INTRODUCTION

Sickle cell disease (SCD) is the most common genetic disease worldwide [1]. SCD is caused by a single mutation in the β-globin gene, which leads to the development of defective sickle hemoglobin (HbS). HbS polymerizes in low oxygen conditions and causes the red blood cells to deform into a sickle shape and become rigid and “sticky.” The sickling of red blood cells can result in occlusion of the microcirculation, causing pain and tissue death from lack of oxygen [1].

Current treatments for sickle cell disease (SCD) are limited. There are drugs, such as hydroxyurea (HU), that increase the concentration of fetal hemoglobin in a patient’s bloodstream. The increase of fetal hemoglobin helps to inhibit the polymerization of HbS, therefore helping to limit many SCD complications, such as episodes of acute pain [2]. HU can lead to a depression of a patient’s immune systems due to a reduced white blood cell count. HU has also been found to cause thrombocytopenia as the drug can decrease platelet count [3]. However, the severity of SCD is not solely dependent upon the fetal hemoglobin concentration, therefore other treatments for SCD patients are required.

The other main treatment for SCD is a healthy donor RBC transfusion, which is more commonly used as a treatment for anemia following a sickle cell crisis than as a preventative measure. Chronic blood transfusions have been found to help greatly reduce the risk for stroke, acute chest syndrome, and spleen issues. However, chronic blood transfusions can lead to increased alloimmunization and dangerously high levels of iron, which can cause liver failure [4]. The alloimmunization can lead also to serious complications, such as hyperhemolysis. While the hemochromatosis can be treated successfully with iron chelation therapy, patients receiving multiple transfusions can develop alloantibodies that make it increasingly difficult and eventually impossible to find a compatible donor. SCD treatment options are very limited, and there is currently no alternative to blood transfusion [5].

As the majority of problems from SCD are caused by the defective sickle hemoglobin, Dr. Kameneva (McGowan Institute for Regenerative Medicine) and Dr. Waters (Magee Women’s Hospital) have proposed to remove the defective hemoglobin from lyed patient’s RBCs and replace it with normal human or polymerized bovine hemoglobin (Pittsburgh University OTM Invention Disclosure, October 2013), thus reducing alloimmunization in patients with SCD in need of blood transfusion.

It has been established that RBCs can be lysed and drained of the hemoglobin inside the cells, then refilled with a hyperosmotic solution to create transparent ghost RBCs [5]. The hyperosmotic solution used to refill the RBCs could contain a drug, essentially making RBCs that are able to distribute drug throughout the body. This method of drug delivery is completely biocompatible and elicits no immune response, as a patient’s own cells are being transfused [6]. It is currently unknown if it is possible to refill ghost red blood cells with hemoglobin.

OBJECTIVE

The objective is to determine if it is possible to refill human ghost red blood cells (RBCs) with either human or polymerized bovine hemoglobin. Refilling solutions of 1%, 5%, and 10% hemoglobin will be tested.

HYPOTHESIS/SUCCESS CRITERIA

Based on microscopic observation, at least 50% of the RBCs should be refilled. A reasonable hemoglobin concentration should able to be measured in the RBCs following the procedure. For the protocol to be considered successful, the RBCs should be refilled 90% of the time.

METHODS

For this study, human blood and polymerized bovine hemoglobin (HBOC, Biopure Co.) was used. To create the ghost RBCs, packed RBCs were washed using phosphate buffered saline (PBS). After washing, the RBCs, refilling solution, lysing solution, and Tris buffer were all placed on ice and cooled to 0°C. For this study, the refilling solution consisted of 5X PBS and hemoglobin. Refilling solutions of 1%, 5%, and 10% were studied.

After the solutions reached the desired temperature, the refilling solution and Tris buffer were added to the RBCs after 5 minutes, and then the solution was placed back on ice overnight. Resealing of the cells occurred the next day by removing the cells from ice and placing them into a hot water bath, which was at 37°C. The cells were then washed using PBS in a high-speed centrifuge at 18,000g. The cells were then able to be collected.

The collected cells were observed under a light microscope in order to determine if they had successfully refilled or not. If a successful refill occurs, the cells rheological properties are then tested using a Linkam shearing stage and a Brookfield cone-in-plate rheometer. These devices measure deformability and viscosity, respectively.

RESULTS

From this study, only one successful RBC refill has been achieved (see Figure 1). Our current protocol does not consistently result in refilled RBCs and therefore needs to continue to be modified in order to achieve better results.
DISCUSSION

It has been determined that it is difficult but possible to refill human RBCs with polymerized bovine hemoglobin. As success has been extremely limited with the current protocol, modifications still need to be made in order to achieve better consistency.

Once this protocol has been finalized, it will then be applied to refill RBCs from SCD patients that contain HbS. The first step will be to ensure that normal ghost RBCs can be made from the SCD cells. If it is possible to refill the cells with PBS, refilling with polymerized bovine Hb will be attempted. These procedures may require further modifications of the protocol as sickle cells may exhibit different sensitivity to osmotic pressure. Finally, various physiological and rheological parameters will be tested to ensure that the modified RBCs are valid for human circulation. The analysis of the refilled sickle cells would then be compared to established rheological data of normal red blood cells to see if the two cell groups behaved in a comparable manner.

Currently, there is no FDA approved polymerized bovine hemoglobin in the United States [6]. Due to this, the source of hemoglobin is a limitation to the study. If FDA approval is not obtained for the hemoglobin, this study would not be applicable clinically. The time it takes to refill the RBCs with hemoglobin is also a limitation. It takes two days to refill the cells using the current protocol, so this time would have to be greatly reduced in order to be used with SCD patients.

The ultimate goal of this study is to develop a new treatment for SCD patients, therefore expanding their currently very limited treatment options. While chronic blood transfusions greatly improve patient quality of life, many patients are unable to continue this vital treatment due to alloimmunization. If their own cells could be refilled with normal hemoglobin, patients could receive fewer transfusions, which would lessen SCD complications and reduce patient morbidity and mortality.

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REFERENCES