Project Description

Title: Synthesis of Artificial Red Blood Cells from Polymersome
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Advisor: Dr. Dan Hammer

Creation of artificial red blood cells could be life-saving for patients suffering from certain diseases like sickle-cell anemia where patients do not get enough oxygen (supplied by red blood cells) to their tissues and organs. Syung set out to synthesize artificial red blood cells from nano-sized vesicles known as polymersomes and succeeded in generating the precursors for the red blood cells. He also carried out simulations to predict the performance of those cells.

Synthesis of Artificial Red Blood Cells from Polymersome

BE492 Fall 2009
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Mentor: Dr. Greg Robins, Department of Bioengineering

Background

- Red Blood Cell related diseases include: Sickle-cell Disease, Thalassemia, Hemolysis and etc.
- 1 in 5,000 African Americans are affected by SCD in the United States, according to National Institute of Health.
- Problem of patients = insufficient oxygen supply to necessary tissue and organs
- Synthesizing Artificial Red Blood Cells from Polymersomes such as PEO-PBD.
- Provides adequate oxygen supply to organs in patients with RBC related diseases.

Hypothesis, Objective, and Goal

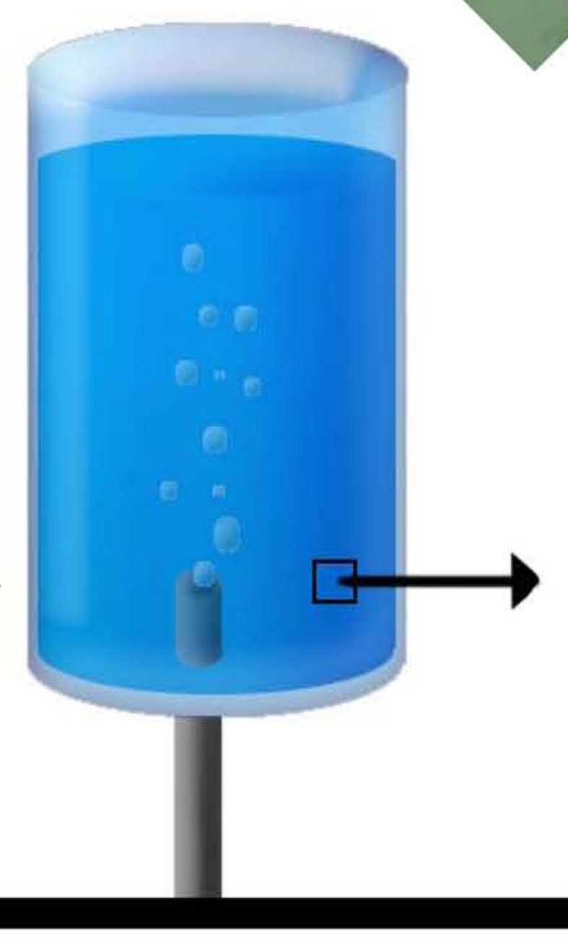
- Synthesize and characterize nano-sized polymer vesicles encapsulated with human hemoglobin.
- Develop the simulation system that generates the vesicle membrane permeability value, using the oxygen concentration measured in experiment.
- Polymersome membrane tunings such as increasing membrane thickness or cross linking the polymers will result in less permeable, but more stable vesicles.

Project Illustration

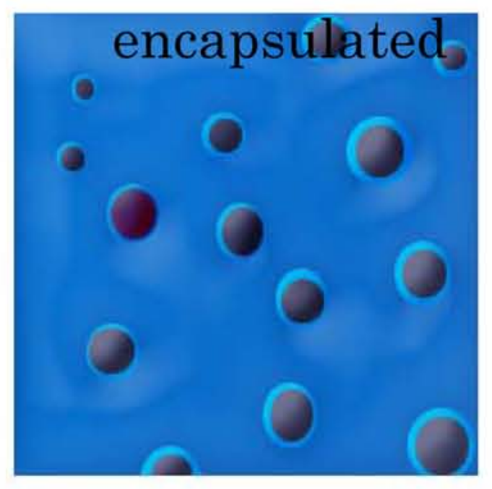
Vesicle formation using sonicator

Sonicator

Vesicle membrane permeability measurement experiment



Nano-sized polymersome vesicles with hemoglobin molecules encapsulated



Experimental Procedure

- Extract hemoglobin from blood → Hemoglobin purification → Measure concentration of hemoglobin. (e.g., Gel electrophoresis, UV testing, MetHb Concentration.)
- → Make vesicles from polymersomes → Encapsulate hemoglobin in vesicles → Measure encapsulation percent after lyses / Measure size of vesicles
- → Centrifuge and discard unencapsulated hemoglobins → Bubble in nitrogen gas until vesicles are fully deoxygenated. Then, bubble air until vesicles are fully oxygenated. Bubble nitrogen gas again until vesicles are fully deoxygenated. (Use oxygen microelectrode)
- → Use simulation model to construct an equation, and use data gathered to obtain the membrane permeability

Results

$$V_s \cdot \frac{d[O_2]_s}{dt} = P_i([O_2]_a - [O_2]_s)(A_i + A_b) + P_v \cdot A_v([O_2]_v - [O_2]_s)$$

$$\frac{d[O_2]_v}{dt} = (\frac{P_v \cdot A_v}{V_v})([O_2]_v - [O_2]_s) - \frac{d[Hb]_o}{dt}$$

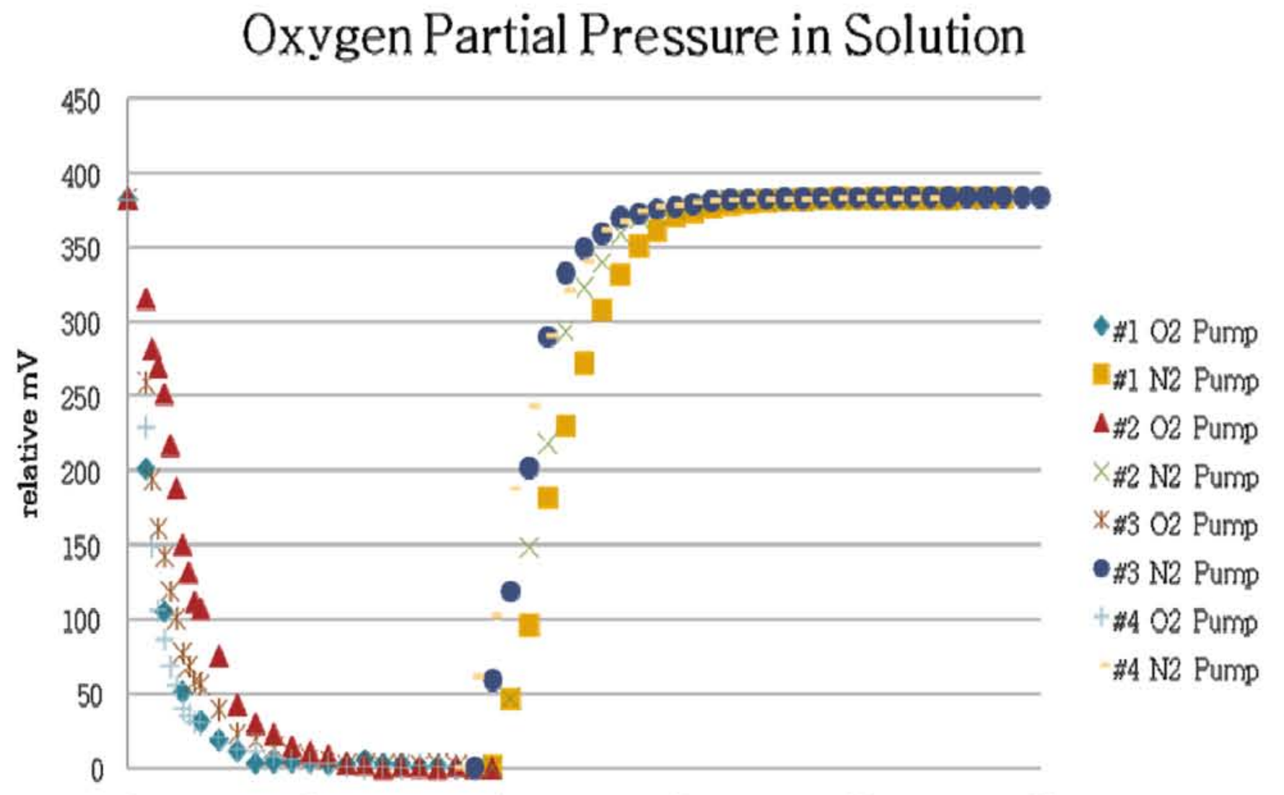
$$\frac{d[Hb]_o}{dt} = K_{on}[Hb]_v[O_2]_v - K_{off}([Hb]_0 - [Hb]_v)$$

Radius of vesicles	75-100nm	**Concentration of oxygen in**	0.273mmole/L
% composition of vesicle	4% experimentally	**K_{on}***	40,000/(M*s)
Concentration of total hemoglobin	70mmole/L	**K_{off}***	1,000/s

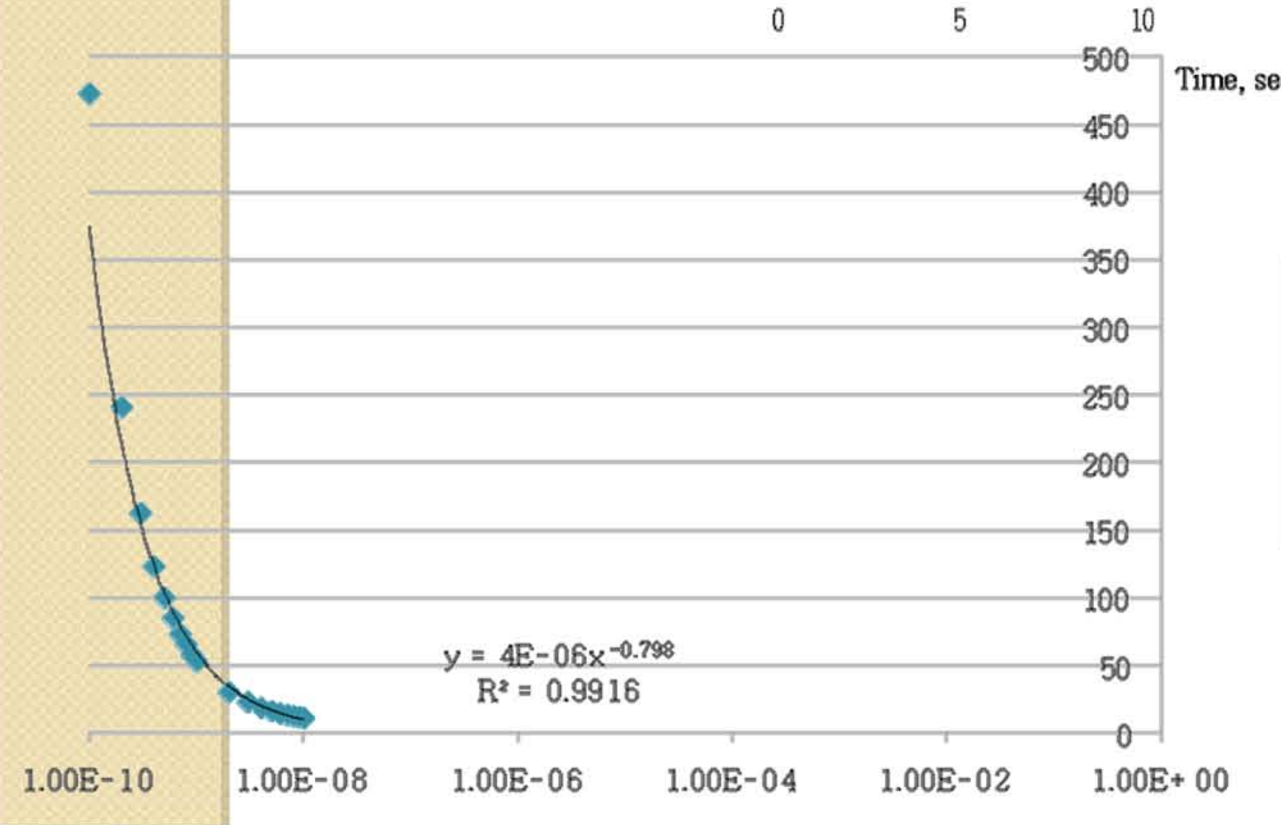
* Leow, M. Configuration of the hemoglobin oxygen dissociation curve demystified. *Adv Physiol Educ 31:198-201, 2007.*

	Trial 1	Trial 2	Trial 3	Trial 4
$T_{1/2}$	93 sec	85.8 sec	87 sec	91.2 sec
Membrane Permeability	1.0468822E-07 m/sec	1.1164369E-07 m/sec	1.1041268E-07 m/sec	1.0633438E-07 m/sec

Results



Vesicle membrane permeability measurement using the oxygen microelectrode



Simulation model usage to obtain the membrane permeability value from the measurement above.

Experimental Adjustment

- Inducing condition for creation of nano-sized vesicles with hemoglobin molecules encapsulated was from high temperature to room temperature.
- Separation of unencapsulated hemoglobin was done by different method of using filter tubes of 300K.
- Percent of vesicles in total solution has been increased in the simulation model.

Conclusion, Recommendations and Acknowledgement

- Hemoglobin was extracted from human blood and purified.
- Stable nano-sized vesicles encapsulated with hemoglobin were synthesized.
- Simulation model was established to predict vesicle permeability from oxygen partial pressure measurements in solution.
- Different polymersome vesicle testing is still under progress.