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Encapsulation of Glucose in Core-Shell Microcapsules for Improved Red Blood Cell Storage

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Introduction:

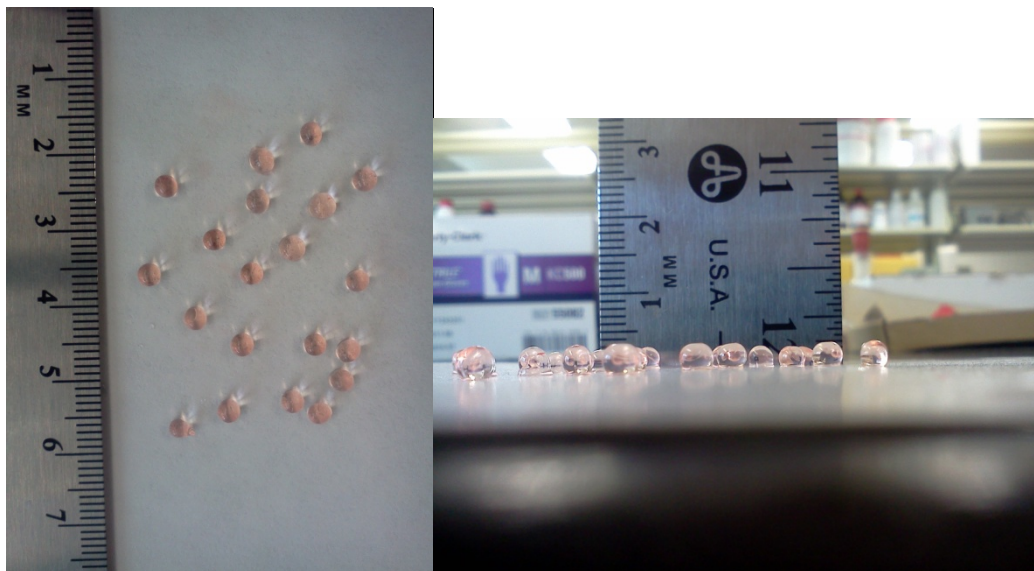
Blood transfusions have always been justifiably associated with a number of complications stemming from the transfused blood, itself. Many of these adverse effects results from the transfusion medium the blood is stored in prior to the transfusion process taking place¹. The medium used for storing the blood depends on the length of time the blood is being stored for; for example, in rare cases, blood can be stored for a maximum of 10 years using the process of cryopreservation where the blood is stored in a glycerol solution and kept at subzero temperatures in order to keep any cellular activity from taking place. However, such long storage comes with a number of complications such as the formation of ice crystals in the solution and is only used for rare blood samples. Most commonly, cryopreservation is used for storing other cells such as sperm cells, oocytes, embryonic cells, and ovarian and testicular tissue. The two most common methods of storing blood results in storage for up to 35 or 21 days depending on the solution and environment the red blood cells are being stored in. When red blood cells are being stored in a solution of citrate-phosphate-adenine (CPDA1) solution, it can be stored for up to 35 days. However, if storage is in acid-citrate-dextrose (ACD), citrate-phosphate-dextrose (CPD), or citrate-phosphate-double dextrose (CP2D) at around 5 degrees Celsius, maximum storage is for about 21 days². There are other methods that can store red blood cells for up to 42 days while keeping them alive; however, for blood that is permitted for transfusions, this length of time does not maintain the cells healthy enough for being used in transfusions. The issue with these solutions is that in essence the blood is being stored in a medium that is completely saturated with dextrose, which is what red blood cells, erythrocytes, feed on. These solutions contain approximately 4.8 femtomoles of glucose per red blood cell when in euglycemic conditions there is only about 1.2 femtomoles per red blood cell. This poor glycemic control causes the erythrocytes to be exposed to a hyperglycemic environment due to the constant exposure of about 4 times more glucose per red blood cell than in the human body. Typically, erythrocytes have the lifespan of about 120 days; however, due to the number of complications resulting from transfusions, 35 days is current length of time blood can be stored safely and still be used for transfusions³. Some notable similarities that transfused whole blood and diabetic blood share include lower concentrations of 2,3-DPG (2,3- diphosphoglycerate) which lowers the red blood cells ability to release O₂, increased microparticle budding on cell membrane which are usually filtered out by the liver; however, there is nothing to filter out transfused blood, and lastly, increased levels of Advanced Glycation endproducts (AGE) which are believed to play a causative role in the vascular complications of diabetes mellitus⁴.

One method of approaching this is to expose the erythrocytes to the ratio of dextrose in accordance with a euglycemic environment by controlling the release of dextrose being exposed at a time instead of drowning the RBCs with excess dextrose in order to minimize the risk of specific complications such as hyperglycemia⁵. A few approaches to controlling the glucose release is through

the use of core shell microcapsules. The idea is to place a glucose-enriched microcapsule into the solution containing the RBCs with a semi-permeable membrane that will only allow enough glucose to be released as the RBCs need to continue their normal metabolic activity⁵. This should contain the RBCs in a more natural environment and decrease the chance of hyperglycemia and other adverse effects since there will not be a large excess of dextrose being exposed. The goal is to increase the lifespan of stored RBCs to over 30 days while still maintaining the cells in a healthy state.

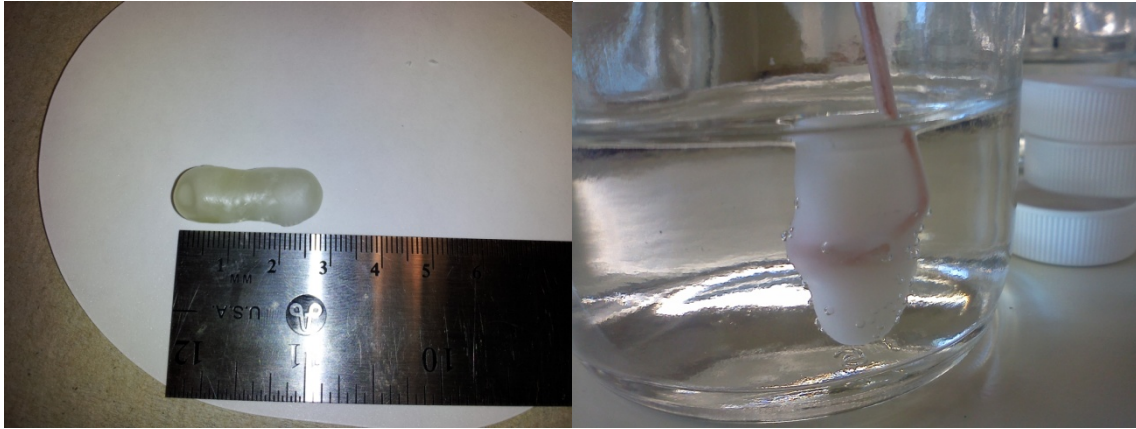
Experimental:

There are many different recipes and approaches that we have or plan on studying as attempts to control the release of glucose. At the beginning of the summer, I began studying the use of dextrose enriched hydrogels through the process of reacting sodium alginate with calcium chloride. In essence, the partial (-) charge found in the chains of sodium alginate are connected to each other by bonding to Ca^{2+} . Since the calcium has a +2 charge, it can bond to any two chains and thus the wall is formed when an excess of calcium is provided. This wall is a random network of chains connecting the polymer backbones and thus creating a hydrogel. Initial concentrations of sodium alginate hydrogels had release rates that were too high; however, lately I have been incorporating chitosan into the sodium alginate to strengthen the membrane in hopes to lower the release rate of dextrose. This method did prove more effective than the original hydrogels; however, not effective enough.



Sodium Alginate Capsules dyed with Congo Red

Therefore, another approach has been used in lab is through the use of gelatin capsules, which were incorporated for a number of reasons. There is ample space to work with inside them, a multitude of sizes, and the ability to use multiple layers when encapsulating. Unfortunately gelatin is water soluble and thus, the initial results were discarded since the capsules swelled up and broke. This has been solved by encapsulating the gelatin in a water insoluble wax and only exposing a small portion of the gelatin which also lowers the surface area for the dextrose to release from.



Gelatin Capsules Encapsulated in Wax

Dextrose is an extremely small monosaccharide and thus is very difficult to maintain inside a shell and control outward release at desired rates. Due to this nature of the molecule, the idea of using a larger starting molecule sparked the use of enzymatic reactions as a new approach to controlling glucose release. This way the molecule on the inside of the capsule could be a disaccharide or polysaccharide and as the enzymes break them down, the monosaccharide portions broken off could freely travel out of the capsules. For the experiments ran in lab, the first enzyme used was Lactase with the disaccharide sugar, lactose. When these two react with each other lactose is broken down into glucose and galactose in a 1:1 ratio. Enzymatic reactions have given us the most steady and consistent release thus far.

In addition to studying enzymatic reactions, I am currently working with a new hydrogel using PVA and Glutaraldehyde as the cross linking agents. This new hydrogel is has a high level of biocompatibility to ensure it is safe to use; however, an initial problem that arose is that the synthesis of the hydrogel involves a significant amount of sulfuric acid as the catalyzing agent. This problem was eliminated by adding a final step to the synthesis which was to place the hydrogels in a buffering solution once they had fully formed. This was important to take into consideration because this project is dealing with the hopes of using a hydrogel that will be placed in a solution of red blood cells; due to this nature of the project, every hydrogel that can be considered must be as biocompatible as possible with the human body. I began by synthesizing varying sizes of the gel as well as concentrations of the crosslinking agent. Two approaches were primarily used to load the solute into the PVA hydrogels. The most effective method was allowing the gel to soak in a dextrose-enriched solution overnight. The samples were then placed in their testing mediums and showed consistent release rates for only about one week before the release became too unpredictable. Testing with this hydrogel has not yet finished as more tests are being taken expanding from the data from the original set of samples.



PVA Hydrogels

¹ Colleen Koch, Liang Li, Daniel Sessler, Priscilla Figueroa, Gerald Hoeltge, Tomislav Mihaljevic, Eugene Blackstone, 2008, Duration of Red-Cell Storage and Complications after Cardiac Surgery, *New England Journal of Medicine* 358: 1129-1239.

² Circular of Information for the use of Human Blood Components, 2010, *AABB, ARC* 1: 16.

³ Blood and Blood Disorders, *MedlinePlus and the National Institute of Health*.

⁴ Xiong, Z. *et al.* Red Blood Cell Microparticles show altered inflammatory chemokine binding and release ligand upon interaction with platelets. *Transfusion* DOI: 10.1111/j.1537-2995.2010.02861.x (2010).

⁵ Conan McCaul, Ciaran Moran, Donal O'Cronin, Finola Naughton, Michael Geary, Edmund Carton, James Gardiner, 2003, Intravenous Fluid Loading with or without Supplementary Dextrose does not prevent Nausea, Vomiting and Pain after Laparoscopy, *Canadian Journal of Anesthesia* 50: 440-444.