A. JAMES CLARK SCHOOL OF ENGINEERING

THE FISCHELL DEPARTMENT *of* BIOENGINEERING

UNIVERSITY OF MARYLAND

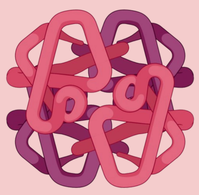
Team B5: Optimizing Erythromer Testing Methods

Daniel Coile, Sophia D'Amico, Hannah Lee, Fiona Sheridan, Abhishek Malhotra, and Casey Byrne

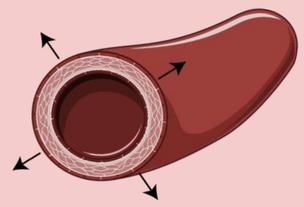
Advisor(s): Dr. Alisa Clyne, Department of Bioengineering, UMD-College Park & Dr. Alan Doctor, UM-School of Medicine

Background:

Blood shortage crisis in the US → Blood alternatives interfere with NO in arterioles → EM could serve as an effective blood substitute



Erythromer (EM) is a **nanoparticle** composed of a **lipid shell around hemoglobin (Hb).** EM can **effectively carry oxygen** and **can be lyophilized** for long-term storage. However, EM, like other blood substitutes, scavenges nitric oxide (NO) in the bloodstream, leading to harmful vasoconstriction. Coating EM with a layer of **polyethylene glycol (PEG) may reduce NO scavenging**.



*NO is a **vasodilator**, important for **blood regulation** in arterioles.*

Project Objectives:

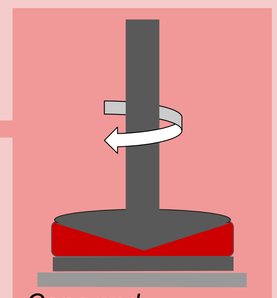
- Optimize **research methods** for PEGylated EM
- **Quantify** how PEGylated EM **alters blood viscosity**
- **Determine** how PEGylated EM **centralizes in the blood flow**

Methods: Rheometry

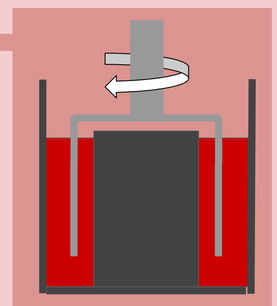
Initial Method: Cone and Plate
Problem: Samples were evaporating at edges, causing unrealistically high viscosities at low shear rates

Final Method: Concentric Cup Double Gap
Solution: Creates a humid environment for the sample. The method also improves accuracy for lower viscosity fluids such as blood, via increased shear surface area.[1]

Testing: EM with 2k and 5k PEGylation, with PEG densities per particle at 0.3%, 3%, and 5%. Tests performed were controls of phosphate buffer solution (PBS), whole blood (WB), 1:1 PBS:WB, 1:1 WB:PBS solution of EM [$2.6*10^{13}$]

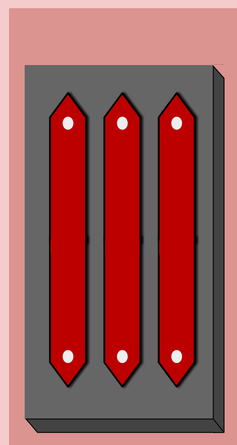


Cone and Plate



Concentric Cup Double Gap

Methods: Microfluidic Flow Modeling



Microfluidic Chip 3 channels (red) and inlet/outlet (white)

Initial Method: Confocal Microscopy
Problem: The imaging acquisition was too slow, leading to high standard deviation. The particles were suspended in PBS leading to particles sinking

Final Method: Spinning Disk Confocal Microscopy
Solution: Allows for rapid imaging at z slices, allowing better visualization of particles in flow and suspended particles in viscous Polyvinylpyrrolidone (PVP) to prevent sinking.[2]

Testing: To determine if this method can visualize centralized flow, polystyrene beads (PB) labeled with FITC in 2:1 PB:PVP for 0.2μm and 3:1 for 7μm (equal particle count) to test method before moving on to EM testing.

Results: Rheometry

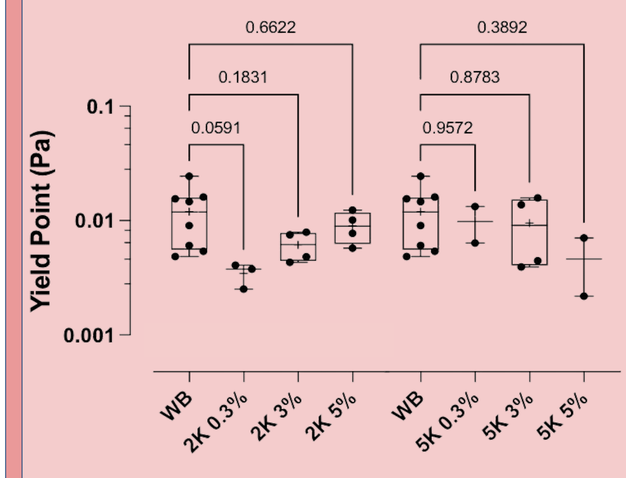


Figure 1: *Comparison of yield points using ANOVA with EM-PEG 2k and 5k at 0.3, 3, and 5% PEG against WB. All EM samples were suspended in a WB:PBS-EM 1:1 mixture with $2.6*10^{13}$ particles of EM/mL PBS.*

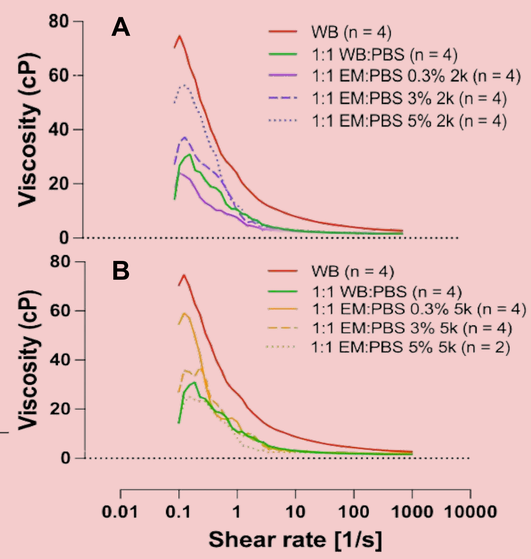
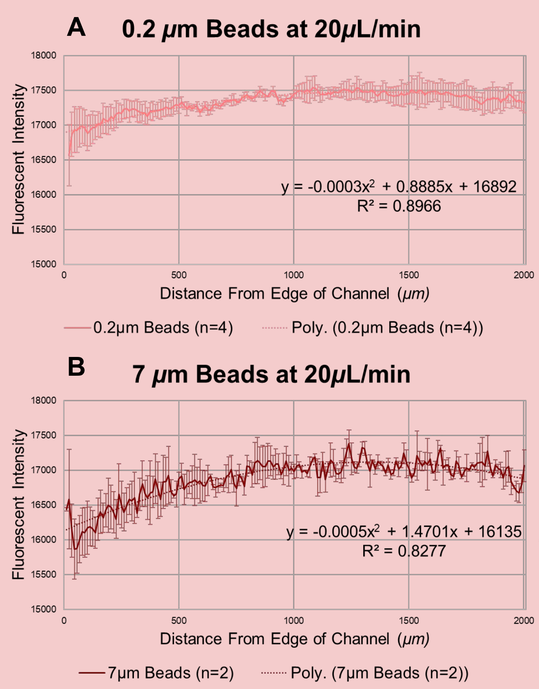


Figure 2: *Effect of EM PEGylation density on viscosity, tested at 2k [A] and 5k [B] with $2.6*10^{13}$ particles of EM/mL PBS. Mean curves plotted without error bars. Hematocrit of rabbit blood normalized to 40% ± 1% with an average of 41.11%.*

Results: Microfluidic Flow Modeling



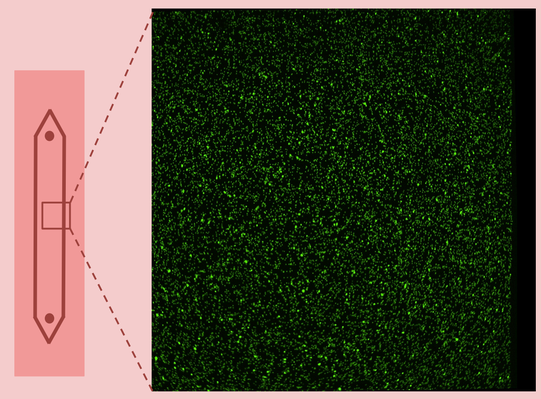


Figure 3*: Fluorescent Intensity across the channel width (total width of 4000 μm) viewed at 4x magnification. The beads were ran at 20 μL/min with 0.2μm diameter beads (diameter of EM) [A] and 7μm diameter beads (diameter of Red Blood Cell) [B]*
Figure 4: *Example of visualization of region of interest on 7μm bead images [top right]*

Conclusion:

- For **PEG 2k**: as EM PEGylation increased, the **viscosity increased** as expected, with EM PEG 2k 5% having only a **3.3% difference** to WB at 0.5 s^{-1} (physiologically relevant microcirculation shear rate[3]). For **PEG 5k**: as EM PEGylation increased, the **viscosity decreased**, likely due to decreased density from the 5k PEG replacing the Hb in EM.
- When comparing the yield points between WB and all experimental trials, all **p values > 0.05**, see *Fig. 1*, therefore there was **no statistical significance** of PEGylation on the yield points.
- **PB** sizes showed **increased centralized flow** for the **7μm** vs. the **0.2μm** beads, as expected, determined by comparisons of their second derivative (-0.0003 and -0.0005). Indicating that this method could be adequate for visualizing EM in the future.

Bioethical Implications:

Animal Considerations: Animal use in experiments

Insurance Coverage: Cost for general public

Future Testing: In vivo: animals/humans

Future Work:

Rheology:
1) Test rheology of EM in **human serum** albumin, to **replicate** actual **transfusion conditions**

Microfluidic:
1) Testing with fluorescent beads of **differing sizes** at the same time
2) Testing **EM** using **varying PEG** length

In vivo testing with **PEGylated EM** in **animal models**

References:
1. Ghanbari A, Mousavi Z, Heuzey MC, Patience GS, Carreau PJ. Experimental methods in chemical engineering: Rheometry. Can J Chem Eng. 2020;98(7):1456-1470. doi:10.1002/cjce.23749
2. ZEISS Microscopy Online Campus | Introduction to Spinning Disk Microscopy. Accessed April 22, 2024. https://zeiss-campus.magnet.fsu.edu/articles/spinningdisk/introduction.html
3. Merrill EW, Gilliland ER, Cokelet G, Shin H, Britten A, Wells RE. Rheology of blood and flow in the microcirculation. J Appl Physiol. 1963;18(2):255-260. doi:10.1152/jappl.1963.18.2.255