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# Erythrocyte Deformability in Response to Glucose Using Liquid Crystals

An undergraduate honors thesis

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College of Engineering

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By

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**Abstract**

The worldwide prevalence of diabetes mellitus is rapidly increasing with about 9.3% of the adult population living with the disease. People with diabetes have trouble regulating their blood glucose levels which typically leads to hyperglycemia. Under normal physiological conditions, erythrocytes can undergo deformations in response to shear stress when passing through capillaries with a smaller diameter. Poorly managed hyperglycemia can lead to the glycosylation of erythrocyte membrane proteins and hemoglobin. This glycosylation leads to increased rigidity of the cells along with decreased deformability in response to mechanical stress; therefore, these cells have a higher susceptibility of getting stuck in the microvasculature leading to a greater chance of cardiovascular complications. Currently, several methods can assess the deformability of red blood cells, but many of the methods don't utilize a solvent that accurately mimics blood plasma, and they tend to be time-consuming. This study utilizes liquid crystals (LC), specifically disodium cromoglycate (DSCG), which are highly organized molecules that exhibit anisotropy when in the nematic phase. When red blood cells are transferred from a pure aqueous solution to the nematic phase of LC, they exhibit shape changes from a biconcave disk to an elongated oval. The use of DSCG in this study along with microscopy could serve as a novel method for the assessment of erythrocyte deformability when exposed to different glucose concentrations. Results were gathered using ImageJ to extract aspect ratio data from microscopic images. The results were compared between the varying glycemic states (5 mM, 45 mM, 100 mM) to confirm the trend of increased glucose concentrations leading to decreased cell deformability. The data obtained indicated this trend, yet more will

need to be collected for this to be considered a viable method. Additionally, the incorporation of machine learning will enable this method to become a novel diagnostic tool for assessing blood glucose concentration.

## Introduction

The worldwide prevalence of diabetes in adults (individuals over the age of 18) has increased dramatically over the years [1]. In 2019, it was estimated that approximately 9.3% of the worldwide adult population had diabetes mellitus [1]. This amounts to about 463 million individuals living with diabetes. Diabetes mellitus is a metabolic disorder that affects the way an individual regulates their blood sugar. Individuals with this disorder experience abnormalities with a hormone called insulin. Insulin is a hormone secreted by the beta islet cells of the pancreas in response to elevated glucose levels in the bloodstream which is typically the case after a meal. Under normal physiological circumstances, insulin acts to stimulate GLUT4 transporters on the surface of cells, typically muscle and adipose tissue, which allows them to uptake the excess glucose in the plasma [2]. These cells are then able to utilize this glucose for their metabolism through the glycolytic pathway. There are two main types of diabetes, Type 1, and Type 2. Type 1 diabetes, the less common of the two types, is an autoimmune disorder in which the body's immune system attacks the beta cells in the pancreas [3]. This causes the pancreas to lose its ability to produce insulin sufficiently which prevents the uptake of glucose by the body's cells. The other main type, Type 2, is caused by insulin insensitivity in which cells don't respond as they should when exposed to insulin [3]. Both types result in a higher-than-normal amount of glucose within the individual's

blood. This can lead to a variety of cardiovascular complications. One such complication that this paper will focus on is the glycosylation of erythrocytes (red blood cells). Glycosylation is the addition of a glucose moiety to a protein. When exposed to high glucose concentrations in the blood, erythrocyte membrane proteins, as well as hemoglobin, become glycosylated which leads to decreased membrane fluidity and deformability [4] – [6]. Under normal physiological conditions, erythrocytes have the capacity to deform in response to mechanical stress [5], [6]. This feature allows erythrocytes, which typically have a diameter of 7-8 microns, to pass through capillaries, which are estimated to have a diameter anywhere between 4-9 microns, without getting stuck and causing a clot [5], [6]. Glycosylated erythrocytes tend to have difficulty passing through these microvessels due to their decreased deformability which can lead to clots and ultimately localized ischemia, or low blood perfusion [5], [6]. In diabetic individuals, this could present itself as diabetic retinopathy, a complication that affects vision, or foot ulcers [5].

Based on how high blood glucose influences the deformability of erythrocytes, assessing their deformability could be beneficial in determining an individual's current glycemic condition. Once determined, intervention could be implemented so that the individual doesn't experience the adverse consequences of this pathology. There have been several studies dedicated to various methods of assessing the deformability of red blood cells in response to elevated blood glucose levels. One such study involved assessing their deformability using microporous membrane filtration [6]. In this study, a membrane was created that had pores that simulated capillaries by having a similar

diameter to that of these vessels [6]. An erythrocyte suspension was created and was forced through the membrane using a pressure-driven flow that mimicked typical blood pressure [6]. The amount of time that it took the suspension to pass through the membrane was recorded [6]. Red blood cells that were suspended in a solution with a higher concentration of glucose had a longer passage time through the membrane due to their decreased deformability [6]. Another study assessed the deformability of the erythrocytes using optical diffraction patterns or ektacytometry [6]. When going from stagnant to flow conditions in a microchannel or rheoscope, the erythrocytes changed shape from a biconcave disc to an elongated ellipse [6]. This change in shape was measured by a shift in diffraction pattern [6]. The extent to which the shape of the erythrocytes changed was assessed using a parameter called the elongation index [6]. An alternative study observed the deformability of red blood cells using optical tweezers [6]. The optical tweezers trapped the erythrocytes using lasers and drug them through a viscous medium to assess how much deformation they had undergone [6]. All the aforementioned methods tend to be time-consuming and use a buffer, phosphate-buffered saline (PBS), that isn't indicative of human blood plasma [6]. The method proposed in this paper has the potential to be a more rapid process with the incorporation of machine learning as well as the use of a salt solution that more closely simulates the solute concentration of blood plasma.

The method addressed in this paper incorporates liquid crystals (LCs) which are crystalline compounds that have the physical properties of a liquid. In other words, they are highly organized molecules but have properties that allow them to behave like a

liquid and undergo flow. A typical property that makes LCs so desirable in research is their ability to generate an anisotropic, fluidic environment [7]. Anisotropy is a description of a physical property that indicates it is not consistent in all orientations. Isotropy is just the opposite and means that the physical properties are equivalent in all directions. The reason being able to create an anisotropic environment is so beneficial is that doing so provides a method for inducing mechanical stress in a single direction [7]. Disodium cromoglycate (DSCG) was the type of LC used in the study. The main reason for the use of this specific LC is that, unlike most other LCs, DSCG is not a significantly amphipathic molecule [7]. Therefore, DSCG has particularly weak chemical interactions with biological membranes making it ideal for this study [7]. There are many different phases of LC arrangement, but the phase that was utilized in this study was the nematic phase. The nematic phase is simply the phase at which DSCG molecules arrange themselves by stacking on top of one another and forming columns [7]. These columns inherently generate an anisotropic environment due to the anisotropy of the columnar shape [7]. The nematic phase is reached once the concentration of DSCG has reached a certain threshold. In a previous study, this concentration threshold was determined to be 17.3% by weight [7]. The nematic phase of DSCG was exploited in the study to mimic the mechanical shear stress experienced by red blood cells such as when traveling through microvasculature in vivo. The application of constant shear stress along with microscopy allows for the assessment of red blood cell deformability under various concentrations of glucose.

The study aims to utilize DSCG liquid crystals in conjunction with microscopy as a novel method to assess erythrocyte deformability in response to varying concentrations of glucose. The assessment will be conducted by collecting aspect ratio data using image analysis software. The data will be analyzed by comparison of experimental groups with various glucose concentrations to identify if there is any significant change in erythrocyte deformability between groups. By doing so, the paper strives to understand the correlation between blood glucose concentration and deformability. Additionally, the paper aims to determine if this novel method would be an effective diagnostic tool to determine the glycemic state of diabetic individuals.

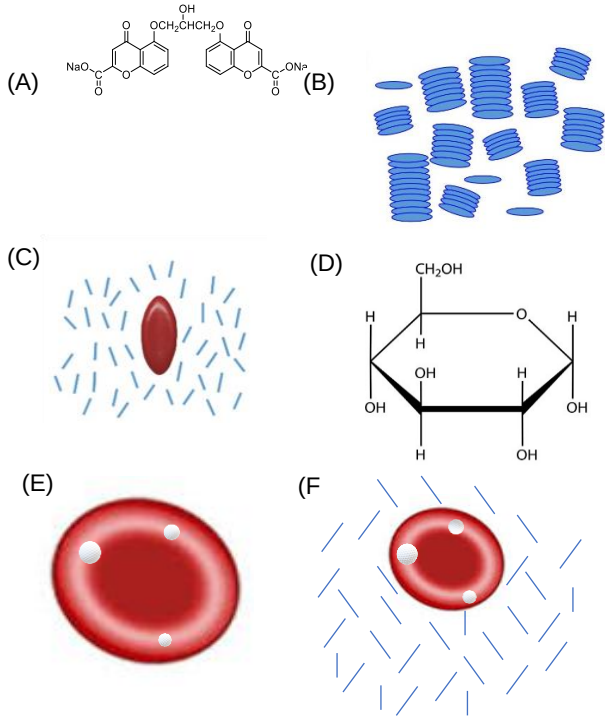


**Figure 1:** (A) The molecular structure of DSCG. (B) Schematic showing the self-assembly of DSCG molecules into columnar aggregates that exhibit order in the nematic LC phase. (C) Schematic showing erythrocyte deformation by the nematic LC phase of DSCG. (D) The molecular structure of D-glucose. (E) Normal biconcave shape of an erythrocyte. (F) Schematic showing undeformed erythrocyte suspended in the isotropic LC phase of DSCG.

## Materials and Methods

*Isotonicity Experiment*

Before beginning the assessment of erythrocyte deformability, it is essential to ensure there are no confounding variables within the experiment. Adding glucose to the solution could have an impact on the tonicity of the solution, and thus it would influence the shape of the erythrocytes. Based on previous literature, glucose has been shown to not illicit any osmotic effects on erythrocytes [8]. This is because erythrocytes have a constitutively active glucose transporter called GLUT1 [8], [9]. GLUT1 acts using facilitated transport, a form of passive transport, to bring glucose inside the erythrocyte whenever the concentration gradient is established [8], [9]. Erythrocytes need to have a constitutively active method of glucose transport because they rely solely on glucose for their energy requirements since they lack the mitochondria to perform oxidative phosphorylation. To be meticulous though and ensure this is the case, a preliminary experiment was conducted. To begin, a salt solution needed to be created to mimic the plasma solute concentration present in human blood. In a previous paper, it was determined that a salt solution of 154 mM NaCl closely mimicked human blood plasma since erythrocyte swelling or shriveling was not observed in a solution of this concentration [7]. The solution was made by adding .09 g of NaCl salt to 10 mL of deionized water in a centrifuge tube. The solution was mixed using the vortex for 5 minutes. After making the salt solution, 3000 µL of the solution was added to a separate centrifuge tube with 700 µL of human erythrocytes. 3.3 mg of D-glucose was added to this solution to create a glucose concentration of 5 mM which is the normal, physiological blood glucose concentration [10]. The solution was put on a vortex for 2

minutes to allow for sufficient mixing. Microscope slides were then prepared to visualize the erythrocytes. The slides were created by using a glass cutter to cut 2 smaller rectangular pieces from a larger glass slide. 2 pieces of double-sided tape were applied to the glass slides and the slide fragments were attached on top of one another. 10 µL from the 5 mM solution was taken and placed between the stacked slides and the double-sided tape. Grease was applied to the open ends of the slides to ensure that none of the solution leaked out while imaging. Images were taken periodically every 15 minutes using an Olympus BX41 microscope with a 40x objective lens for 3 hours. Procedures for additional concentrations of glucose were not performed since it is well documented that glucose has no osmotic effect on erythrocytes[8], [9].

*Preparation of DSCG Solution*

To begin the assessment of erythrocyte deformability, a liquid crystal solution was prepared. Based on a previous study, it was determined that a 17.3% by weight solution of disodium cromoglycate (DSCG) causes the liquid crystals to form an anisotropic environment called the nematic phase [7]. To create the solution, .173 g of cromolyn disodium salt hydrate was added to 1000 µL of deionized water in a centrifuge tube. The solution was mixed in the centrifuge tube utilizing a vortex for 2 hours. Once 2 hours had passed, the DSCG solution was removed from the vortex.

*Erythrocyte Imaging without DSCG*

3 different experimental groups were created each with a different glucose concentration. The experimental groups were 5 mM, 45 mM, and 100 mM of glucose.

The 5 mM group was made in prior steps and served as a control for this experiment. The 45 mM group was meant to simulate slightly high blood glucose levels, and the 100 mM group was intended to simulate conditions of hyperglycemia like what would be seen in individuals with diabetes [10]. 3000 µL of the NaCl solution generated in the previous experiment was added to 2 separate centrifuge tubes along with 700 µL of human erythrocytes. For the 45 mM group, 30 mg of D-glucose was added to one of the centrifuge tubes, and for the 100 mM group, 67 mg of D-glucose was added to the other centrifuge tube. These 2 tubes were placed on the vortex for 2 minutes. 3 slides were prepared in the same manner as the previous experiment. For each of the 3 slides, a 10 µL sample was taken from each experimental group and imaged using an Olympus BX41 microscope with a 40x objective lens.

*Erythrocyte Imaging with DSCG*

Next, 5 µL was taken from each of the experimental groups and placed in 3 separate microcentrifuge tubes. In each of the tubes, 40 µL of the previously made DSCG solution was added. Microscope slides were created similarly as they were previously, instead this time, before attaching the smaller pieces, the surface of the pieces was scratched with sandpaper in a single direction. This was done to ensure that, when DSCG was added to the solutions, it would be in the nematic phase and form an anisotropic environment. 10 µL were again added to the 3 additional slides from each of the solutions containing DSCG. The slides were again imaged using an Olympus BX41microscope with a 40x objective lens. Unlike in the previous images, polarized light was applied to the microscope to ensure that the LCs were in the nematic phase.

Under polarized light, the nematic phase can be recognized when the background becomes monochromatic.

*Fluorescent Imaging*

To determine if the red blood cells were becoming glycosylated when exposed to high glucose levels, fluorescence imaging was conducted. A commonly used fluorophore used for glucose imaging is fluorescein isothiocyanate (FITC) [11]. In this experiment, FITC was dissolved in ethanol with a concentration of 20 $\frac{mg}{mL}$. 2 µL of the solution was added to 200 µL of erythrocytes suspended in a 154 mM NaCl solution with 45 mM glucose concentration. This concentration of glucose was chosen because this group is made to represent slightly high blood glucose and therefore will indicate, via fluorescent imaging, that the cells have started to become glycosylated. The mixture generated was stirred gently and stored in a freezer at 4 ºC for 4 hours before imaging. The fluorescent images were gathered using an Olympus BX51 microscope, and they were analyzed to detect the presence of glycosylation of the red blood cells.

*Image Analysis*

All the images in the experiments using the Olympus BX41 microscope were analyzed using software called ImageJ. Using the software, aspect ratio data was collected from each of the experimental groups. The data was collected by finding the ratio of the long axis of the erythrocytes compared to the short axis of the erythrocytes. In the collection of this data, a line plot was generated showing the aspect ratio of red blood cells over time when suspended in glucose. Histograms were created showing the distribution of

varying aspect ratios of erythrocytes exposed to different glucose concentrations. The line plot was analyzed to determine that there is no significant difference at the various time points in aspect ratio indicating that glucose imposes no osmotic effects on erythrocytes. The histograms were analyzed to determine the extent to which the erythrocytes were able to deform in response to the anisotropic environment generated by the DSCG LCs in their nematic phase.

## Results

*Isotonicity Experiment Results*

The preliminary experiment in the previous section was conducted to determine if glucose has any osmotic effects on erythrocyte shape. Once proven to be true, there would be no apparent confounding variables affecting the assessment of erythrocyte deformability under varying glucose concentrations. Figure 2 shows the results of this experiment. It illustrates the average aspect ratio of cells within the microscope frame at 15-minute intervals for 3 hours.

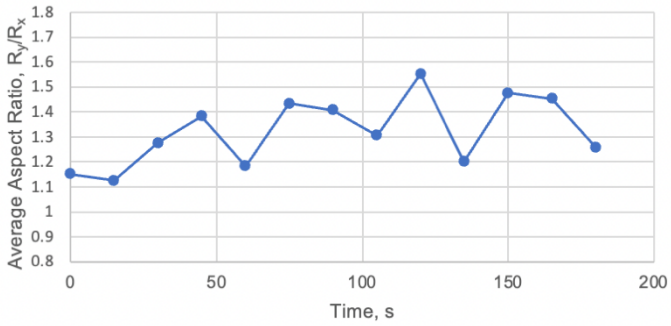


**Figure 2:** Line plot showing the average aspect ratio of all erythrocytes seen in a microscope frame at 15-minute intervals over the span of 3 hours.

*Aspect Ratio Deformability Assessment*

The main experiment was conducted to determine the relationship between blood glucose concentration and erythrocyte deformability. The microscope images obtained were analyzed using ImageJ software to assess their deformability by collecting aspect ratio data. Figure 2 shows images for each of the experimental groups without the presence of DSCG while Figure 3 shows images for each of the experimental groups exposed to DSCG. Also pictured in Figure 2 is the method for calculating aspect ratios. An aspect ratio is a parameter that illustrates the size of an object by comparing its height to its width. The major axis of an elliptical object is defined as being the longer diameter with the minor axis being defined as the shorter diameter. In Figure 2, the major and minor axes are indicated by $R_y$ and $R_x$ respectively. The aspect ratio data was collected by using ImageJ software and logging the four points for each of the erythrocytes with one point being on one end of either the major or minor axis. $R_y$ and $R_x$ were calculated using the distance formula which can be seen in Equation 1. The x's and y's corresponded to the x and y coordinates of the points along the blood cell. Points 1 and 2 were the endpoints of the major axis while points 3 and 4 were the endpoints of the minor axis.

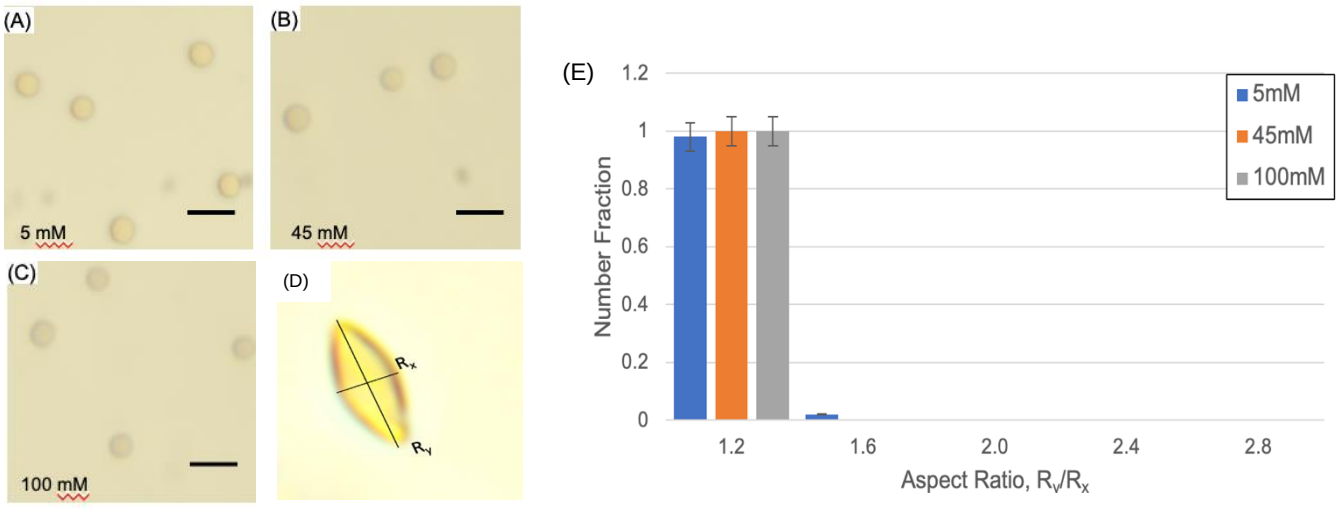


**Figure 3:** (A) 40x objective microscopic image showing human erythrocytes suspended in 5mM glucose solution. (B) 40x objective microscopic image showing human erythrocytes suspended in 45mM glucose solution. (C) 40x objective microscopic image showing human erythrocytes suspended in 100mM glucose solution. (D) Schematic demonstrating how the aspect ratio data was collected with $R_x$ indicating the minor axis and $R_y$ indicating the major axis. (E) Histogram comparing each iteration of glucose concentration (5mM, 45mM, and 100mM) in the absence of DSCG with each bin representing the average aspect ratio.

$$R_y = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}$$

$$R_x = \sqrt{(x_3 - x_4)^2 + (y_3 - y_4)^2}$$

$$Aspect\ Ratio = \frac{R_y}{R_x}$$

**Equation 1:** Equations showing how the length of the major and minor axes were determined along with the aspect ratios of each of the erythrocytes.

Aspect ratio data was collected for 50 cells from each experimental group. After the aspect ratio data was recorded, histograms were generated using Microsoft Excel. Each bin of the histogram was divided into an average aspect ratio with the height of each bar indicating the number fraction, or the fraction of total sampled cells. A histogram was created comparing each of the experimental groups in the absence of DSCG and another was created comparing the experimental groups in the presence of DSCG.

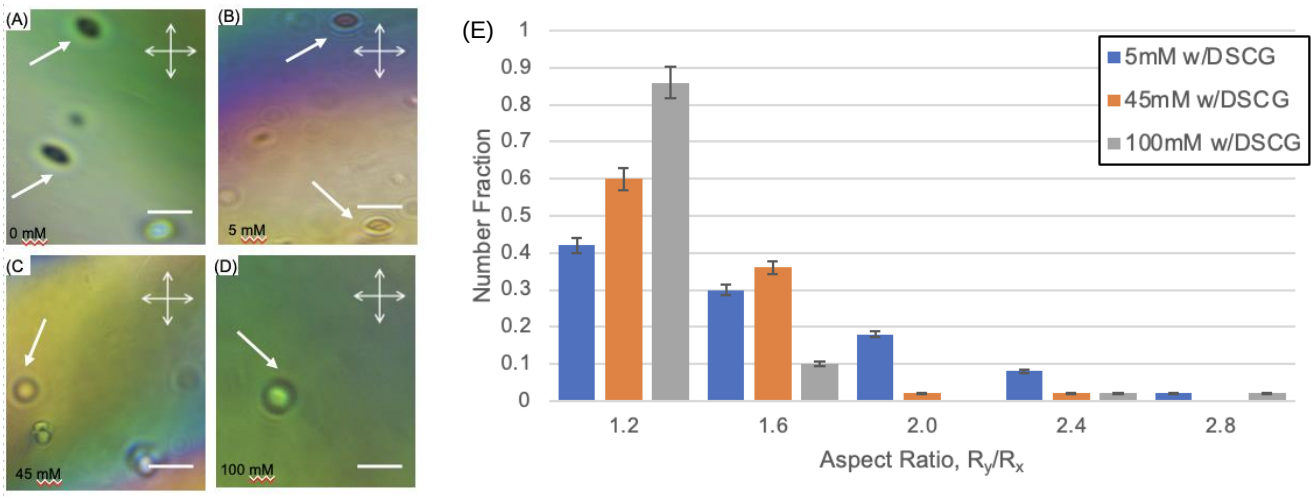


**Figure 4:** (A) 40x objective microscopic image of human erythrocytes in glucose-free solution with 17.3% by weight DSCG. (B) 40x objective microscopic image of human erythrocytes in 5mM glucose solution with 17.3% by weight DSCG. (C) 40x objective microscopic image of human erythrocytes in 45mM glucose solution with 17.3% by weight DSCG. (D) 40x objective microscopic image of human erythrocytes in 100mM glucose solution with 17.3% by weight DSCG. (E) Histogram comparing each iteration of glucose concentration (5mM, 45mM, and 100mM) in the presence of DSCG with each bin representing the average aspect ratio.

*Fluorescent Images*

Shown in Figure 5 are the fluorescent images gathered to determine if the red blood cells were becoming glycosylated. The images were analyzed by detecting the presence of green fluorescence in the images within the structure of the cell.

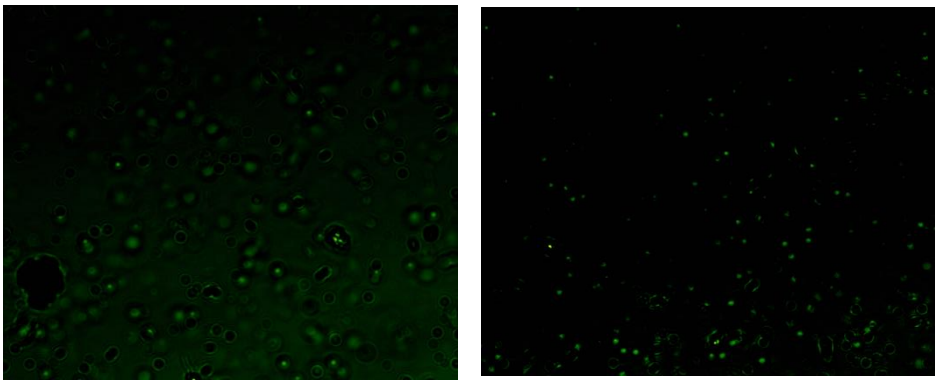

**Figure 5:** Images gathered with an Olympus BX51 microscope using FITC to detect the presence of glucose within the cells indicating the glycosylation of the erythrocytes in a 45 mM glucose environment.

## Discussion

*Isotonicity Experiment Analysis*

The results from the preliminary experiment were analyzed to determine if glucose had any impact on the osmosis of erythrocytes. The data was analyzed using a population standard deviation to determine how spread out the data is. The equation for population standard deviation is shown in Equation 2. $\sigma$ represents the population standard deviation, $\mu$ is the population mean, $n$ is the number of samples, $X$ is the sample mean, and $\Sigma$ indicates a summation.

$$\sigma = \sqrt{\frac{\Sigma(X-\mu)^2}{n}}$$

**Equation 2:** Equation showing how to calculate the population standard deviation. Population standard deviation indicates the spread of the data.

The calculated standard deviation for the average aspect ratio data was .1316. This is a relatively small standard deviation. This means that the values of the aspect ratios are relatively close together, so it was concluded that the addition of glucose has no osmotic effect on the red blood cells.

*Analysis of Erythrocytes without DSCG*

Each of the experimental groups was also analyzed to determine the effects of glucose on red blood cell deformability. All the groups without DSCG were compared to determine if the different concentrations of glucose affect the aspect ratios of the red blood cells. As can be seen in Figure 4, most of the experimental groups fall into the 1.2 category. From this, it was concluded that there is no significant difference between the

experimental groups. It was also concluded that since the aspect ratio of each of the groups is so small there was no deformation of the erythrocytes. This result is consistent with the results of the preliminary experiment as the glucose didn't cause any swelling or shrinking of the cells. Also, since the cells aren't in the presence of DSCG, there should be no observed deformation which was the result that was observed.

*Analysis of Erythrocytes with DSCG*

The experimental groups with DSCG were compared among the varying glucose concentrations. From the histogram in Figure 4, it is evident that the 5 mM glucose group shows the greatest amount of variation in aspect ratio with a larger proportion of its cells showing a significant amount of deformation. The 5 mM glucose group had the most cells that had an aspect ratio in which its length was at least twice that of its width. The results from the 5 mM group closely followed the results in previous assessments of deformability because this group is made to represent normal physiological blood glucose levels; therefore, these cells should have the capacity to deform a significant amount. The 45 mM glucose and 100 mM glucose group show almost the same amount of distribution in terms of the proportion of significantly deformed erythrocytes. Where these groups differ significantly is the number of cells that are slightly deformed. The 45 mM group showed a higher percentage of cells that were slightly deformed (average aspect ratio of less than 1.6 and greater than 1.2) when compared to the 100 mM group. Therefore, it follows that the 100 mM group showed a greater percentage of little to no deformation (average aspect ratio of less than 1.2) when compared to the 45 mM group. These results were also similar to previous research that found that erythrocyte

deformability decreases as blood glucose increases. An interesting result that arose in the results of the experiments was the presence of significantly deformed erythrocytes which had an aspect ratio over 2 in the 100 mM glucose experimental group. This was an unexpected result because, in theory, this group should exhibit the most rigidity among all groups due to its representation of hyperglycemic conditions which should lead to a significant amount of glycosylation. A simple explanation of this could be the fact that these cells are outliers, and if a greater number of cells were sampled, they would present themselves as so. If it turns out that these were not outliers when more cells are sampled, then another potential explanation for this result is the fragility experienced by erythrocytes when exposed to hyperglycemic conditions. Osmotic fragility is a common cause of hemolysis, or red blood cell lysis, in diabetic individuals and is believed to be caused by hyperglycemia [6], [12]. As stated previously, hyperglycemia causes the glycosylation of membrane proteins. One such protein that can become glycosylated is the sodium-potassium pump [12]. Once this pump becomes glycosylated, it becomes inactivated and the cell is no longer able to maintain a proper ion balance leading to increased osmosis and lysis [12]. Another common pathology of erythrocytes exposed to high concentrations of blood glucose is mechanical fragility. Mechanical fragility. as the name suggests, is the reduced capacity for a red blood cell to withstand mechanical stress, typically shear stress, without lysing [13]. The hyperglycemic conditions could have caused increased mechanical fragility which led to lysis when shear stress was applied via the DSCG LCs.

*Fluorescent Image Analysis*

Based on the images shown in Figure 5 it is evident that the cells had become glycosylated due to the presence of green fluorescence in the framework of the erythrocytes. This was due to the nature of high blood glucose causing the glycosylation of membrane proteins as well as hemoglobin. This result confirms that there is a link between glycosylation of the cells and their deformability when exposed to shear stress.

## Conclusion

The study aimed to propose and test a new method of assessing erythrocyte deformability utilizing liquid crystals to induce shear stress. The inspiration behind the proposed method was to improve upon the pitfalls of current methods which are time-consuming and don't properly mimic blood plasma. The study improved upon the blood plasma downfall by utilizing a 154 mM NaCl solution which allowed for a simulated plasma solute concentration. The method however was still time-consuming due to the nature of calculating each cell's aspect ratio. The method proved to be effective in its assessment because the red blood cells' deformability followed the trend observed in previous literature. This trend is predicated on the fact that cells became more rigid when in the presence of higher concentrations of glucose. There were some unexpected results, particularly in the very high blood glucose experimental group with the presence of extremely deformed erythrocytes. This was attributed to either a small sample size, osmotic fragility, or mechanical fragility. Further testing should be done to confirm the results attained in this study so that the method proposed can be considered beneficial and novel. The method could be improved with the incorporation

of machine learning to integrate aspect ratio data to determine blood glucose levels. This could serve as a novel diagnostic tool for diabetic individuals to be able to assess their glucose concentration and mitigate any cardiovascular complications.

**Acknowledgments**

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## References


[1] P. Saeedi *et al.*, "Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition," *Diabetes Research and Clinical Practice*, vol. 157, Nov. 2019, doi: 10.1016/j.diabres.2019.107843.

[2] S. Huang and M. P. Czech, "The GLUT4 Glucose Transporter," *Cell Metabolism*, vol. 5, no. 4. pp. 237–252, Apr. 04, 2007. doi: 10.1016/j.cmet.2007.03.006.

[3] M. Cnop, N. Welsh, J.-C. Jonas, A. Jö, S. Lenzen, and D. L. Eizirik, "Mechanisms of Pancreatic-Cell Death in Type 1 and Type 2 Diabetes Many Differences, Few Similarities," 2005. [Online]. Available: http://t1dbase.org/cgi-bin/enter_

[4] Y. Wang *et al.*, "The Relationship between Erythrocytes and Diabetes Mellitus," *Journal of Diabetes Research*, vol. 2021. Hindawi Limited, 2021. doi: 10.1155/2021/6656062.

[5] Y. I. Cho, M. P. Mooney, and D. J. Cho, "Hemorheological Disorders in Diabetes Mellitus Introduction and Background," 2008. [Online]. Available: www.journalofdst.org

[6] M. Singh and S. Shin, "Changes in erythrocyte aggregation and deformability in diabetes mellitus: A brief review," 2009.

[7] K. Nayani, A. A. Evans, S. E. Spagnolie B , and N. L. Abbott, "Dynamic and reversible shape response of red blood cells in synthetic liquid crystals", doi: 10.1073/pnas.2007753117/-/DCSupplemental.

[8] K. Lindmark and K. G. Engström, "D-Glucose additive protects against osmotic-induced decrease in erythrocyte filterability," *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 60, no. 6, pp. 473–482, 2000.

[9] L. Livshits *et al.*, "The role of GLUT1 in the sugar-induced dielectric response of human erythrocytes," *Journal of Physical Chemistry B*, vol. 113, no. 7, pp. 2212–2220, Feb. 2009, doi: 10.1021/jp808721w.

[10] J. Viskupicova *et al.*, "Effect of high glucose concentrations on human erythrocytes in vitro," *Redox Biology*, vol. 5, pp. 381–387, Aug. 2015, doi: 10.1016/j.redox.2015.06.011.

[11] H. Kataoka, A. Ushiyama, H. Kawakami, Y. Akimoto, S. Matsubara, and T. Iijima, "Fluorescent imaging of endothelial glycocalyx layer with wheat germ agglutinin using intravital microscopy," *Microscopy Research and Technique*, vol. 79, no. 1, pp. 31–37, Jan. 2016, doi: 10.1002/jemt.22602.

[12] N. Rownak, S. Akhter, M. Khatun, S. Baksh, S. Rafiq, and M. Rahman, "Study of Osmotic Fragility Status of Red Blood Cell in Type II Diabetes Mellitus Patients," *European Journal of Environment and Public Health*, vol. 1, no. 2, Dec. 2017, doi: 10.20897/ejeph/77094.

[13] G. Lippi, M. Mercadanti, R. Aloe, and G. Targher, "Erythrocyte mechanical fragility is increased in patients with type 2 diabetes," *Eur J Intern Med*, vol. 23, no. 2, pp. 150–153, 2012.