

Regina Adao, PhD¹, Stephen Rogers, PhD¹; William McGhee, PhD²; Mary Brummet, MS¹; Esmā Alp, PhD²; Elizabeth McAslan², Ujjal Didar Singh Sekhon PhD³, Sana Syed, PhD³, Baylee Traylor³, Elaine Haynes, RPh², Anirban Sen Gupta, PhD⁴, Christa Pawlowski, PhD³, Michael Bruckman, PhD³, Allan Doctor, MD¹.

¹Center for Blood Oxygen Transport and Hemostasis, University of Maryland, School of Medicine, Baltimore, MD, ²KaloCyte, Inc., Baltimore, MD, ³Haima Therapeutics LLC, Cleveland, OH ⁴Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH

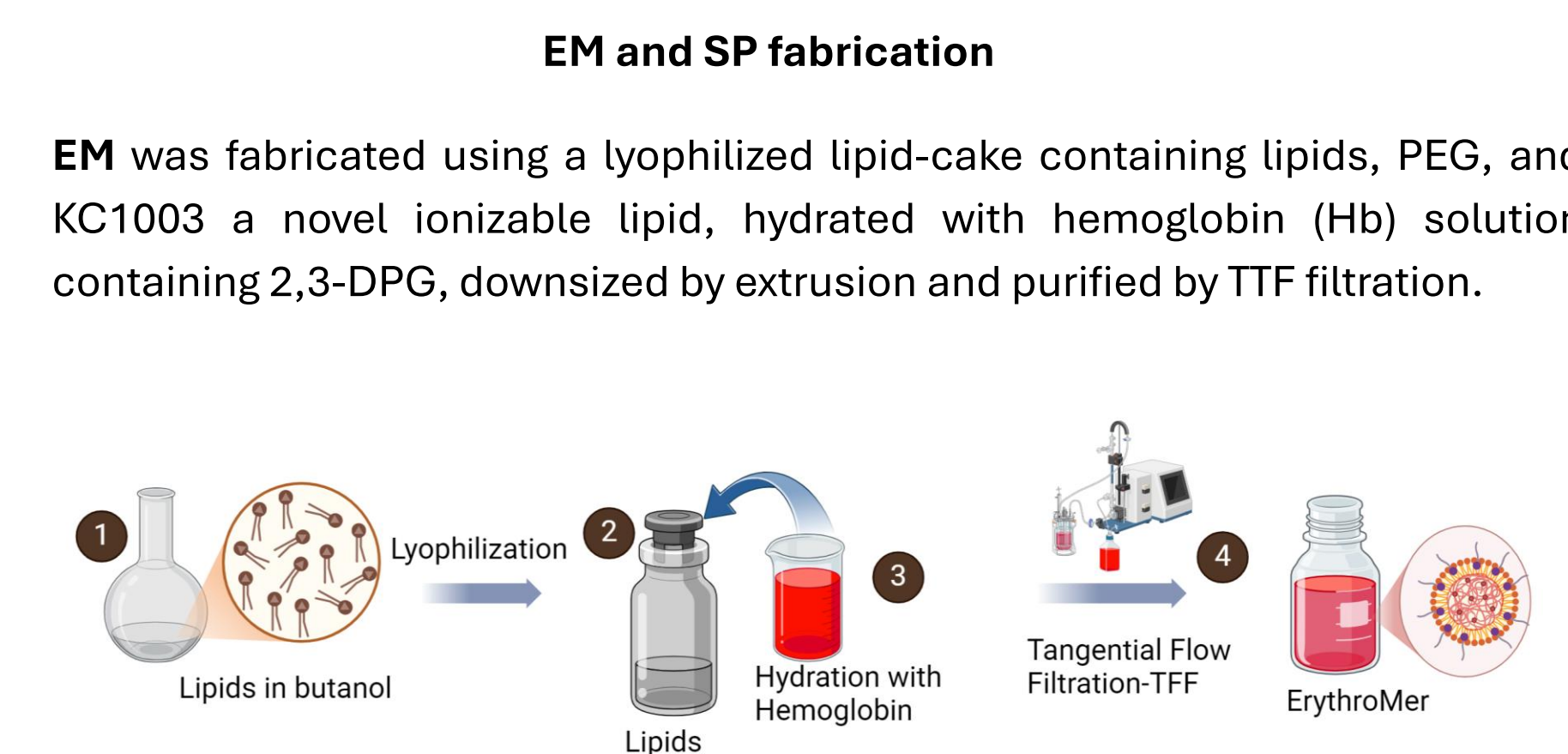
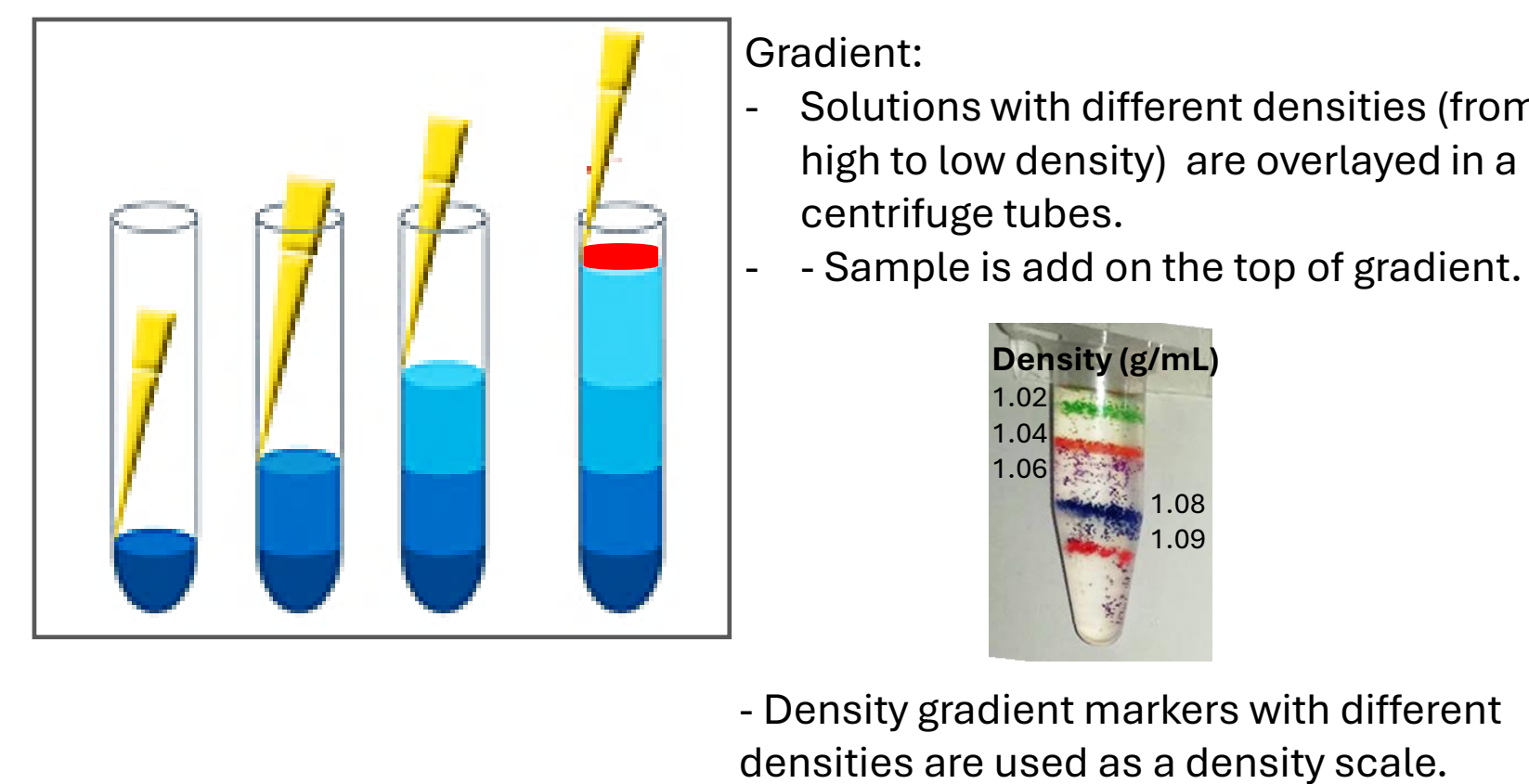
BACKGROUND / AIM

- Hemorrhagic shock is the leading cause of preventable traumatic fatalities with 30,000 deaths/year in the US among civilians and military Americans from 1 to 46 years old.
- Hemorrhagic shock, characterized by the loss of Hb and loss of oxygen carrying capacity takes only 2 hours from the injury onset to death.
- Data from the U.S Department of Defense about mortality in battlefield, demonstrated need for improvement of prehospital hemorrhage control, and oxygen carrier supplies, since bank blood is not available in remote settings, battlefields, and catastrophes.
- To mitigate this need, we are developing an unrivaled Whole Blood analogue (WBA) to emulate key elements of whole blood: **ErythroMer (EM)**, an oxygen carrier that mimics red blood cells, **SynthoPlate (SP)** to mimic platelets and **Freeze-dried Plasma (FDP)**.
- Key features of our WBA include compatibility with all blood types, freeze-dried for room temperature stability, and long self-life.

AIM: Since ErythroMer and SynthoPlate are lipid-based nanoparticles, we performed **density gradient** to separate EM and SP from engineered WBA and tested its **biocompatibility**.

METHODS

| Samples |
|---|
| <ul style="list-style-type: none"> EM (50 µL) SP (50 µL) EM+ SP (1:1 and 2:1 mixtures – 100 µL) Control (density gradient markers – beads with different densities) |
| Gradient |
| <ul style="list-style-type: none"> Isotonic Ficoll solution with sucrose Isotonic Ficoll solution with sucrose/PBS (to adjust the desired density) |
| Centrifuge |
| <ul style="list-style-type: none"> Ultracentrifuge Rotor: F50L-24x1.5 – K-factor = 33 Speed: 23100 rpm (60 000 x g), Angle 45° Time: 90 min |



SP was manufactured using a lipid thin-film-rehydration method followed by extrusion and lyophilization.

The WBA components were reconstituted and mixed at fixed ratios and then separated using an optimized density gradient method.

Aliquots of SP and EM were characterized before and after separation to compare their properties:

Physicochemical parameters:
 Hemoglobin Payload (EM)
 - Hydrodynamic particle diameter
 - Particle concentration
 - Surface charge

Physiological Performance:
 - Oxygen (O₂) affinity (release/uptake)
 - Nitric oxide (NO) trapping
 - NO vasoactivity in a vascular ring bioassay

REFERENCES AND ACKNOWLEDGEMENTS

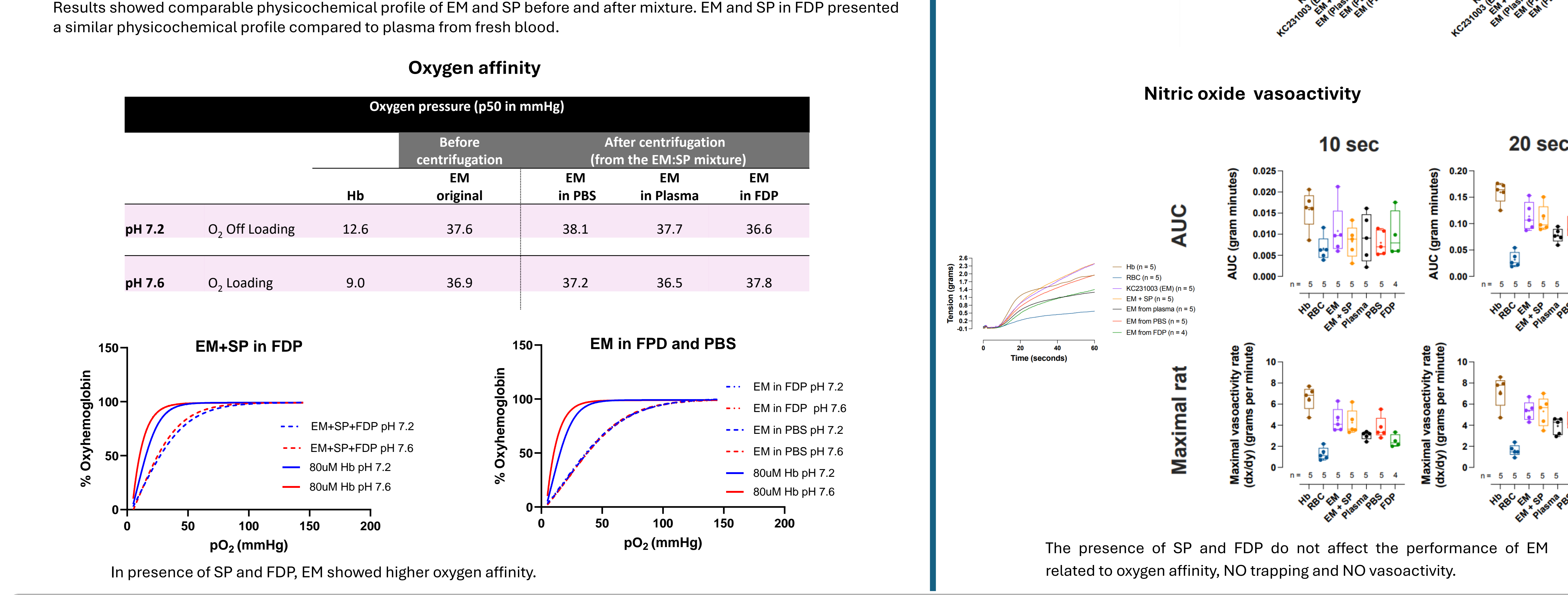
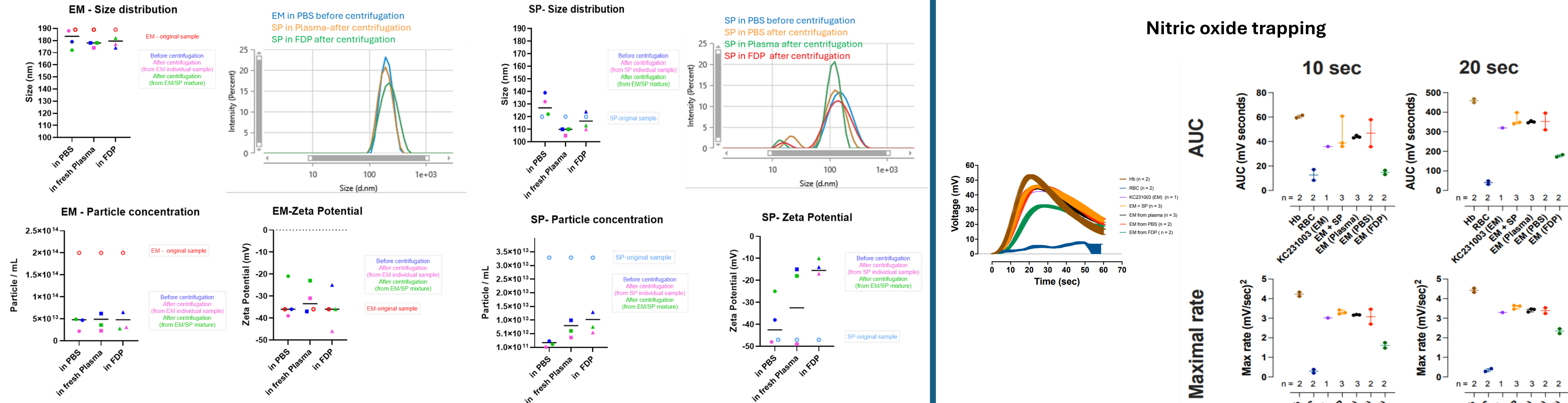
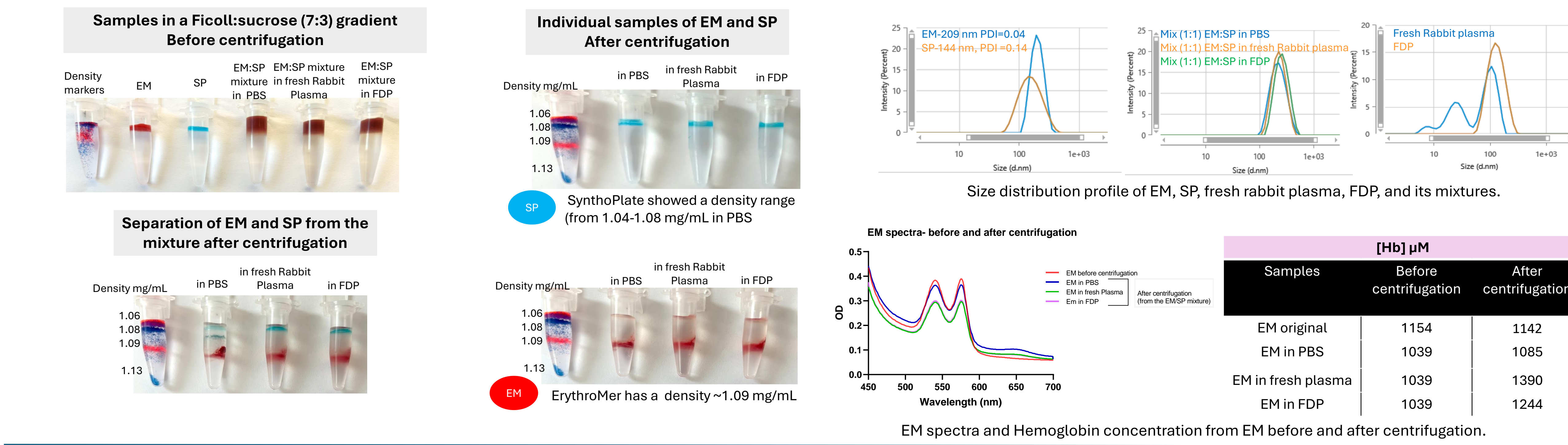
-Figure 1- Frontiers | Imaging Platelet Processes and Function—Current and Emerging Approaches for Imaging in vitro and in vivo - <https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2020.00078/full>.

-Figure 2- SynthoPlate- <https://www.haimatherapeutics.com/synthoplate>.

-Figure 3- <https://kaloocyte.com>.

Research-grade freeze dried plasma (FDP) was provided, in kind, by Vascular Solutions LLC, Minneapolis (a wholly owned subsidiary of Teleflex, Inc.) for purposes of this research opportunity only. Research-grade FDP is a pooled product that it is not approved by the US Food and Drug Administration or available for sale in the US.

RESULTS



CONCLUSION

- Density gradient is a suitable method to separate EM and SP from a mixture using PBS and rabbit plasma and FDP as diluent media.
 Density range:
 EM: 1.08-1.09 mg/mL
 SP: 1.02-1.04 mg/mL

- The compatibility of EM, SP and FDP, and the physicochemical function of EM after separation by density gradient method suggests their ability to participate in the biophysical and biochemistry processes of blood. Our shelf-stable, bio-inspired blood analogue is an innovative and added value to pre-hospital hemorrhage control and transfusion in the future.