Purification of Hemoglobin from Red Blood Cells Using Tangential Flow Filtration (TFF)

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Background

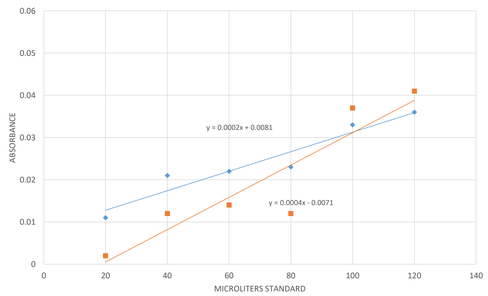
- Donor blood is vital to the success of organ transplants, transfusions, and other invasive surgeries. These patients often need fresh blood immediately, especially when used for military patients.
- Clean blood is necessary but it is not the optimal solution as there is a shortage of donor blood and it only has a shelf life of 42 days.
- A good substitute for blood is **purified hemoglobin (Hb)** derived from blood.
  - ❑ Performs the basic function of blood; delivering oxygen to cells and bringing carbon dioxide to the lungs.
  - ❑ Has a shelf life of about 1+ years (more research needed to find exact time).
  - ❑ Universally accepted meaning it has no "blood type".
- Hemoglobin is found in red blood cells (RBC) with 270 million Hb in each cell, and there is between 20-30 trillion RBCs in a human body.
- A process for the extraction of Hb needs to be developed that minimizes product loss and doesn't compromise the solution with contaminates or exposure to oxygen.

Methodology

- We observed the ultrafiltration system setup in the lab that was already designed for our project.
  - ❑ From these observations we found issues with air bubbles, pressure rates, and the bags holding solutions.
- First, the blood is run through a system to get the clots out of the blood.
- Then, to prevent bacteria from growing, the blood is placed in an ice box and shaken by an orbital shaker.
- Membranes were used to separate material from the blood in order to get a solution that was only Hemoglobin.
  - ❑ One tangential flow filtration membrane with a 100 kD membrane for the initial blood source, which filters any material less than 100 kD into the next filtration system, and the material greater than 100 kD into the original blood bag to be refiltered.
  - ❑ Another tangential flow filtration membrane with a 30 kD membrane is used for the second filtration of the blood, filtering the material less than 30 kD into a waste container and the material greater than 30 kD back to the second bag to be refiltered.
  - ❑ Hemoglobin in blood is between 100 and 30 kD, creating a solution of only hemoglobin.
- Three pumps were used in the system to retain pressure in the proper direction during the filtration process.
  - ❑ Two peristaltic pumps were used; one to move the saline into the blood bag and one to push the purified Hb through the system.
  - ❑ A centrifugal pump was used to drive the original blood through the system.
- Pinchcocks were used to regulate the flow of material through the system and make sure the pressure pushes the solution in the correct direction.

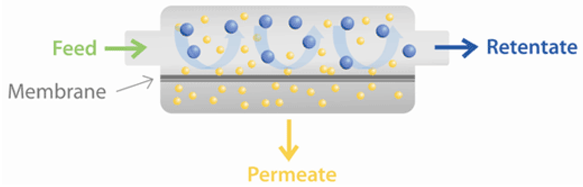
Experimental Process Images

Absorbance vs. Microliters Stock



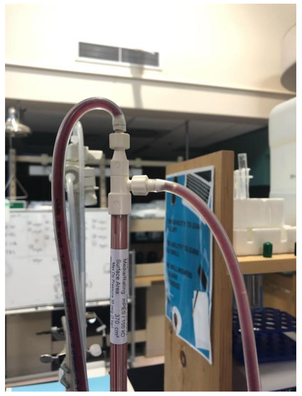
This is the calibration curve we used to calculate our concentration of hemoglobin from the measured absorbance of the solution.

How does a TFF membrane work?



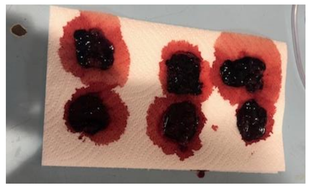
This is a tangential flow membrane. It separates one solution into two different solutions that are greater than, and less than the given membrane kD.

Blood Filtered Through the TFF



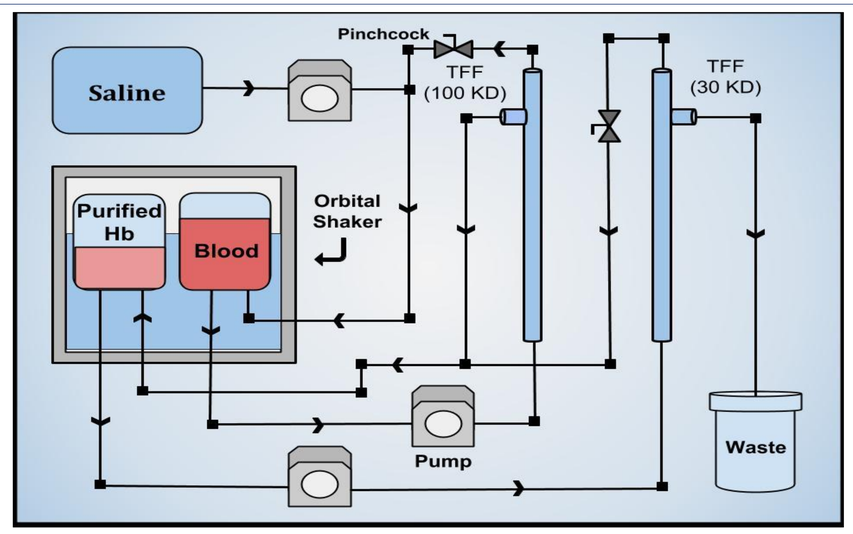
The difference in color of filtrate and retentate can be clearly seen here. The lighter the solution, the higher the concentration of hemoglobin.

Removing Blood Clots



These are the clots we filtered out of the blood before we ran it through the TFF system.

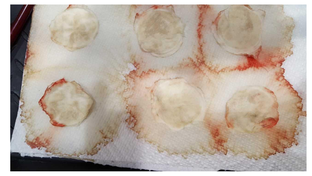
The Purification Process



The setup of our tangential flow filtration system (TFF) used to separate hemoglobin from the blood.

Results

- We are able to get a visibly purer solution of hemoglobin
  - ❑ The intensity of red in the blood solution indicates proteins, so a less red solution means you have removed some of the proteins and driven up the concentration of the hemoglobin.
- For production purposes, an automated system with pressure gauges and controller should be set up to control the system flow rates using the pumps. An optical sensor can indicate when the solution is at the desired concentration.



Clearly seen above, after ultrafiltration the blood solution no longer clots.

Conclusions

- Separating hemoglobin from blood is the easy step; our setup was able to isolate the hemoglobin from its bigger and smaller counterparts. The challenge lies in keeping the blood pure and oxygen-free.
- Shaking the bags in an ice bucket prevents unwanted bacteria from growing and also reduces the possibility of clots forming.

Future:

- Develop a way to keep air completely out of the system, allowing us to produce purer hemoglobin that is not saturated with oxygen.
- Measure flow rates in and out of each bag, allowing us to consistently add the correct amount of saline.
- Create a bag that has outlets and inlets that are placed apart from each other, preventing saline from flowing straight through the bag.
- Find a way to fill the second bag, which is initially empty, with a solution that will not contaminate the blood. We want to do this to keep oxygen out of the bag and from binding to the oxygen.

Acknowledgements

Innovation Scholars Program, College of Engineering and Physical Sciences, Devahn Dembro, Shadi Zamani

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