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Sickle Cell Adhesion To Blood Vessels

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Senior Project Submitted to
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by

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DEDICATION

I am mainly dedicating this project to all the people that have helped me throughout the way. Firstly, I want to thank my closest friend Afua. She inspired this whole project and I want to just give her all the appreciation in the world. She is the kindest and one of the most understanding people I know and has been there with me since I was 11. Secondly, Ajshe for just being there for me and showing up 1000% of the time even when I had my most ridiculous ideas. I want to thank all my other friends who I cannot mention by name but have had a huge impact on me throughout my time at Bard. I care for all of you deeply and appreciate your support. I want to thank all my roommates for the support they have given me while writing this. I want to thank my brother Euler for all his help keeping me on task during my years at Bard. I would not have completed most of my assignments without you. I want to thank my sisters for all the back and forth driving they have done for me to be here today. I appreciate them for spending a huge chunk on toll money just to get me here. I would also like to appreciate my mom for always giving me something to look forward to when I got home. I want to add my eldest sister to the list because of all the phone calls we had with an 8-hour time difference. We love to see commitment.

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Lastly, I want to show appreciation for my mind and body for pulling through when there was no end in sight.

ABSTRACT

Sickle cell disease is a hemoglobin disorder that occurs when there is a mutation on the hemoglobin gene. This leads to an irregular shape in sickle cells as compared to regular red blood cells. Sickle cells are also stickier than regular red blood cells because they contain higher levels of P-selectin, an adhesive molecule. The stickiness of Sickle cells can lead to them sticking in blood vessels, which in turn blocks oxygenated blood from reaching different parts of the body and eventually leads to a crisis. A crisis event causes extreme pain in the person experiencing it. A recent study shows that decreasing the concentration of P-Selectin in sickle cells lessens the adhesive properties of the cells. I created an agent-based model of Sickle cells to determine whether crises events would occur faster: 1) because sickle cells were colliding to cells that were already stuck in the blood vessel due to high P-Selectin or 2) because sickle cells were getting stuck in the blood vessels as single cells since they had higher P-Selectin levels. I conducted 20 computational trials when P-Selectin ranged from 0-0.001 and 0-0.01 to check whether crisis time was affected by collisions or by sickle cells getting stuck because they had a high P-Selectin. The results showed that crisis time is more affected by the number of collisions than a higher concentration in P-Selectin.

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1.INTRODUCTION

In 1910, a dental student with pulmonary symptoms became the first person to be diagnosed with a disease called Sickle Cell Disease (SCD). SCD is caused by a mutation in DNA that affects the shape of red blood cells. Hemoglobin, a protein in red blood cells, is needed to transport oxygen. Hemoglobin is made up of an alpha and beta chain which are both needed for transport.

The Hemoglobin Subunit Beta Gene (HBB) is used to encode proteins for the beta chain ("Genecards, "2021) and people who have SCD have mutations in the HBB gene. For people with sickle cell the mutations occur because one of the beta-globin is replaced with hemoglobin S, which is known as HBS. In the homozygous form (HbSS) of sickle cell, both hemoglobin's are replaced with hemoglobin S and in the heterozygous form only one is replaced.

1.1 *Cellular Level interactions*

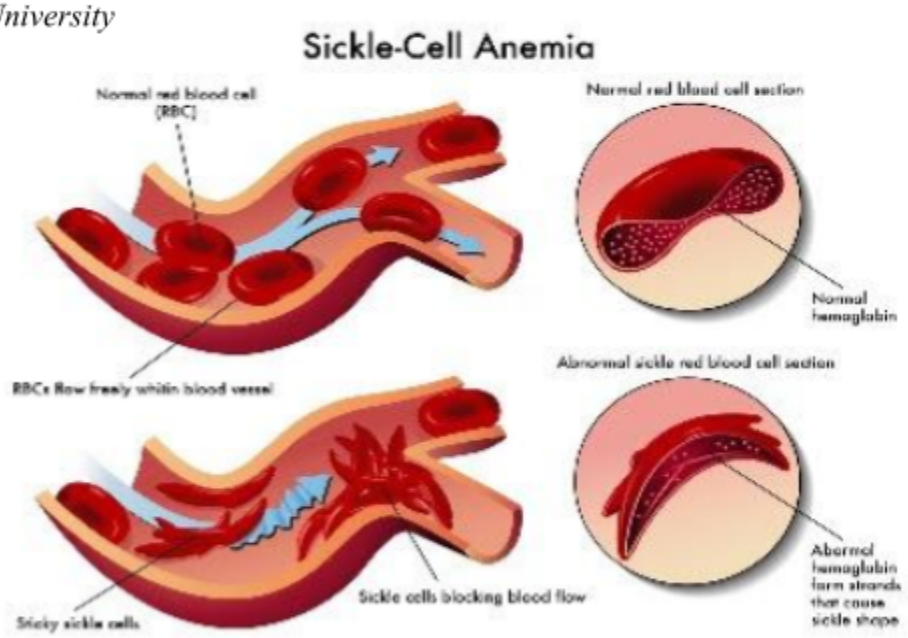
In the encoding for the beta chain in people with SCD thymine(T) is replaced with adenine(A). Instead of a G(Guanine)T(Thymine)G(Guanine) sequence, the sickle cell has a G(Guanine)A(Adenine)G(Guanine) bond. The substitution for A instead of T creates valine instead of glutamic acid on the second nucleotide on the 6th codon. Unlike glutamic acid, valine is hydrophobic and uncharged ("HBB Gene",2017). The hydrophobic nature of valine causes stickiness in sickle cells.

1.2 Structure

Due to the mutation that produces valine, sickle cells have a higher electric potential. This higher electric potential means that the positive charges, cations (+), easily escape the sickle cell. The electric potential is higher when the sickle cell is deoxygenated ("ASH Sickle Cell Disease Initiative", 2021). The increase in electrogenic passage of cations gives the sickle cell its sickle shape when it is deoxygenated and contributes to the quick dehydration of the cell.

Unlike regular red blood cells that are smooth and whose shape remains largely unaffected by the changes in oxygen levels, sickle cells are stiff and sticky, see *Figure 1*. The stiffness and stickiness of sickle cells lead to sickle clumping up together and sticking to each other and to the walls of the blood vessel (called vasso-occulation) ("Sickle Cell Disease", 2021). In addition, the shape of the sickle cells leads to a quicker death of the blood cell compared to regular shaped red blood cells. Sickle cells have a shorter lifespan (10-12 days) compared to regular red blood cells (120 days) ("Sickle Cell Disease | Cedars-Sinai", 2021).

Figure 1. *Sickle-Cell Anemia [Online Image].Howard University*



Sickle cell is commonly found in people with African ancestry (Sickle Cell Disease | Cedars-Sinai, 2021). The mutation itself has been shown to be helpful against contracting malaria. Many believe that the sickle cell mutation occurred as a response to the malaria ridden mosquitos in equatorial climates. In America, 1 in 13 black children are born with sickle cell. Although sickle cell is prominent in people with African ancestry it can affect anyone if both parents are carriers. There are multiple variations of the sickle cell mutation. There is also a 50% chance that the children will be carriers of sickle cell (Spector, J., Kodippili, G. C., Ritchie, K., & Low, P. S., 2016). If both parents are carriers of SCD then there is a 25% chance that their child will have sickle cell. When both parents are carriers of SCD their children are more likely to have the homozygous form of SCD (HbSS). The homozygous form of sickle cell (both the Bs in hemoglobin are replaced by S) is the worst version overall due to its severe symptoms. If you are a heterozygous carrier then the mutation only occurs on one of the beta-globin.

1.3 Signs and Symptoms

Sickle cell symptoms are so severe that the average lifespan is 42 years for men and 48 years for women. The homozygous form of sickle cell comes with many symptoms including:

- **Anemia, Stroke, Jaundice, Acute Chest Syndrome:** Occurs in sickle cell because of the high turnover rate in sickle cells. Sickle cells die quickly resulting in a shortage of cells to transport oxygen throughout the body. The shortage of cells leaves patients susceptible to anemia.

- **Spleen dysfunction:** The spleen is responsible for removing dead red blood cells from the bloodstream. The spleen does much of the "cleaning up" when it comes to sickle cell. Given that the lifespan of sickle cells is shorter than that of regular red blood cells, their overproduction increases the cleaning up burden of the spleen.The overproduction of sickle cells leads to more cells for the spleen to get rid of. Therefore, the spleen must take more time cycling the sickle cells out than tending to the other issues it has (Booth, C., Inusa, B., & Obaro, S. K. 2010). In people with the homozygous version of sickle cell, the spleen is more likely to get infected because of the sluggish movement of sickle cells through the spleen.

- **crisis:** A sickle cell crisis is one of the most painful experiences for a person. A crisis can be described as a series of pain inside one's bones. A crisis is triggered when sickle cells block the blood vessels. The blockage can occur in the joints especially, in the arms, legs and knees. This pain feels like an itch in the body that you cannot get

to. A crisis can be caused by one's environment. If a person that is suffering from sickle cell stays in an area that is either too cold or too warm, then a crisis can occur because the person is unable to get enough oxygen to stop the cells from being sickled. Crisis can last for unpredictable amounts of time and often leave people bed ridden for days. There is no way to estimate when a crisis is going to happen next so there are no current methods to prevent one (Sickle Cell Disease | Cedars-Sinai,2021). Most people with SID experience 2-4 crises in a year. If a crisis is not properly managed it can lead to all the symptoms mentioned beforehand. Currently the only treatments available to target the crisis as it is happening are opioids and blood transfusions ("ASH Sickle Cell Disease Initiative", 2021).

1.4 Treatment Options

There are limited treatment options for people that suffer from sickle cell because sickle cell remains uncured. The only options people suffering from SCD have are a blood transfusion, opioids to manage the pain, or a bone marrow transplant ("ASH Sickle Cell Disease Initiative", 2021).

1.5 Agent Based Modelling

Modelling uses technology to represent naturally occurring phenomena. Agent based models can be used to model trends such as migration, disease and even animal behaviors. Agents, when they are autonomous, live by a set of rules and can make their own decisions. "In agent based modelling the objects created can interact with their environment as they store references to the

objects around them?" (Daniel Shiffman, Nature of Code). Autonomous agents do not have a leader, there is no one object in charge that controls movements. Autonomous agents also take in information from their environment and make choices based on that information. Agent based modelling allows us to represent complex systems because all the parts interact with each other and their environment. Agent based modelling is not limited to using experimental data and can be used to represent experiments and trials that would otherwise be impossible to conduct. With agent-based modelling we can see the effects of something without going through the arduous task of doing actual physical trials ("Agent-Based Modeling | Columbia Public Health", 2021). Through simulations we can first test out the hypothesis under multiple conditions to see if there is any proof that supports one assumption prior to starting the physical trial. This saves time and energy because we are easily able to predict/hypothesize the outcome of different situations.

1.6 What Causes the Stickiness?

The focus of my model is to simulate the sickle cell crisis because it is one of the defining effects of SCD. Due to mutations in sickle cells, this leads to their quick creation, known as polymerization, and their quick destruction, known as denaturation (Spector, J., Kodippili, G. C., Ritchie, K., & Low, P. S. 2016). During the creation of sickle cells," Under hypoxic conditions, HbS polymerases and causes red cell sickling, a rise in intracellular Ca2+ and exposure of phosphatidylserine (PS)" (Lu, D. C., Wadud, R., Hannemann, A., Rees, D. C., Brewin, J. N., & Gibson, J. S. 2021). Once the sickle cells have been destroyed, they attach themselves to the Band 3 protein. Band 3 is a protein found in the blood vessels that aids in the transportation of Chlorine (Cl) and Bicarbonate (HCO_3). As more and more sickle cells die, they attach

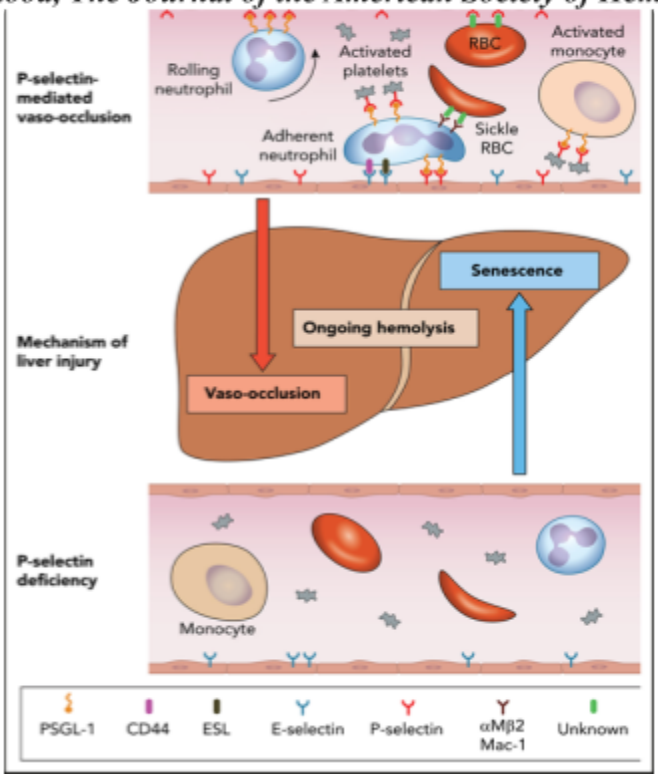
themselves to the Band 3 protein and create clusters. The Band-3 protein gathers the membrane of sickle cell erythrocytes. Erythrocytes, also known as red blood cells, are responsible for the transportation of oxygen and carbon dioxide through the body. The Band-3 sickle cell structures then stick to the endothelium and limit blood flow. Additionally, the cell structures are recognized by the body's immune system and targeted for removal. The Band 3 structures that are formed are sticky and make up the sticky characteristic that we associate with most sickle cells.

1.7 *When does a crisis occur?*

The crisis occurs mainly because of the stickiness of sickle cells. After the sickle cells lose oxygen, they become sticky and adhere themselves to the walls of the blood vessels. When sickle cells adhere to themselves to the inside of a blood vessel, a crisis takes place. A study that analyzed the flow of blood cells through vessels showed that the more sticky-cells cause more blockages in the cellular walls. .The study looked at whether lowering the stickiness caused by p-selectin would lead to lower adhesiveness in sickle cells (Man, Y., Goreke, 2020). *Figure 2* shows the process of sickle cells sticking in the blood vessels and how it affects the liver.

In the study the level of P-selectin was lowered using Crizanlizumab to produce the following

Figure 2. P-selectin and sickle cell disease: a balancing act.[Online Image].] Blood, The Journal of the American Society of Hematology, 2021



stickiness coefficients of stickiness used 0.056, 0.026, 0.016. After being treated with Crizanlizumab the cell's adhesiveness was then measured (Manwani, 2021). The results found that the less p-selecting the sickle cell had the less adhesive the sickle cell was. Thus, I want to model the stickiness of sickle cells to see whether a crisis occurs faster with higher levels of collisions or with lower levels of collisions. My hypothesis is that higher levels of collisions result in a lower time for a crisis to occur. Thus, more collisions will lead to lower time passing before a crisis occurs. I will run three different simulations with three different coefficients of stickiness (0.10,0.01,0.001) and see whether my results match the hypothesis.

2. METHODS

2.1 How will I be modelling a crisis?

The microscopic changes that happen in the blood vessels because of the sickle shape are what controls every aspect of sickle cell disease. Sickle cell interactions mainly affect small blood vessels because of the smaller surface area available for movement. My model documents the processes that occur leading up to a crisis by imitating sickle cell behavior inside a blood vessel. I did not directly replicate a crisis but looked at the changes in cell clumping levels right before a crisis. My model depicts an isolated section of a blood vessel that has sickle cells flowing through it and tests out what happens when the stickiness of the cells is varied. I completed three trials with varying amounts of stickiness and determined how long it took for a crisis to occur. By comparing the motion of sickle cells without an added variable for stickiness I will look at how much slower the cells move when they are able to stick. I analyze whether cellular motion decreases more when the sickle cells attach themselves to walls or stick to each other and form clumps of sickle cells.

2.2 What will I be using to create a simulation?

I will be doing this model using Processing 3.5.4. I chose processing because it is easy to represent autonomous objects in and build models that interact independently with each other. Processing also offers a lot of customization which is essential when making a biological system such as a blood cell.

Sickle Cell Class

I have created two classes: sickle cells and determined how often the sickle cells get stuck inside the walls. After the sickle cells are stuck to all sides of the cell wall then a crisis is declared. I then determined how much time it took for a crisis to be declared with increasing stickiness.

Table 1: Classes

Class	**Attributes**	**What does it do**
Cell *Parent class*	**PVector** location shows the start location for each **PVector v**elocity: the speed of the cell **PVector** acceleration : controls how the cell speeds up or down **float** lifespan: how long it takes the cells to die **Boolean collided:** checks for collision	Cell() Assigns values to the cells' attributes Move() Updates each cells' movements Applyforce() Takes in a PVector and updates the force accordingly Stops movements following a collision Collide() Checks whether two cells are close to each other Drag() Represents the tension of the flow of cells

		Edges() Causes cell to wrap around when out of bounds Update() Decrements the lifespan of the cell at each iteration
Sickle *Child class*	**Float** stickA: inherent stickiness present in each sickle cell lifespan boolean TouchWall boolean stuck	Sickle() Assigns values to the cells' attributes Display() Draws the cells Run() Initializes movement and drag force Decrements lifespan of cell Also contains checkWall Collide() Creates a version of collision checking for sickle cells compares the stickiness level and if it is high the cells collide

		CheckWall() Has the sickle cell gotten stuck on multiple sides of the wall?
Main Tab	ArrayList **Sickle**: Declares an array list with sickle cells **PVector** flow: Controls the motion of the cells **PVector** go: Adds random movement to the cells	Setup() Initializes the array list of sickle cells And the size of the blood vessel Draw() Loops through all the cells and makes sure all the processes are run on them

Measurement ratio

All the measurements when it came to objects in the blood vessel environment were in micro units or nano units. To best represent everything but also depict things in a way in which they can easily be visible I used the following measurements for all my simulations.

Table 2: Measurements

Item	Used measurement in the program				Actual		
	Length	Width	Height	Length	Length	Height	

Red Blood cell					6.2 micrograms		
Collision Width	Not available	N/A	N/A		10 pixels		
Blood vessel	30 pixels	N/A	N/A		30 pixels		
Sickle Cell	850nm	50nm	N/A		10		
Red Blood cell	10.2	N/A	N/A		6.2 micro grams		
Lifespan	20	N/A	N/A		26 days		

2.3 How will it work?

Each sickle cell is created with a certain inherent probability of stickiness. If this probability is equal to 0.001 then the cells start sticking to the blood vessel. The cut-off of stickiness/adhesion is at 0.001. This threshold was created because I am assuming that regular cells have some level of stickiness but are not sticky enough to get stuck. All cells with stickiness equal to or greater than 0.001 will get stuck while those with stickiness lower than that will keep moving. If a cell does not meet the threshold of sticking (being >=0.001) and is 10 pixels away from a cell that is stuck, then it also gets stuck. Below are the classes of my program.

[1] Class Cell

When isolated in the blood vessel, sickle cells have many similarities to regular red blood cells, the only difference is that sickle cells have an inherent stickiness to them and a different shape that makes them more likely to stick to the walls of the cells. With that in mind I wanted all my types of cells (both sickle cells and red blood cells) to have the same attributes except for stickiness. The differences between them are the stickiness coefficient, the shape of sickle cells, the short lifespan, and the high replication rate that is found in sickle cells. Thus, my Cell class represents all the components that are found in any type of red blood cell whether it be sickled or normal. The section below describes the uses of each of the attributes and what the methods do.

Table 3: Class Cell

Attributes

Location

The location PVector is a vector that allows me to keep track of each cell's location. Each cell in a blood vessel has a different movement at a given time, that is why the location of my cells is varied as they move through the blood vessel. Each cell has a given location that is within the bounds of the blood vessel. Any movement the blood cells make is recorded in the location allowing me to keep track of the blood cells with each iteration.

Velocity

Velocity controls the speed of cells within the blood cell. For the blood cell to move the velocity must constantly change and having a velocity attribute makes it easier to control how fast the cells are moving. The blood cells in blood vessels usually move at a constant speed no matter the type of cell so this attribute had no specification and could stay at the same value.

Acceleration

I used acceleration to control how much the speed changes by. To initialize the movement of the cells I needed to create an acceleration component. Acceleration is also useful when handling forces applied on to the cells when forces such as drag or flow that influence the movement of the cells in the blood vessel are added. The cells in the blood cell accelerate at a constant speed so acceleration was necessary to control the aspect of movement.

Collided

Is a Boolean value used to see whether collisions have occurred? It starts out as false because at the beginning of the program no cells have interacted yet. However, when a cell is close (X pixels away) to another cell it becomes true.

Lifespan

Lifespan is how long the cells stay alive for. Sickle cells take 10-20 days to die. While regular red blood cells take 120 days to die. Sickle cells have a lower lifespan compared to regular cells. I wanted to keep track of how many sickle cells remained alive compared to regular red blood cells. However, the sickle cells also have a faster turnover rate which means more of them are created after a shorter period. I wanted to emulate this aspect of sickle cells by having new sickle cells appear after each iteration.

Methods

Cell()

Initialize everything declared in the cell class and gives it a value. It also initializes the cell class itself. All the values chosen for the attributes were as close as possible to the values of red blood cells, see *Table 2*.

Move()

The values of a cell's velocity and acceleration are always changing. To update them correctly I created this class that adds the new value of acceleration to the velocity. Changes in the cell's location are then made as the velocity and acceleration are updated.

ApplyForce()

This method takes in a PVector and changes the acceleration based on the given value of the PVector. I designed this method to specifically take in the flow force that allows the cells to move through the screen. The method is essential because it allows me to control how each added force affects the cells' movement. The method also stops the acceleration of cells whenever there is a collision. To get acceleration you divide force by mass and then update it accordingly. I did this because when sickle cells collide with each other its movement stops which leads to their inevitable sticking.

Collide()

The method compares two different cells and their location to each other. First, the location of one cell is subtracted from the location of the other using PVector subtraction. The subtraction yields a PVector. The magnitude of the PVector created is converted to a float number. The number in the float is then compared with to check whether it is less than 10. If so, then then the cells are close enough to interact and influence each other. It checks whether cells are close enough to each other to interact. If the cells are close enough to interact and one cell has a high sticky value, causing it to stop, then the other cell also stops because it is being influenced by the one with the higher value. This method is supposed to emulate how sickle cells begin to clump together when one of the cells is stuck. The method thus represents the attractive forces between

one sickle cell and another. This version of collide only checks the distance between cells and measures collisions that way. It does not take stickiness into consideration because regular red blood cells do not have a stickiness component to them.

Update()

Checks on the lifespan of the cell. I wanted to include lifespan to show how fast sickle cells die compared to normal cells.

Drag()

To accurately depict the conditions in the blood vessel I need to add drag to counteract the flow of the red blood cells so they could face resistance. Due to a red blood cell being in a liquid environment, it faces resistance from drag. The Drag force takes in the location of a blood cells and applies a force of resistance to it. This force is then applied onto the cell and limits the range of movement a cell can take.

Edges()

I wanted each of the cells to wrap around the size of the e blood vessel. This method looks at the location of the blood cell and checks if it is inside the height and width limits of the size of the screen. If the location of the cells is greater than the height, then the cell is taken back to location (1,1). This keeps the cells in bounds and makes it easier to track them as they move across the screen. Without this method it would be harder to see cell activity because the cells could go anywhere. This method simulates the motion of blood cells within a controlled section of the blood vessel.

Display()

One of the other major differences between regular shaped red blood cells and sickled cells is the shape of the cells. Regular red blood cells have a plump, even cylindrical-like shape that allows them to easily go through blood vessels. While sickle cells are sickle shaped. Display makes sure that each of the cells are drawn on the screen. This display defaults to representing red blood cells as round. It also sets the size of the diameter for a red blood cell.

[2] Class Sickle

Class sickle cell an extension of class Cell. This makes Class Sickle the child class of class cell. Class sickle is unique because it contains all the information that differentiates a sickle cell from a regular cell. This class is supposed to have all the components present in a sickle cell. One of the ways sickle cells are different from regular red blood cells is the way they are shaped. The cells have a sickle like shape that I wanted to express be using this class. I also wanted to include the collision detection and change it to work specifically for sickle cells so there is no confusion as to how it will function later. Below are the attributes and methods for the sickle cell class. This class is containing the primary aspects of sickle cells and is the class that is most influential in sickle cell behavior.

Table 3a: Class Sickle

Attributes

There is an extension call at the beginning of class sickle. This allows sickle to use all the methods of the Cell class and modify some to suit its needs if required. Being that sickle is the child class of Class Cell it contains many of the components already present in the cell class.

This includes attributes of location, velocity, and acceleration. All these attributes thus remained the same for the class Sickle.

PImage Sickle

The PImage function is responsible for rendering the image of the sickle cell.

Float StickA

When creating a stickiness variable, I wanted to meet conditions that were like those in study about different stickiness variables. In the study, different stickiness variables would result in different probabilities of a cell sticking to the walls of the red blood cells. The highest values for stickiness in the study were 0.1, 0.01, and 0.001, with 0.1 being the one with the highest value. The higher the stickiness coefficient the more the cells got clogged in the blood vessels. In this case sticka acts as the stickiness coefficient. I want to look at how different values of stickiness affect how many cells get clogged. Sticka represents the inherent stickiness that each sickle cell has. This variable is used when stopping sickle cells from moving. Each sickle cell has a random value for stickiness that is assigned when it is created.

Float Lifespan

Sickle cells have a shorter lifespan than regular cells. The lifespan of sickle cells is 10-20 days as compared to that of regular cells. I wanted to cycle out the sickle cells after they died so I used a lifetime variable that tracks how long the cells have been alive for and gets rid of dead cells.

Boolean touchWall

The variable checks whether sickle cells are stuck inside the blood vessel walls. When sickle cells get stuck inside blood vessels it leads to a crisis. The touchWall variable helps me easily check when a crisis is happening.

Boolean Stuck

Checks if a sickle cell has stopped.

Methods

Sickle()

Initializes the sickle cell class and gives values to all the components.

Display()

Represents the sickle cell with a sickled shape. The size of the sickle cell is determined at this point in the program. Allows for the sickle cell to be displayed on the screen.

Wallcheck()

This part of the lab is supposed to mimic a crisis. As discussed before a crisis occurs when many sickle cells are blocking the blood vessels. The blockage of the blood vessel results in a shortage of oxygenated blood cells around the body. Eventually the blocked blood vessels lead to the manifestation of physical symptoms that are defined as a Sickle Cell Crisis. WallCheck is a method responsible for looking at the number of places in the cell wall where the sickle cells are stuck. If the number of places the sickle cells touch exceeds 4 then there are too many blockages on the blood vessel walls. I picked four because if more than four cells are stuck inside the blood vessel it makes it difficult for other cells to travel around it. After a crisis has occurred the value of stickA/P-Selectin for sickle cells that have not collided prints onto the

screen. WallCheck() has a touchWall that keeps track of the number of times the wall has been touched; this will be useful later when graphing. The method prints the stickA value for each cell that is stuck inside the wall but has not collided with another cell. I did this to verify the number of cells that collided was correct.

Run()

Initializes move and run if the sickle cell is not stuck. This saves space when running the methods in the mainTab because there is just one method that contains each of the other methods. Run also updates the lifespan of sickle cells during each iteration. I wanted to get as close as possible to showing sickle cell behavior.

Collide()

Sickle cells have an inherent stickiness to them that makes them stick to both each other and the walls of the red blood cells. Stickiness works hand in hand with how far sickle cells are from each other. For one cell to be stuck to another they need to be close enough and one cell needs to have a high enough stickiness for them to collide and stop. This method brings the stickiness of the sickle cell into consideration and allows for the stickiness variable to be used in collision detection. The method takes in one sickle cell and compares its distance to the current sickle cell being checked. When distance is less than 10 and stickiness is present the cell being compared stops.

isTouching()

Returns whether the sickle cell has stopped inside the blood vessel. isTouching() is useful when determining whether a crisis is occurring because we can check the number of times sickle cells get stuck inside the blood vessels.

isStuck()

Checks whether the cell has stopped.

DecreaseLife()

Decrements the lifespan of a sickle cell as the program runs. During each run of the program a sickle cell's lifespan is decreased by 0.1.

IsDead()

Checks if the sickle cell is still alive. Sickle cells have a short lifespan and need to be cleared out when they are dead. A crisis occurs because too many sickle cells are being created and quickly dying without being properly discarded. The death of sickle cells without them being discarded leads to further blockages in the blood vessels. This method keeps track of whether a sickle cell is still living and is later used to display the sickle cells that are alive and clear dead sickle cells.

[3]Main Tab

The main tab is just where everything that is part of the program runs. This class just calls on all the functions and methods that were created before and makes sure they are executed. The main Tab also lets you know where the errors in the program are.

Figure 3c: Main Tab

Attributes

ArrayList <Sickle> cell

Creates an array list of sickle cells. The method controls the initial value of sickle cells that appear on the screen. Through it I wanted to customize the number of cells

that are there to begin with during each round. I chose about 20 to begin with so that they could easily be represented on the screen.

boolean crisis

Is true when a crisis is happening.

boolean running

Is true when there is no crisis. Used to keep track of how long it takes for a crisis to occur.

PVector flow

Flow controls the motion of the cells in the bloodstream. The value from flow is taken and used to update the velocity of the blood cells.

float sticky

Stores the stickA values for all the cells that are alive after a crisis has occurred.

PImage BloodV

Has the visual representation of the background.

Methods

Set up()

Creates the size of a blood vessel.

Initializes the sickle array list.

Creates the total number of sickle cells in the array list and adds a sickle cell.

Draw()

The background color is set to zero in draw. Has a for loop that goes through all the sickle cells and makes sure all the methods are run on them. The method contains an if

statement that checks the value of the sticka variable to see if it is less than random(1). If sticka is greater than the acceleration of the cell is set to 0 and the cell stops moving. The run method is also called inside draw and it accomplishes all the things that are present in a sickle cell. There is also another if statement that takes in other cells that are different from the one being looked at and then runs the collide function on them. This if statement checks whether a collision has occurred. Applyforce on the flow force and the go force are also called within the draw function. Edges method is also called to check for interactions. The amount of time that has passed before a crisis occurs is tracked here. Draw also prints out the pictures of the whole run of the program. The cells are cleared off the screen and new ones are created when in draw.

3. RESULTS

Results from Study Referencing

The study I based my experiment on was testing how adding Crizanlizumab lowered the adhesion of P-Selectin. P-Selectin is highly responsible for the vaso-occlusion of sickle cells. The study was checking to see if lowering P-selecting by using Crizanlizumab would lower the adhesive properties of sickle cells. Thus, blood from sickle cell patients was drawn and different amounts of Cirzanlizunab were added to P-Selectin for dilution. In the study three samples were produced, the control with a p-selectin value of 0.057, another with a P-Selectin value of 0.027

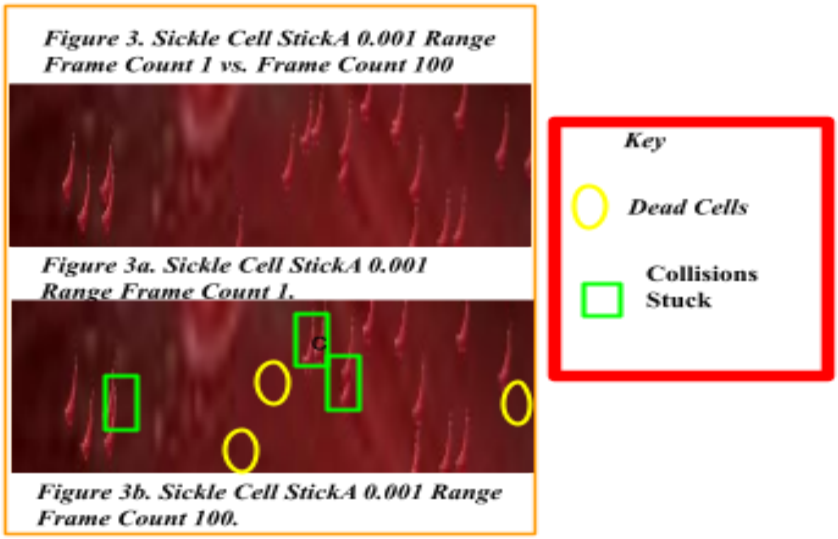
and another with a value of 0.016 (Man, Y., Goreke, U., Kucukal, E., Hill, A., An, R., Liu, S., Bode, A., Solis-Fuentes, A., Nayak, L. V., Little, J. A., & Gurkan, U. A, 2020). In the study the lower the P-selectin value the less adhesion occurred.

3.1 *Data Background*

I have developed an agent-based model for sickle cell disease, including crisis events and varying cell stickiness. Each cell I will be modelling has a sickle shape and has sticky behavior. I conducted 20 trials with the stickA parameter as random(0.001) and random(0.01). Each trial started with twenty sickle cells.

3.2 Sickle Cell Behavior as Program Progresses: StickA random(0.001)

The behavior of cells during the simulation is consistent for cells that have a stickA of 0.001, see *Figure 3*. Initially in the program there are a total of 20 sickle shaped cells that each have a different lifespan, *see Figure 3*. In *Figure 3* the sickle cells at FrameCount 1 are compared to those at Frame Count 100. *Figure 3* shows sickle cells getting stuck in the blood vessel as the



FrameCount goes from 1 in *Figure 3a* to 100 in *Figure 3b*. The sickle cells highlighted by yellow circles are dead while those highlighted by green squares are stuck in the blood vessel.

In *Figure 4* there are multiple cells that have collided together. *Figure 4* compares the number of dead cells and cells that are stuck between Frame Count 100 and 200. As time goes on more cells begin to die as marked by the yellow circles and additional cells stick to each other, *Figure 4b.*

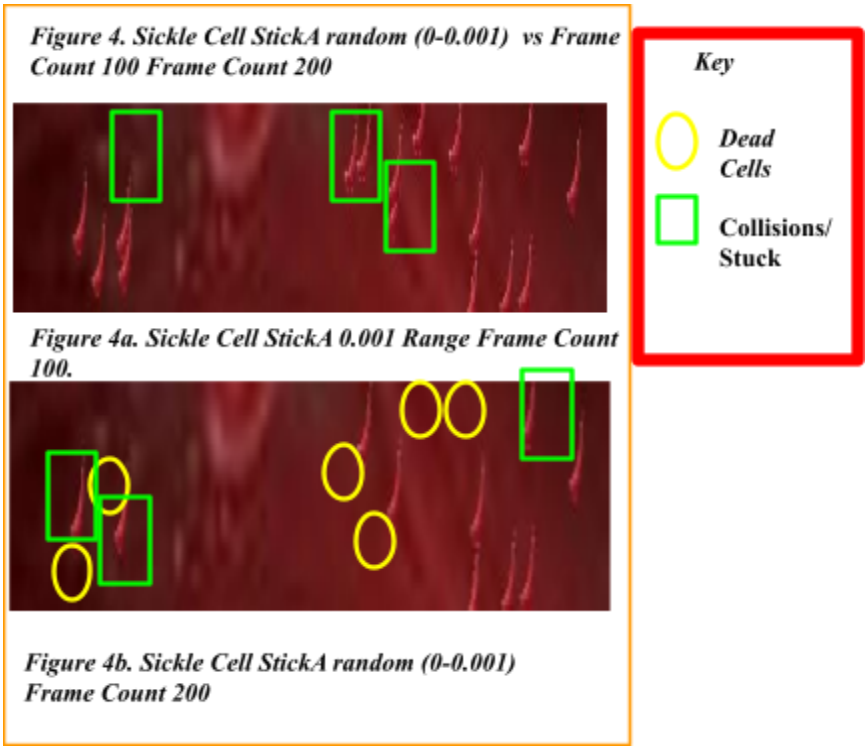


Figure 4. Sickle Cell StickA random (0-0.001) vs Frame Count 100 Frame Count 200

Figure 4a. Sickle Cell StickA 0.001 Range Frame Count 100.

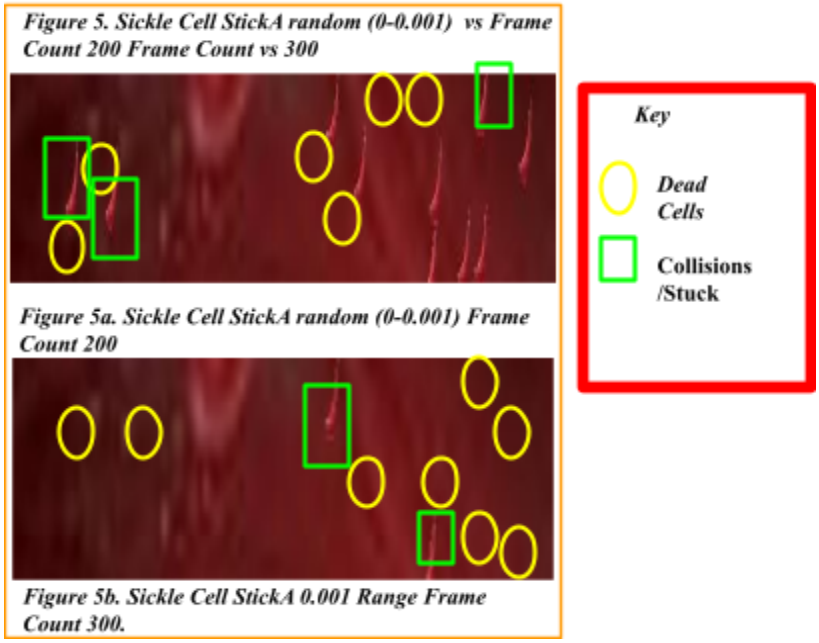
Figure 4b. Sickle Cell StickA random (0-0.001) Frame Count 200

Cells also die, and new cells are created.

Figure 5 shows less than twenty cells on screen because some cells died. *Figure 5b* is taken to show the difference in cell behavior between Frame Count 200 and Frame Count 300. *Figure 5b*, shows a crisis has taken place because a large number of cells have been cleared from the screen

between Frame Count 200 and Frame Count 300. This is evident because cells begin to be cleared from the screen because a crisis is in progress and their lifespan is decreasing, *Figure 5b*.

In *Figure 6*, sickle cell behavior at Frame Count 300 is compared to sickle cell behavior at frame Count 400. In *Figure 6b*, a crisis has happened and there are no new cells being created. All cells



are cleared from the screen following a crisis, see *Figure 6b*.

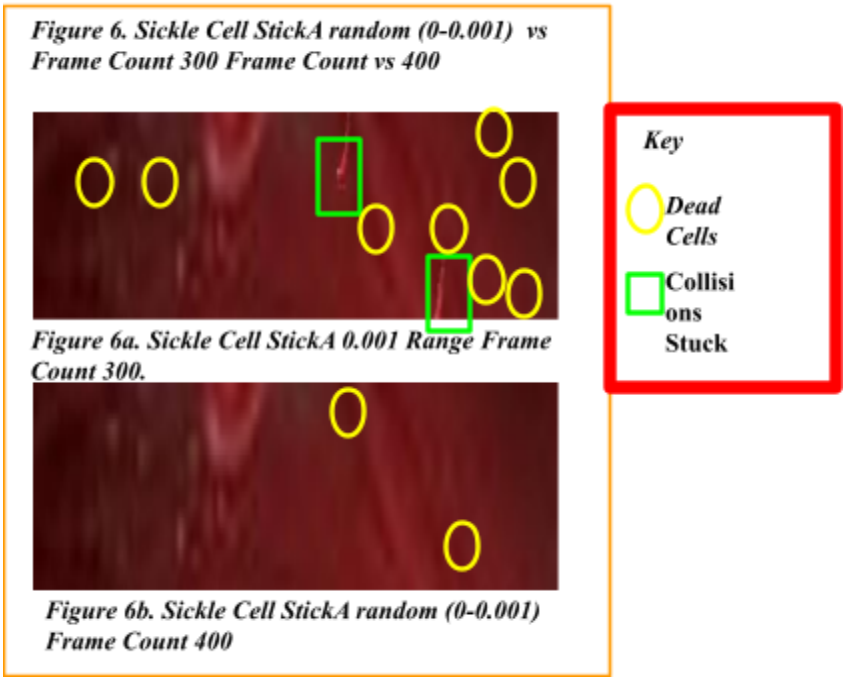


Figure 6. Sickle Cell StickA random (0-0.001) vs Frame Count 300 Frame Count vs 400

Figure 6a. Sickle Cell StickA 0.001 Range Frame Count 300.

Figure 6b. Sickle Cell StickA random (0-0.001) Frame Count 400

3.3 Sickle Cell Behavior as Simulation Progresses: StickA random(0.01)

For my other 20 trials I looked at sickle cell behavior when the concentration of StickA was random(0-0.01). *Figure 7* compares the difference in sickle cell behavior between Frame Count 1 and Frame Count 100. At a frameCount of 1 there are 20 different cells, see *Figure 7a*.Some cells begin to get stuck, see *Figure 7b.* Compared to the sickle cells in *Figure 3* the sickle cells in *Figure 7* are more spread out and have a larger distance between them.

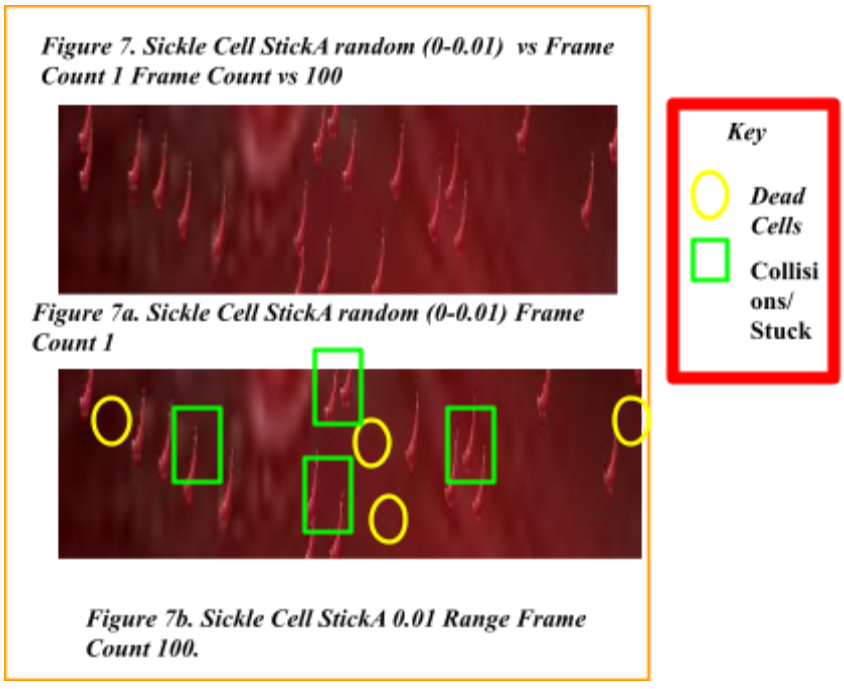


Figure 7. Sickle Cell StickA random (0-0.01) vs Frame Count 1 Frame Count vs 100

Figure 7a. Sickle Cell StickA random (0-0.01) Frame Count 1

Figure 7b. Sickle Cell StickA 0.01 Range Frame Count 100.

Cells also begin to die as shown by the yellow circles highlighting missing cells between the Frame Count of 1 and the Frame Count of 100. Cells also begin to get stuck and stop in the blood vessel as highlighted by the green squares in *Figure 7b*.

In *Figure 8* more sickle cells begin getting stuck. However, unlike when StickA ranges between 0 and 0.001, cells in the 0-0.01 concentration have more distance between them. This means that the cells when concentration is 0-0.01 are getting stuck because of high P-Selectin and not because of collisions.

Figure 8. Sickle Cell StickA random (0-0.01) vs Frame Count 100 Frame Count vs 200

Figure 8a. Sickle Cell StickA random (0-0.01) Frame Count 100

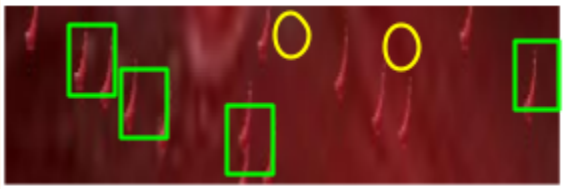
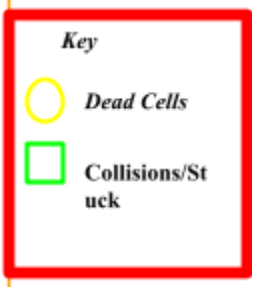


Figure 8b. Sickle Cell StickA 0.01 Range Frame Count 200.



More cells die as the program continues running, *Figure 9*. *Figure 9b* has less sickle cells than *Figure 9a* meaning that a crisis had to have occurred between the two Frame Counts as cells are starting to be cleared out and cells are only cleared off the screen when a crisis has happened.

Figure 9. Sickle Cell StickA random (0-0.01) vs Frame Count 100 Frame Count vs 200

Figure 9a. Sickle Cell StickA random (0-0.01) Frame Count 200

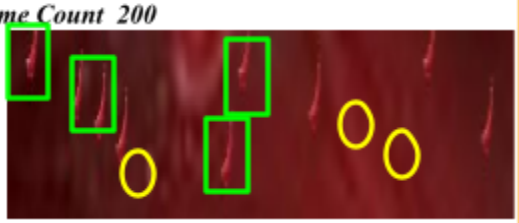


Figure 9b. Sickle Cell StickA 0.001 Range Frame Count 300.

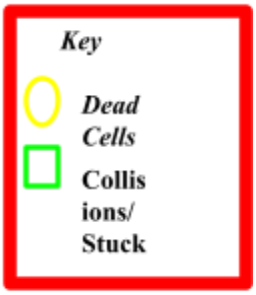


Figure 10 compares sickle cells at Frame Count 300 and Frame Count 400. All 20 cells that remain after the crisis are cleared from screen, *Figure 10b.*In *Figure 10b* the lifespan of the cells continues to go down and the cells are cleared off of the screen after they die.

Figure 10. Sickle Cell StickA random (0-0.01) vs Frame Count 300 Frame Count vs 400

Figure 10a. Sickle Cell StickA 0.01 Range Frame Count

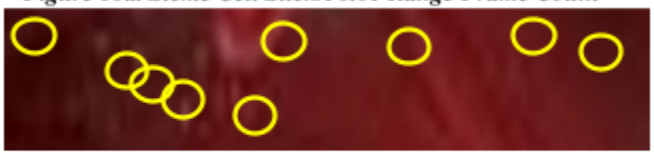


Figure 10b. Sickle Cell StickA random (0-0.01) Frame Count 400

3.4 Data Section

For each trial, meaning each run of the program, I collected the following data :

- The stickA value of twenty cells that were still alive during the crisis
- The time it took for a crisis to take place
- The number of cells that had collided (meaning were stuck to other cells)
- The stickA values for the cells that had not collided (to validate the number collided)
- The total number of cells that were stuck during each round
- The jpeg for the cells throughout the life of the program

3.5 Simulation Trials?

I analyzed data for each of the trials in Excel. Each trial there were 20 different stickA values, one for each cell that was still alive (lifespan>0) following a crisis. I averaged out all 20 stickA values I received and graphed all the data. Following this I calculated the number of cells that were stuck but not collided. I calculated this value by subtracting the total number of cells that had stopped from the ones that had collided. I am defining an uncollided stuck cell as a sickle cell that has stopped because of a high stickA value and not because of hitting/colliding with another cell.

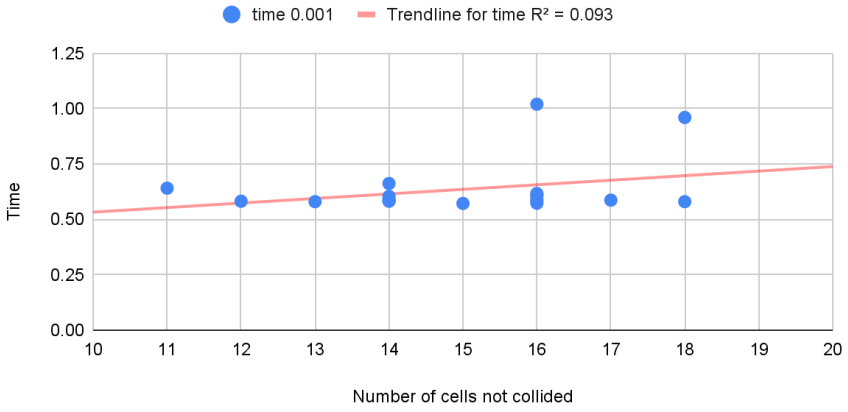
3.6 Graphing Values of Time of Crisis event, stickA average, number of collided cells and uncollided cells

Below are the graphs for each value I received from the data, see Figures 11-21. The first part of the analysis compares the general theme of stickA values, the number of collided cells and the number of uncollided cells. The first graph shows values when stickA random is 0.001 and the other when stickA random is 0.01. These comparisons were conducted to give an overview of the behaviors of sickle cells that are not exclusive to the changes in stickA values.

3.7 Time of Crisis Event vs. Number of Cells Not Collided

A crisis event occurs in my program when 5 or more cells have stopped inside the blood vessel. The cells that have not collided are the cells that have stopped because of their high stickA value and not because they interacted with other cells.

Figure 11:Time for Crisis Event Vs. Number of Cells Stuck but not collided for StickA random(0.001)



The graphs in *Figures 11 and 12* were constructed to highlight the behavior of sickle cells when they are stuck but have not collided with each other.

Figure 12:Time for Crisis Event vs Number of cells not collided for StickA random(0.01)

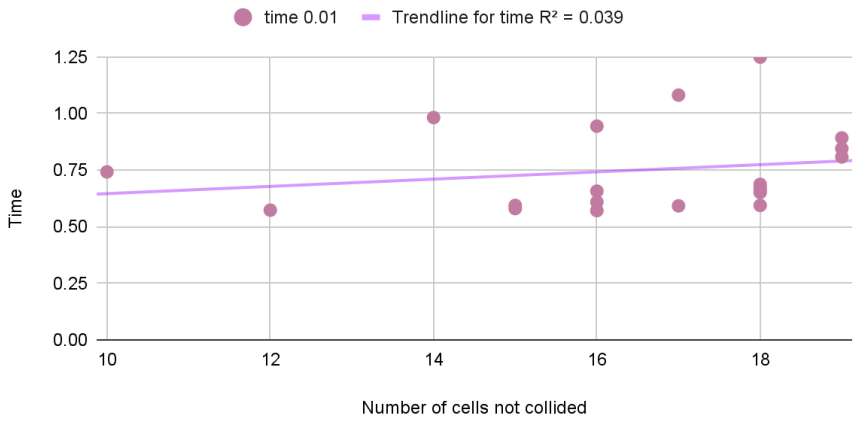
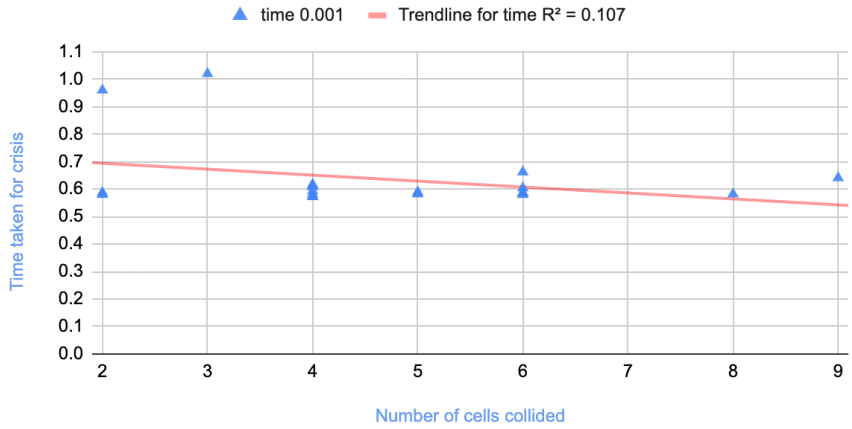


Figure 11 specifically depicts the relationship between time and the number of cells that are alive, have not collided with other cells, and are stuck during a crisis for StickA at < 0.001. The trendline of the chart is pointed upwards meaning that as time goes on the number of cells that have not collided go up. *Figure 11 and Figure 12* show a possible direct relationship between the time taken for a crisis to occur and the number of uncollided cells present. Through *Figure 11 and 12* we see an increase in time accompanied by higher numbers of uncollided cells. This relationship between time and cells colliding is consistent for both stickA values of random(0.01) and random(0.001). *Figures 11 and 12* show that there is a link between crisis time and the number of cells not collided. Although there are a few outliers amongst the trials, the values in *Figures 11 and 12* are consistent with the theme of crisis time being higher when there are less collided cells. The difference between the graphs in *Figure 11 and 12* is that the trendline generally starts higher when the stickA value is random(0.01) than when it is random(0.001). This means that it takes longer for a crisis to occur when the stickA value range is higher in our simulation.

3.8 Time for Crisis Event vs. Number of Cells Collided

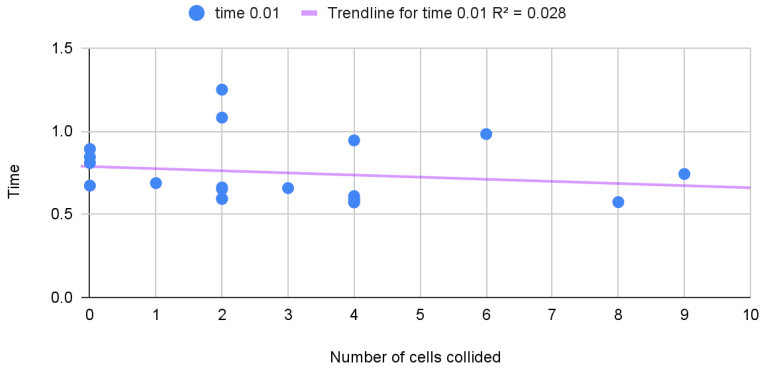
Figure 13:Time for Crisis Event vs . Collisions for StickA random (0.001)



The graph in *Figure 13* shows the total number of sickle cells that have collided during each of the twenty trials. There is an inverse relationship between the number of cells collided and the amount of time taken for a crisis to occur. Cells. The trendline shows that as the number of cells that have collided increases the time it takes for a crisis to happen decreases. This is consistent when stickA has a random value of 0.01 or 0.001. There is a sharp trend downward in time when cells have a greater number of collisions.

However, we have a low R^2 so not all the variation in the data can be accounted for. For the trials with 0.001 the R^2 value is 0.107 meaning that 10.7% of the variance in the relationship between the time taken for a crisis and cell collisions can be explained through the model. The R^2 value for the 0.01 Trial is 0.028 meaning that

Figure 14:Time for Crisis event vs. Number of Cells Collided StickA random(0.01)



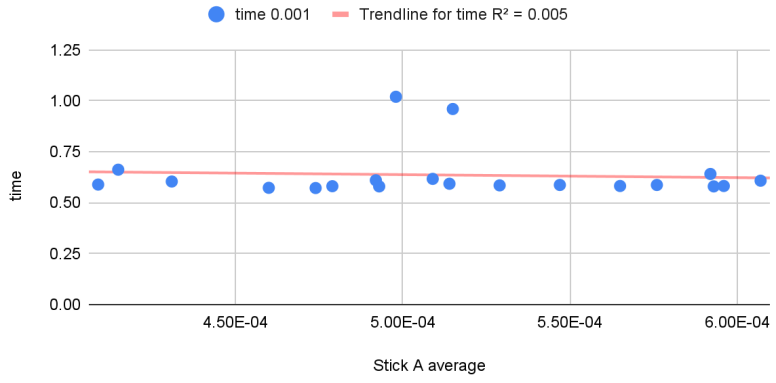
2.8% of the variance can be explained using the model. Thus, we cannot come to a conclusive relationship about the effects of collisions on crisis time.

3.9 Time for Crisis Vs. Average StickA value

Figure 15 and Figure 16 were constructed after averaging out all 20 of the stickA values for each of the trials. The average stickA value received was then graphed against time to see if the value of stickA had any effect on the time it took for a crisis to occur.

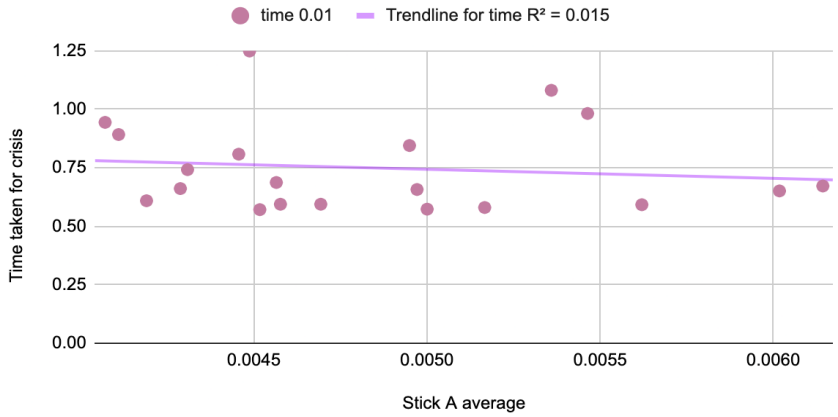
The graph in *Figure 15 and Figure 16* are a representation of stickA values during each trial. The values were graphed against time to check whether an increase in stickA value had any effect on time. As shown in the trendline on the graph when the average stickA value increases the time goes down. This means time and stickA values could have an inverse relationship. The inverse relationship of time and stickA values is consistent with sickle cell behavior because the

Figure 15:Time vs Average value of StickA or StickA random (0.001)



stickier the cells the more collisions are able to occur. There is a slight trend downward, a higher stick rate results in a lower time for a crisis event. A crisis can take place within a shorter period of time due to the higher probability of cells sticking together.

Figure 16: Time for Crisis Event vs. Average StickA value for StickA random(0.01)



However, the data also has a low R^2 value which means there is little certainty that the variance in the data can be explained by the relationship between time and stickiness. The R^2 for 0.01 trials is 0.015 which means that 1.5% of the variance can be explained by the relationship between time and average stickA values. The R^2 for 0.001 is 0.022 meaning 2.0% of the variance can be explained by the relationship between time and average stickA values. It is not possible to make a conclusion on whether the time it takes for a crisis event to happen is related to changes in average stickA values.

3.10 Comparison of Trial with StickA as random (0.01) and (0.001)

This section looks at the individual differences in time, number of cells collided, number of cells not collided and average StickA values compared for stickA of random(0.001) and random(0.01).

3.11 Number of Cells Collided under different conditions of stickiness

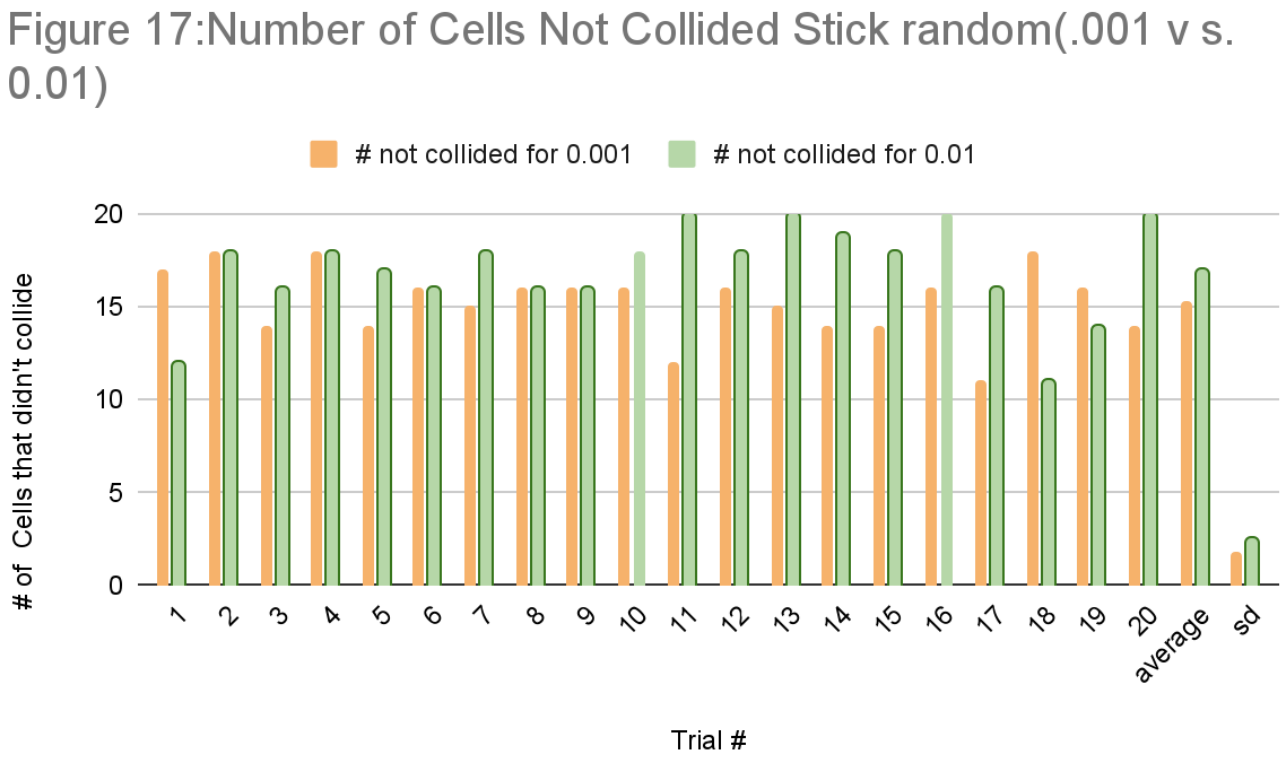


Figure 18 shows the values of cells that were stuck but did not collide. The graph has values of stuck cells from cells with a stickA value of random (0.001) and random (0.01). I calculated the values of cells that were not stuck by subtracting the total number of stuck cells in each trial by the number of cells that had collided. I then graphed these values alongside each other to show which stickA value resulted in more cells getting stuck without colliding. The last column of the bar graph is the average of the total cells that did not collide in each of the 20 trials. The results show that a higher number of cells do not collide when the value for stickA ranges between 0 and 0.01.

3.12 Number of Collided Cells

Collided cells are cells that interact with other cells that cause them to stop.

Figure 19:Number of Cells Collided StickA random(0.001 vs 0.01)

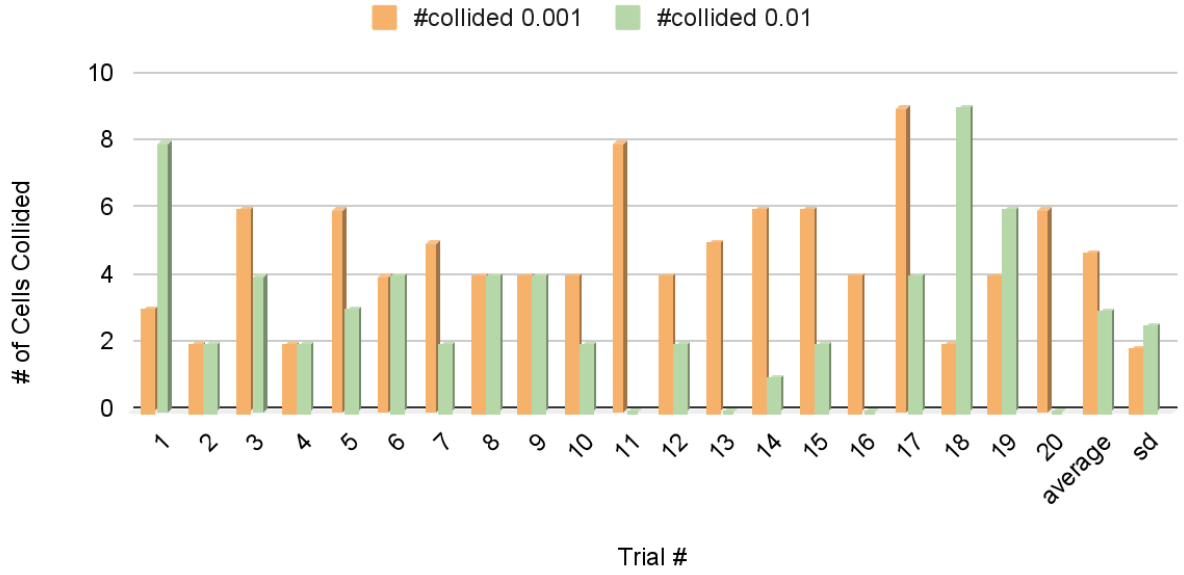


Figure 19 represents the total number of cells that collided with a stickA of random (0.001) and (0.01). I graphed the data of these cells and put them together to check the difference in collisions between 0.001 and 0.01 values of stickA. On average when the value of stickA is at a lower range the number of collisions is higher, i.e., they have an inverse relationship.

3.13 Average stickA value of Cells

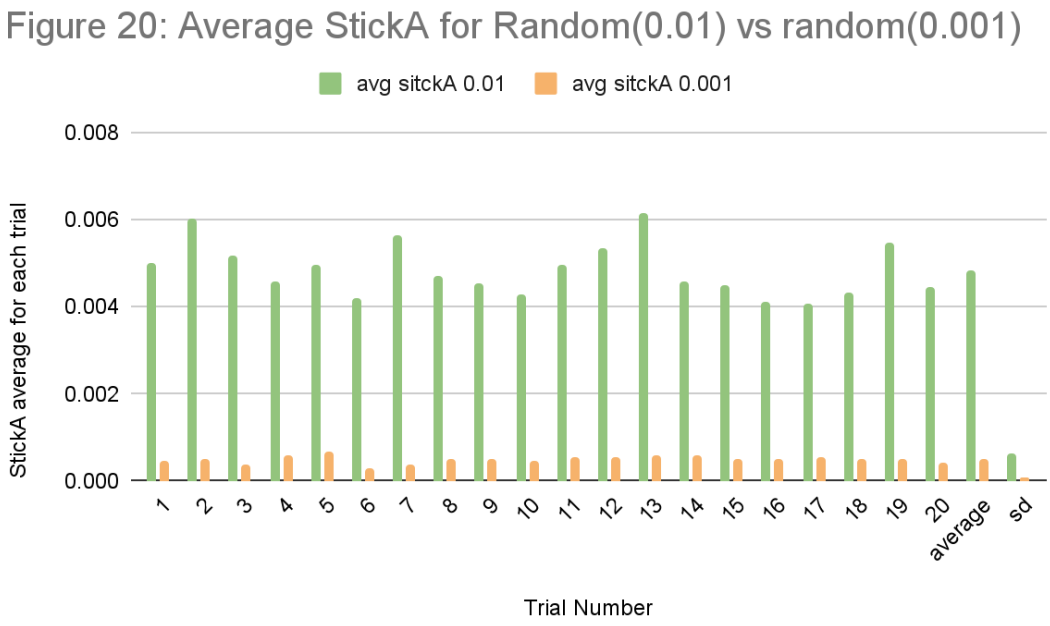


Figure 20 represents the values of stickA for random (0.001) and random (0.01).To create the graph stickA values for twenty cells that were alive at the end of the crisis were averaged and then graphed. In *Figure 20*as the range for which StickA can be increased so do the values of the average stickA. For *Figure 20* average stick A values for 0.01 are larger than those for 0.001. The difference in stickA values makes sense because when the range is 0.01 there are higher values that stickA can be. However, when limited to 0.001 it makes sense that the range of stickA values during a crisis would be lower.

3.14 Time taken for crisis to occur

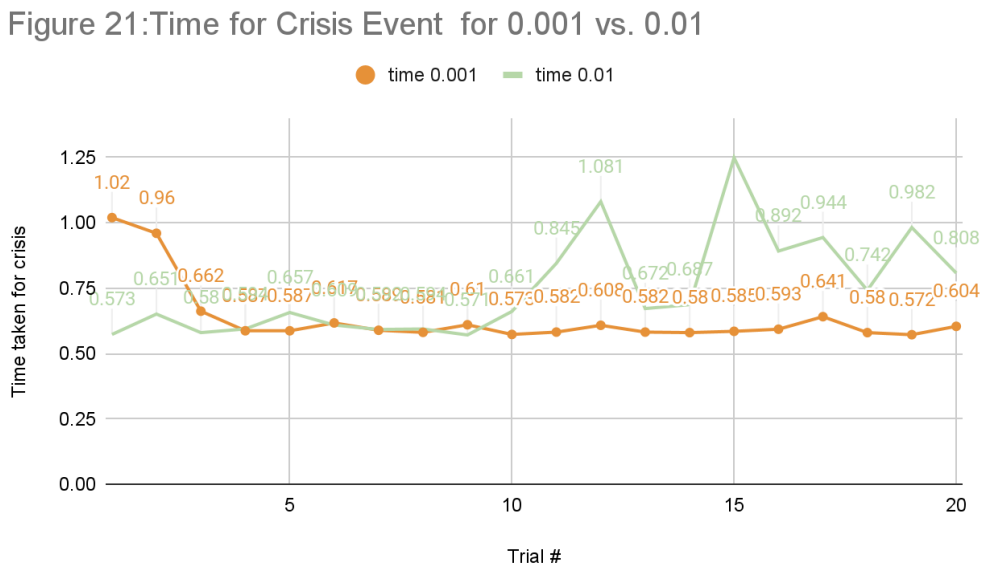


Figure 21 was created to check whether there were significant changes in time when stickA was random(0.001) vs when stickA was random (0.01). I took all the values of final time for each trial and graphed them as shown above. The average time for stickA range of 0-0.001 is 0.63565 and 0.7492 for the stickA range of 0-0.01.

3.15 Calculation Section

I wanted to know whether there is a difference in the number of collisions that take place when stickiness is higher and when stickiness is lower. Thus, I compared two different stickiness values to see if a crisis would occur more because of the higher stickiness or because of the cells colliding.

I decided to do an independent sample t-test to check whether there is a significant difference between the mean when stickA has a random value between 0-0.001 and when stickA has a random value between 0-0.01. In the two sample t-tests the null hypothesis is that there is no significant difference in the means of the times when stickA is between 0 and 0.001 and when stickA is between 0 and 0.001. While the research hypothesis is there is a significant difference when stickA is between 0-0.001 and 0-0.01. According to the results from the two sample t-tests, there is a significant difference in time between the means of the stickA value with a p-value of 0.034.

T-Test Results

The standard deviation or SD in this case looks at how spread apart the data is from the mean. The mean is another term for the average value of the data. For the twenty trials where the P-Selectin concentration was 0.01 (M=0.7492 SD=0.1926) compared to trials with a P-Selectin concentration (M=0.6356, SD=0.1236783924). The result of the independent t-test showed that t(38)=2.21, p=0.03365.

```
Welch Two Sample t-test for Time Taken for Collision

data:  datatime0.001 and datatime0.01
t = 2.2185, df = 32.392, p-value
= 0.03365
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 0.009344032 0.217755968
sample estimates:
mean of x mean of y
  0.74920   0.63565
```

To see if there is any significant difference in the mean of collisions that took place when stickA has a value of 0-0.001 vs when stickA has a value of 0-0.01 I conducted a two-sample t-test. The t-test took in collision values and checked them for differences.

```
---------------------------------------------------------------------------------------------------------------------------------
Welch Two Sample t-test for Number of Collisions

data:  collided0.001 and collided0.01
t = 2.4936, df = 35.004, p-value
= 0.01752
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 0.32531 3.17469
sample estimates:
mean of x mean of y
     4.70      2.95
---------------------------------------------------------------------------------------------------------------------------------
```

In this t-test the null hypothesis is "There is no significant difference in means between collisions when stickA is between 0-0.001 and stickA is between 0-0.01". While the research hypothesis is "There is a significant difference in means between the means. "According to the results from the t-test there is a significant difference in collisions between the number of cells that collided with stickA of random(0-0.001) and those with stickA of random(0-0.01).

T-Test Results

The standard deviation or SD in this case looks at how spread apart the data is from the mean. The mean is another term for the average value of the data. For the twenty trials where the P-Selectin concentration was 0.01 (M=2.95, SD=2.523051619) compared to trials with a P-Selectin concentration (M=4.7, SD=1.866604009). The result of the independent t-test showed that t(38)=2.4936, p=0.01752.888.

4.0 Statistical Results

I wanted to recreate this study to specifically see how the p-selection value or in my case the stickiness of cells affected how Sickle cell Crisis occurred. Specifically, I wanted to see if a crisis occurred faster when cells had a high stickA/P-selection value or when cells stuck to each other. I tested two groups one with a stickA of 0.001 and the other with stickA of 0.01.

According to my results, there is a significant difference between the time taken to reach a crisis when the value of stickiness ranges from 0-0.001 vs when it ranges from 0-0.01. This difference is shown by the t-test checking whether the mean of time from 0.001 is independent from that of 0.01. Surprisingly, the average time it took for a crisis to occur when there is a high stickA concentration was greater than the time it took for a crisis to occur with a lower stickA concentration .

Moreover, the average time taken for a crisis to occur was 0.64 when stickA was random(0.001) and 0.75 when stickA was random(0.01). Thus, on average, when stickA was 0.001 there was a decrease of around 0.1 in the time taken for a crisis to occur. In the *Figure 21* that puts the values of time for both stickA random 0.001 and 0.01 the difference in time is quite clear. The stickA random 0.001 plot has a lower time in general as compared to that of 0.01.

4.1 Collisions vs non-Collisions

Figure 13 compares the values of cells that have not collided to time and shows that a higher number of cells without collisions leads to higher crisis times. T The stickA random(0.001) experienced more collisions than stickA (0.01). The average collisions present in stickA random

(0.001) was 4.7 compared to 2.95 from stickA random (0.01). Thus, on average the stickA random(0.001) had 1.75 more collisions than stickA. I did a two-sample t-test for collisions and it showed that there is a significant difference between the two stickA value ranges. For collisions the P-Value was p=0.01752.888. When stickA ranged between 0 and 0.01 there were on average two more cells that had not collided as compared to cells that collided when stickA was a lot lower.

5. DISCUSSION

My experiment was based on a study that looked at three different concentrations of the p-selectin molecule and how they affected the adhesion of sickle cells. The goal of my experiment was to check whether a crisis occurred more often because of the inherent stickiness of the cells or because of sickle cells colliding with each other and sticking together. For my experiment I wanted to look at how different levels of p-selection or in my case stickA affected the time it took for a crisis to occur. The hypothesis was that a crisis would occur faster when there were more cell collisions than when cells had a high inherent stickiness. I looked at two stickA value ranges for the experiment, one of the ranges is 0-0.001 and the other is 0-0.01. Through the simulated experiment, I found that higher concentrations of stickA resulted in less collisions. More cells under the 0.01 range constraint got individually stuck and did not collide with each other. However, the cells with stickA values that ranged from 0 to 0.001 had more collisions and got stuck because they interacted with each other. Finally, the overall trend in time showed that trials with higher collisions led to lower times for a crisis to occur. There was also a difference in the means of time between the sample with stickA of 0.001 and that of 0.01. My results align with my hypothesis because the time it took for a crisis to occur was lowered when there were more collisions. This was consistent for both samples when stickA/P-selectin was 0.001 and when it was 0.01.

Why did the higher concentration take longer to reach crisis levels?

When cells had higher P-selectin/stickA values there were less collisions that happened. Most cells had higher stickA values when the range was 0.01 and thus, they got stuck because of their high stickA value. When a cell has a high StickA/P-Selectin concentration it is more likely to get stuck. This means at higher P-Selectin concentrations cells get stuck in place without having the opportunity to move towards each other and collide. The high stickA value results in less collisions because the cells get stuck before they can even move. This explains why the values for cells that had not collided were higher for the stickA range of 0.01 than they were for that of 0.001. The time to reach crisis for the cells with higher P-selection was slower because it takes longer for cells to meet the threshold to get stuck in place than it does for them to move around and interact with cells that are stuck. In my program a crisis occurs when there are four or more cells that are alive and have stopped within the blood vessel. Each cell starts out with a different lifespan and the lifespan is constantly decreasing. Each time a cell reproduces, the daughter cells are assigned a new stickA value. Due to the inability to reach crisis levels because the cells are either dying or are not meeting the threshold for crisis, the 0.01 ranged cells take longer to get stuck. Thus, the simulation results are consistent with the results of the experimental results.

5.2 Why did the higher concentration take longer to reach crisis levels?

The cells that have the lower concentration of P-selectin had more collisions taking place. To reach a crisis a cell needs to both be alive and have a stickA >0.01 or stick to another cell. In this range, it is hard for cells to meet the threshold possible to get stuck by themselves, so they can move around more until some of them meet the criteria to stop moving and get stuck. The lifespan decrement also applies to cells with low concentration, so they too have a limited time to interact with the cells around them. However, the cells with lower concentration of P-selectin/stickA have the advantage of movement and can easily

interact with cells that are around and already stuck. This leads to more cells being stuck because they have more free movement and are less predisposed to sticking than those with higher P-selecting concentrations. Thus, it makes sense that these cells experienced lower times when it came to having a crisis. It also makes sense that the low concentrated cells had more collisions because they could move easier than those with higher concentration. It takes less time for a crisis to occur because when cells collide, they begin to clump together, this clumping attracts other cells to the site leading to multiple cells stopping inside the blood vessel.

5.3 Why were more concentrations not represented?

My original plan was to test whether crises occurred more often when cells collided with each other and got stuck or stopped moving because of having a high stickiness coefficient. I was going to complete trials for 0.001, 0.01 and 0.1. However, I was only able to complete trials for 0.001 and 0.01. I couldn't complete a trial for 0.1 because the value of the stickiness coefficient was too high and led to inconsistent data when I was running the program. The runs that I tried doing when stickA was 0.1 resulted in the incorrect documentation of collisions by the program. For each run in the 0.1 concentration there were three less cells that had collided being reported. I think as the values for stickiness get higher it becomes more difficult to distinguish when cells collided because they bumped into each other or because they had a high stickiness coefficient that led them to automatically sticking. Thus, there was always a discrepancy between the total cells that were stuck and the number of cells that had collided. For the future, I would like to make the program compatible with high values of stickA/P-selection.

5.4 Other possible causes of time discrepancy?

I was running all these trials on a 2017 ASUS laptop and there were multiple applications running in the background. The age of the laptop and the fact that there were background processes running might have

led to longer crisis times for some values. Also, there were instances while I was running the trials when the laptop stopped responding and took a long time to reload after. This explains why some of the trials have outlier crisis times that are as high as 1 second and some are as low as 0.5 seconds. After doing 10 trials the processing window started running slower than it was before. I think some of the trials might have used a lot of power from my laptop. One of the other causes of the spikes in time could have been the battery percentage that my laptop was on because it runs slower when the battery is lower.

5.5 Changes for the future?

I want to create an updated version of the program that would also work on regular cells. My current program only displays and works with sickle cells which are half of the problem when it comes to SCD. Creating a more accurate model would require the addition of regular cells. One of the main problems caused by SCD is that regular red blood cells are unable to reach the destination they need to reach because the sickle cells clog up the blood vessels. To accurately depict a crisis, I would need to add red blood cells and see how they interact with sickle cells. Would crises occur quicker when there are more cells present in the bloodstream? Is a question I would need to answer to understand the whole piece of the puzzle. The regular cells would be blocked from moving if they were close to sickle cells that had stopped moving.

Additionally, I would like to include a lag in the clearing process of sickle cells. Sickle cells die and clump up in blood vessels because the spleen struggles to clean them out because of their short lifespan and their ability to get stuck. In the future I would like to add a method that takes longer to clean the blood vessel out when there are more sickle cells in it.

5.6 *Updates on Sickle Cell*

Since October 5, 2021, news has come out about a new treatment available for sickle cell.(Manwani, D. 2021) The treatment available is the crizanlizumab, which was mentioned earlier in the experiment. The introduction of crizanlizumab, to someone that has sickle cell lessens the adhesive nature of sickle cells. This helps reduce the number of crises that happen by reducing the amount of P-selectin in the cells. Prior to working on my project and doing the research, crizanlizumab was not announced as a possible solution for sickle cell so it is exciting now that a drug will be available in the market soon.

6. References

Agent-Based Modeling | Columbia Public Health, 2021

ASH Sickle Cell Disease Initiative. (2021). Retrieved 23 November 2021, from https://www.hematology.org/advocacy/sickle-cell-disease-initiative#SCDresearch

Booth, C., Inusa, B., & Obaro, S. K. (2010). Infection in sickle cell disease: a review. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases*, *14*(1), e2–e12. https://doi.org/10.1016/j.ijid.2009.03.010

Daniel Shiffman. (2012). Nature of Code. D. Shiffman.

HBB Gene. (2017). GeneCards. Retrieved 11/30/21, from https://www.genecards.org/cgi-bin/carddisp.pl?gene=HBB&keywords=sickle,cell,anemia

Kennedy, J. (2002). Modulation of sickle cell crisis by naturally occurring band 3 specific antibodies - a malaria link. Retrieved 23 November 2021, from https://www.medscimonit.com/download/index/idArt/420855

Loiseau, E., Massiera, G., Mendez, S., Martinez, P. A., & Abkarian, M. (2015). Microfluidic study of enhanced deposition of sickle cells at acute corners. *Biophysical journal*, *108*(11), 2623–2632. https://doi.org/10.1016/j.bpj.2015.04.018

Lu, D. C., Wadud, R., Hannemann, A., Rees, D. C., Brewin, J. N., & Gibson, J. S. (2021). Pathophysiological Relevance of Renal Medullary Conditions on the Behaviour of Red Cells From Patients With Sickle Cell Anaemia. *Frontiers in physiology*, *12*, 653545. https://doi.org/10.3389/fphys.2021.653545

Manwani, D. (2021). P-selectin and sickle cell disease: a balancing act. Blood, The Journal of the American Society of Hematology, 137(19), 2573-2574.

Man, Y., Goreke, U., Kucukal, E., Hill, A., An, R., Liu, S., Bode, A., Solis-Fuentes, A., Nayak, L. V., Little, J. A., & Gurkan, U. A. (2020). Leukocyte adhesion to P-selectin and the inhibitory role of Crizanlizumab in sickle cell disease: A standardized microfluidic assessment. *Blood cells, molecules & diseases*, *83*, 102424. https://doi.org/10.1016/j.bcmd.2020.102424

Sickle Cell Anemia. (2021).Retrieved 23 November 2021, from

https://www.mayoclinic.org/diseases-conditions/sickle-cell-anemia/symptoms-causes/syc-20355876

Sickle Cell Disease | Cedars-Sinai. (2021). Retrieved 23 November 2021, from https://www.cedars-sinai.org/health-library/diseases-and-conditions/s/sickle-cell-disease.html

Sickle Cell Disease. (2021). Retrieved 23 November 2021, from https://www.hopkinsmedicine.org/health/conditions-and-diseases/sickle-cell-disease#:~:text=Red%20blood%20cells%20with%20normal,crescent%2C%20like%20the%20letter%20C.

Sickle Cell Information. (2021).Sickle-Cell Anemia [Online Image].Howard University Hospital.http://huhealthcare.com/healthcare/hospital/specialty-services/sickle-cell-disease-center/disease-information

Spector, J., Kodippili, G. C., Ritchie, K., & Low, P. S. (2016). Single Molecule Studies of the Diffusion of Band 3 in Sickle Cell Erythrocytes. PloS one, 11(9), e0162514. https://doi.org/10.1371/journal.pone.0162514

Yeruva, S., Varalakshmi, M.S., Gowtham, B.P., Chandana, Y.H., & Prasad, P.E. (2020). Sickle Cell Disease - A Comprehensive Study and Usage of Technology for Diagnosis. International Blood Research & Reviews.