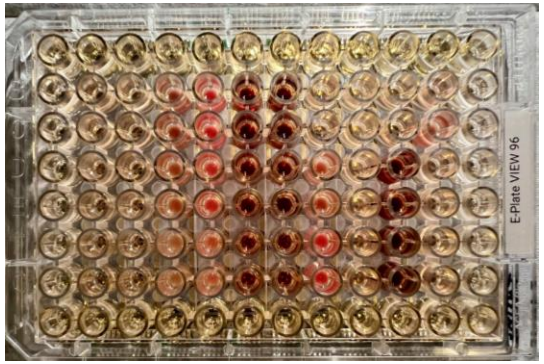
EXPERIMENTAL DESIGN

Day 4

Day 0	Day 2	Day 3	Time 0 hr	Time 0.5, 2, 4 hr	Day 5
Seed primary Human Umbilical Vein Endothelial Cells (HUVECs; 15K cells/well)	Media Change	Stabilization	Treatment	Sample Collection/ Outcomes	Sample Collection/ Outcomes

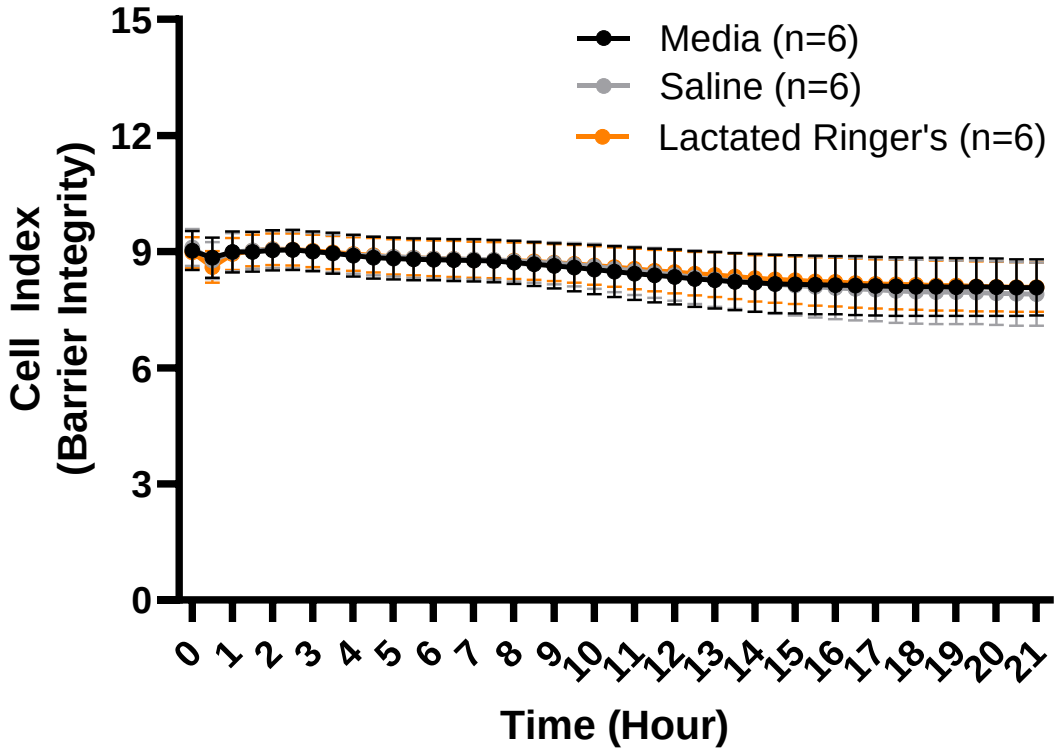
Experimental Groups

Controls: Media, Saline, Lactated Ringer's Solution (LR)
Standard Comparator: Stored Whole Blood (Stored WB; 26 ± 3 days)
Products: WBA, Single Components (EM, lyophilized EM, FDP, SP)

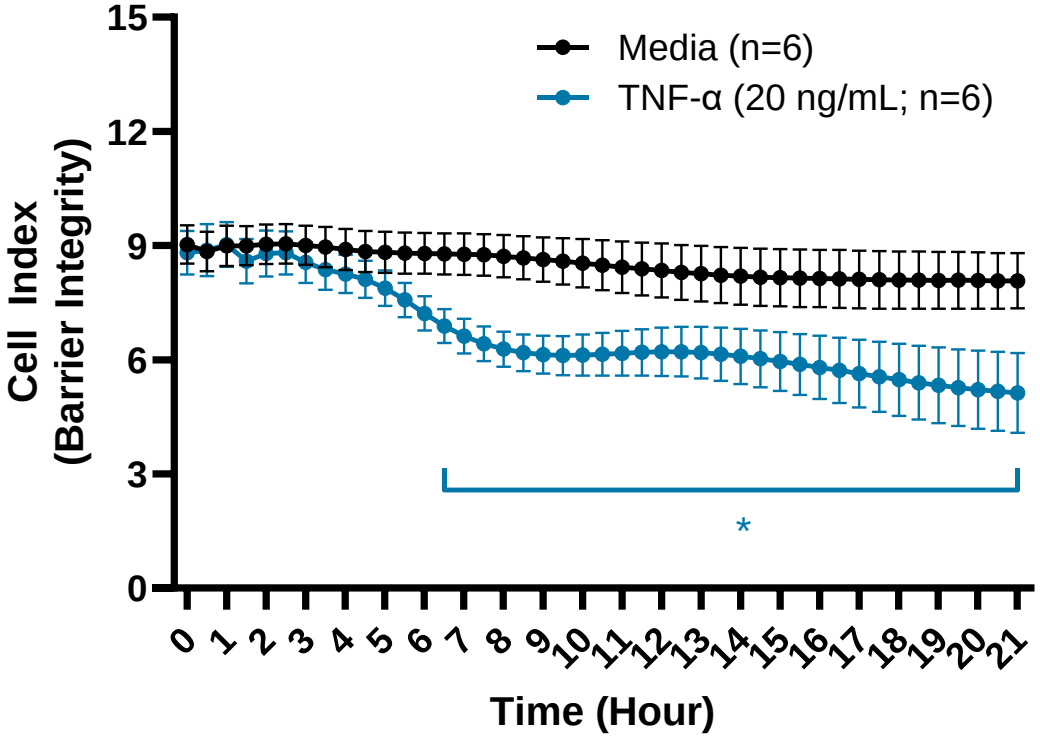


Assay Controls

Saline and Lactated Ringer's

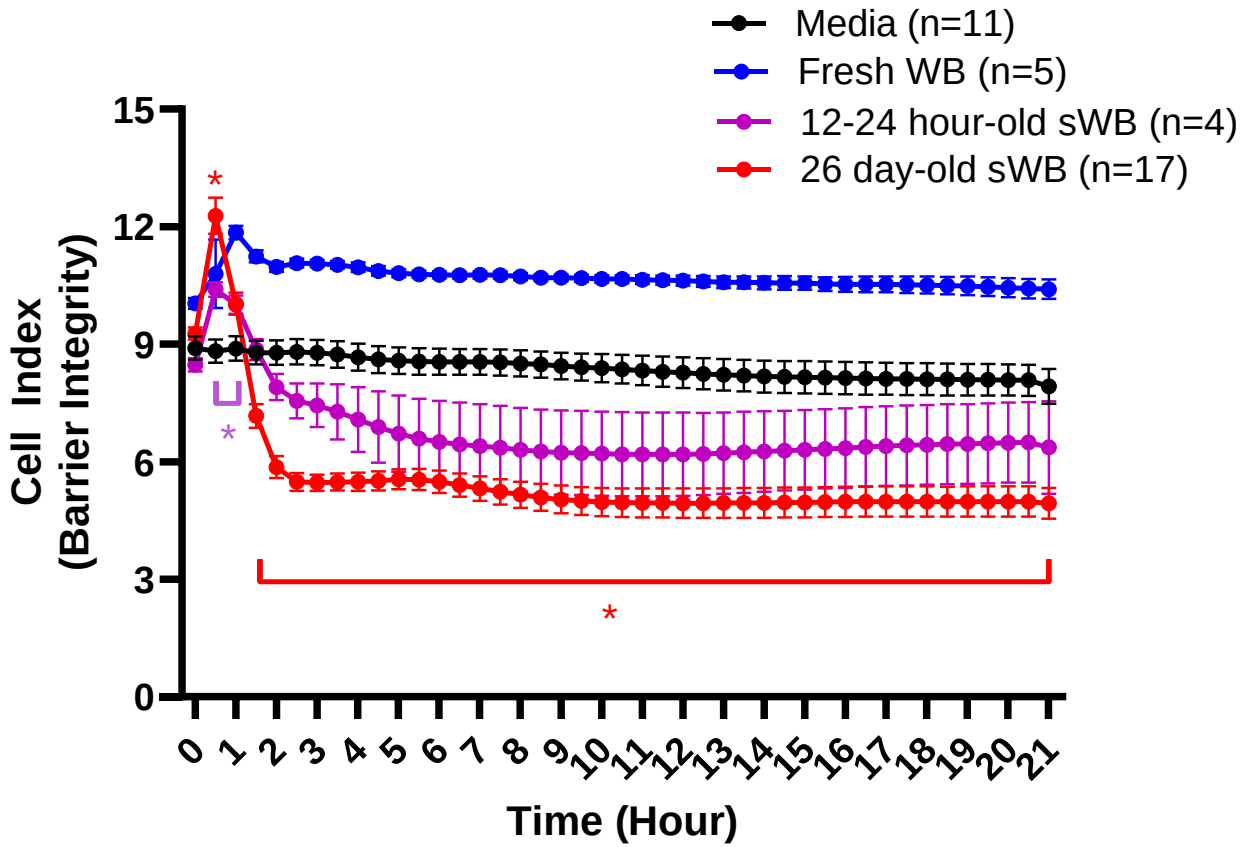


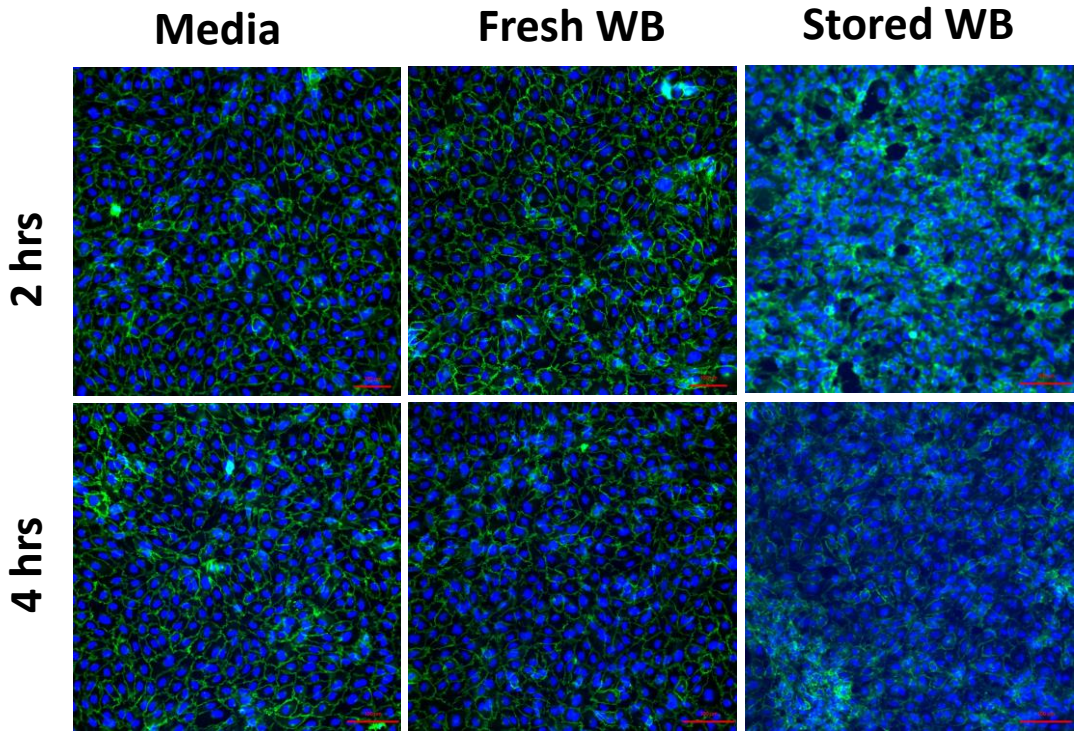
Tumor Necrosis Factor (TNF)-α



Two-way ANOVA with Tukey's post hoc; *p < 0.05 compared to Media

Whole Blood Testing



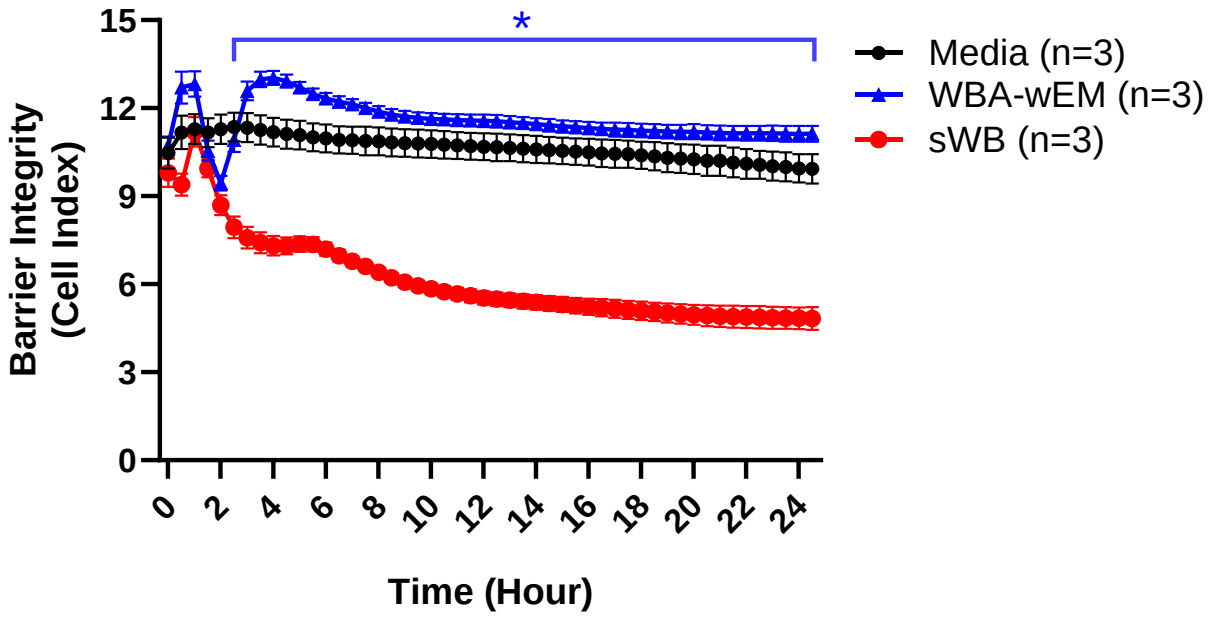


Representative images of HUVECs after treatment; VE-cadherin (green), and nuclei (blue)

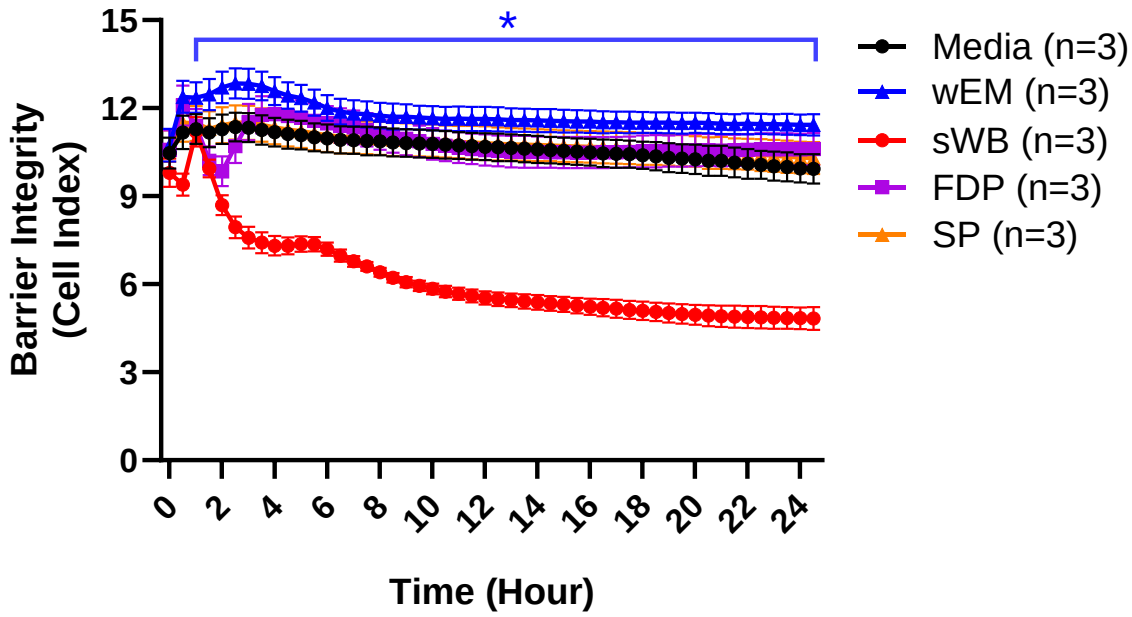
Two-way ANOVA with Tukey's post hoc; *p < 0.05 compared to Media

WBA-wet ErythroMer (wEM)

Whole Blood Analogue



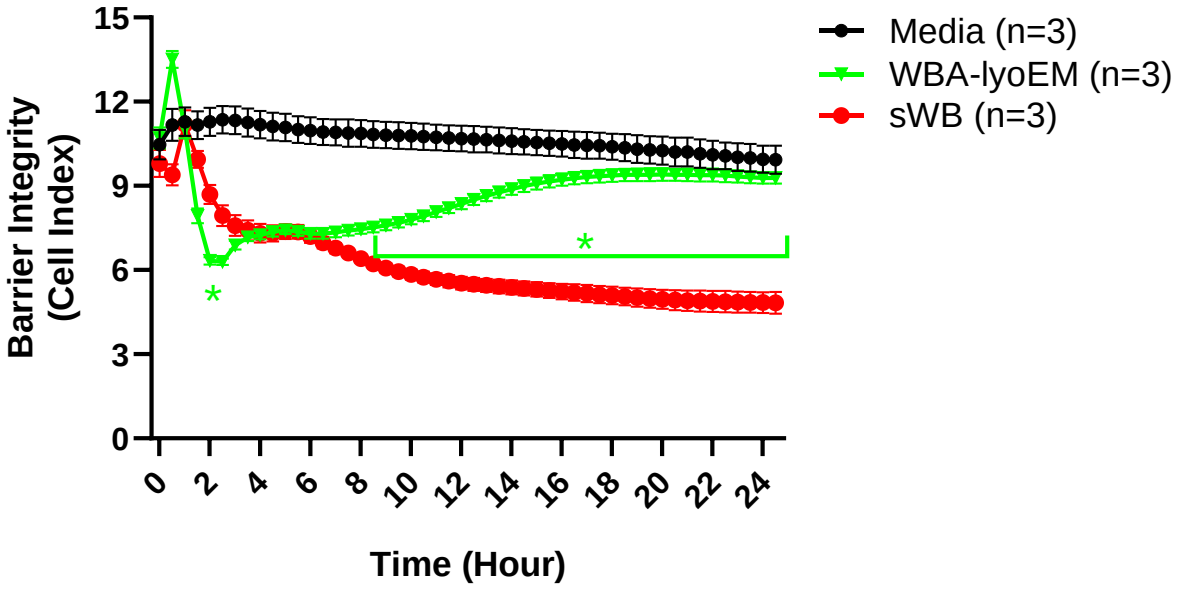
Single Components



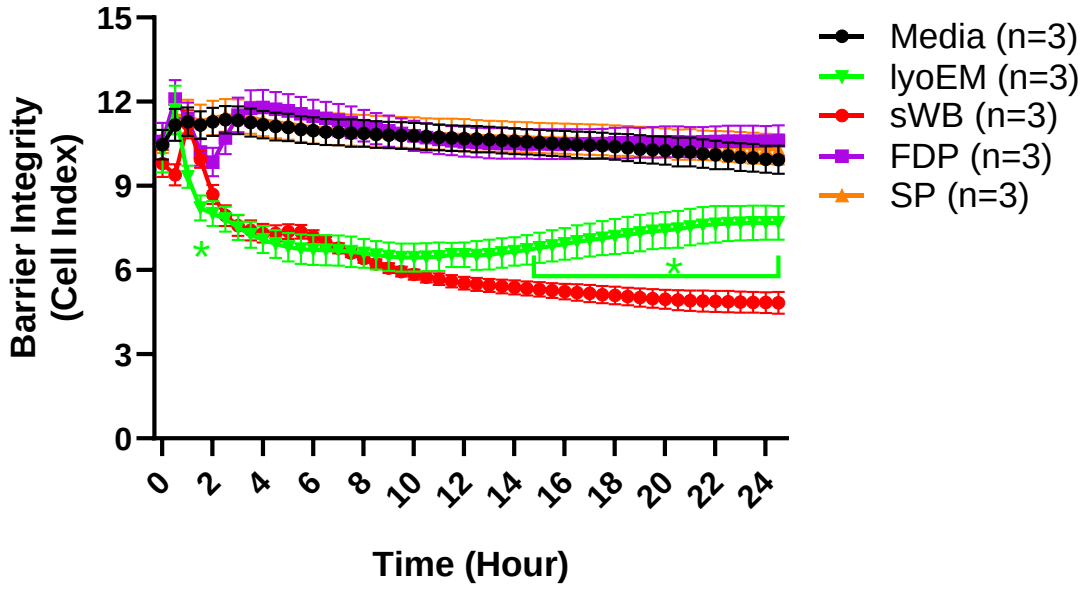
Two-way ANOVA with Tukey's post hoc; *p < 0.05 compared to sWB

WBA-lyophilized EM (lyoEM)

Whole Blood Analogue

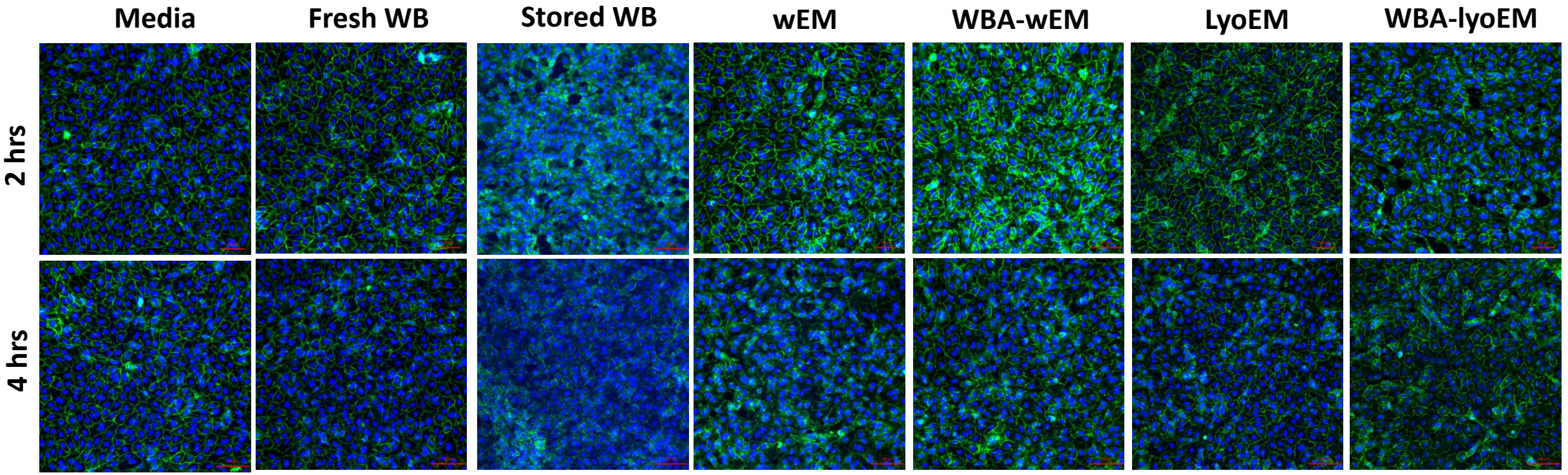


Single Components



Two-way ANOVA with Tukey's post hoc; *p < 0.05 compared to sWB

Immunocytochemistry: Vascular Endothelial (VE)-cadherin



Representative images of HUVECs after treatment; VE-cadherin (green), and nuclei (blue)

CONCLUSIONS

- Development of WBA has focused on formulation of a safe and efficacious HBOC, that is compatible with FDP and SP for use in battlefield scenarios.
- In vitro evaluation demonstrates that wEM or WBA-wEM does not adversely affect in vitro endothelial cell function.
- The effort to improve stability of wEM by lyophilization can cause molecular changes that may negatively impact vascular endothelial health.

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