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Appendixes

1. Abstract

The initial scope of our project was intended to study HL60 cells as a model for neutrophil chemotaxis through a microfluidic maze race. Due to regulations preventing the handling of human cell lines by undergraduate capstone students however, we have shifted the focus of our project to utilize NIH 3T3 murine fibroblasts as a model for wound healing using a chemotaxis assay kit for validation. Once we pivoted our project scope and cell line of choice, we developed new trade studies for our genetic targets in the NIH 3T3 cells to optimize chemotaxis and decided on PDGFR alpha and beta subunits. We also opted to continue with lipofection as our method of delivery for genetic material. We then drafted protocols for each phase of our project and sent them to be approved by the Bioengineering Lab Safety department. Ultimately, we were able to run the chemotaxis assay as planned with the PDGFRbeta modified cells. We observed no significant difference in percent invasion between the WT and modified cells however there was a visual trend that favored the modified cells in terms of percent invasion. Given more time, we would have optimized our ratios of transfection reagents and harvested more plasmid to improve the transfection efficiency of our cells. The work done in this project remains important for laying the groundwork for future research into the mechanisms of fibroblasts in wound healing.

2. Background

2.1. Motivation for studying chemotaxis and methods for enhancing or impeding it.

Chemotaxis is the ability of cells to migrate along a concentration gradient. This mechanism is essential in many vital processes, including embryogenesis, angiogenesis, and the immune response [1]. In all these processes, if cells fail to migrate to their designated location, then the cells can die (from lack of stimulation) or even the entire organism can die [1], [2]. This fate imposes natural selection by which chemotaxis has evolved over billions of years to become a finely tuned and intricate mechanism that functions seamlessly in a healthy organism. However, various injuries and diseases threaten the functionality of the perfected chemotaxis mechanism in immune cells.

Some instances where chemotaxis is negatively altered are in patients suffering from major burns, physical traumas, or cancer [3], [4]. One study isolated neutrophils from major-burn patients along with neutrophils from healthy control patients. Subjecting these neutrophils to microfluidic channels along a concentration gradient, the neutrophils from burn patients were found to have significantly poorer directionality and slower migratory speeds relative to the

healthy control cells (close to two standard deviations lower for these statistics) [3]. The reduction in successful and swift migration of neutrophils to burn sites can be problematic as infections are more likely to develop in burn sites leading to sepsis and eventually death. Moreover, neutrophils lost around the body are more likely to spontaneously activate effector mechanisms and damage distant and unrelated host tissue [4].

A similar phenomenon is observed in patients who experience physical trauma. Studies on neutrophils from patients following traumatic brain injury, penetrative trauma, or blunt chest trauma have been shown to downregulate cell surface proteins (L-selectin, CXCR1, CXCR2) that are required for circulating neutrophils to find and latch on to epithelium at injury sites and extravasate into the tissue to aid in wound healing [5]. This is a drastic consequence of physical trauma as the absence of neutrophils in the wound increases the likelihood of infections and reduces healing time. Cancers are another instance where enhancing cellular migration is desirable. Unfortunately, certain cancers are skillful in evading the immune system and preventing immune cell infiltration, providing another reason for studying cellular migration with hopes of discovering ways to augment immune cell migration to tumors and subsequent infiltration [6].

Indeed, enhancing chemotaxis to specific sites in the body proves important for various disease treatments and therapies, yet there are also instances where *impeding* chemotaxis is preferable. Literature suggests that pulmonary pathology associated with acute respiratory distress syndrome (ARDS) could be due to an over-abundance of neutrophils in alveolar tissue causing hyperinflammation [7]. The trend of accumulating excessive amounts of immune cells in sites where they should not be is common of autoimmune diseases (lupus, arthritis, etc.) and thus provides more applications for any research pertaining to cellular migration in these disease states [8].

Overall, chemotaxis is an intricate, complex, and fine-tuned mechanism that cells use to home to a target site. In states of disease or injury, there is much to be learned regarding how to restore chemotaxis to promote wound healing and treat certain diseases. If we can understand cellular migration to the point where we can successfully modify human leukemia (HL60) cells in-vitro to navigate through a complex maze more efficiently than originally possible, then we will demonstrate the potential ability of immune cells to be modified in-vivo to navigate more efficiently to sites of injury or infection. Hopefully this project will uncover new aspects of cellular migration that have promising applications for various therapies.

2.2. Literature Review

2.2.1. HL60 Cell Features

2.2.1.1. Movement

HL60 (human leukemia) cells refer to peripheral blood lymphocytes that were isolated from a patient suffering from acute promyelocytic leukemia. They are pluripotent and can be differentiated to have similar properties as human neutrophils. HL60 cells are commonly differentiated since neutrophils are characterized by short life spans by which they can

experience apoptosis within the frame of 6 to 12 hours after isolation, providing difficulty in conducting sufficient research with them. Neutrophils move by actin-based protrusions, or amoeboid motion. This is when projections called pseudopodium reach out and briefly extend an arm like projection, stretching the cell. This is followed by contractions of filaments in the cytoplasm to drag the cell along the surface it is traversing[9]. These cellular protrusions are called polarized morphology, which is the cell having a distinctive leading edge and trailing edge. At the leading edge, actin is polymerized preferentially in the presence of a chemoattractant. The cell asymmetrically morphs into a polar shape in response to a chemoattractant but also there is internal signaling at play causing the cell to morph. One hypothesis is this mechanism depends on the activity of one or more Rho guanosine triphosphates (GTPases) and most likely includes activation of phosphatidylinositol 3-kinase (PI3K). Another line of thinking is the protein Cdc42 plays a role in polarization and actin polymerization at the leading edge of the neutrophil. In this study, these methods were not definitively confirmed, however researchers concluded one or both reasonings are the case[10].

2.2.1.2. Chemotaxis/Thermotaxis

Another key feature of HL60 cell movement, and other lymphocytes, is chemotaxis. Chemotaxis is the directed migration of neutrophils and neutrophil-like cells, guided by intracellular signaling and external chemical gradients. Lymphocytes have temporal and spatial regulation of signaling pathways that allow them to be more sensitive to chemical gradients. This allows the cells to rapidly migrate towards the higher concentration of the chemoattractant within the gradient[11]. This is crucial for understanding how HL60 cells migrate. First, we must identify the specific receptors and proteins within the cells that make them more sensitive to these external chemical gradients. Understanding this will help us narrow down how neutrophils are able to respond quickly and effectively to chemical cues within the body. In turn, this will help for the experiment when deciding how to modify the HL60 cells. The calcium dependent protease calpain is thought to play a role in regulating neutrophil chemotaxis. Studies have shown that calpain inhibition increases the cell migration speed of neutrophils. Cells treated with calpain inhibitors, CID-TAT, were shown to promote formation of pseudopods, the projections from the polarized leading edge of neutrophils while moving. This suggests that calpain activity in resting neutrophils may suppress movement. Researchers hypothesized that calpain activity is regulated asymmetrically throughout the cell during chemotaxis. Meaning, during chemotaxis calpain inhibition on the leading edge of the neutrophil may cause an increase of Rac and Cdc42 protein expression, leading to increased movement and speed. Simultaneously on the trailing side of the cell, calpain expression is normal and inhibits expression of movement promoting proteins[12]. Another gradient differentiated HL60 cells have exhibited sensitivity to is temperature. When the body is cut or inflamed, the temperature at the site increases, leading to the idea that neutrophils could also respond to temperature gradients and migrate towards higher temperatures. Through subjecting differentiated HL60 cells to different temperature gradients, researchers found there are two subpopulations, positive and negative thermotaxis. Assuming the cells were not thermotactic, the cells would migrate roughly evenly between high temperature, low temperature, and perpendicular to the gradient. However, researchers found that ninety

percent of adherent and mobile cells migrated either towards the heat source or heat sink. Cell migration speed increased when traveling towards higher temperatures as well[13]. These correlations led to the conclusion that differentiated HL60 cells, and likely neutrophils, are sensitive to temperature and chemical gradients.

2.2.1.3. HL60 Cell Signaling, Motility, and Speed

HL60 cells rely on signaling mechanisms that direct their movement to target locations. These mechanisms follow a methodology of receiving signaling input from specific receptors from the leading edge, known as the lamellipodium, and maintaining an overall cell polarization at the trailing edge, which follows in response to the receptor input. They rely on G-protein-coupled receptors to conduct this process. This signaling mechanism provides further insight into their efficiency in response to directional signaling and reprogramming during cell movement. In a study conducted by Hadjitheodorou et al., researchers investigated the mechanism and potency of receptor inputs compared to the rear polarity of the cell and how these signaling pathways can be modified when directing HL60 cells through microfluidic channels and directional changes [14]. It was identified that receptor input polarization is much stronger in redirecting overall weak polarization in cells compared to cells with strong polarization enabling new receptor inputs. Due to these findings, an approach was taken to focus on modifying receptor input polarization to increase sensitivity to the trailing edge of the cell and improve direction reversal for migration efficiency. For example, these researchers took an approach to adjust the RhoA/ROCK/myosin II pathway in a neutrophil-like cell by targeting modifications in activity of myosin II. This was performed since this pathway is located in the trailing edge of the cell, which means the polarization signals from the rear of the cell would inhibit new receptor inputs. Therefore, researchers wanted to target a method for altering this mechanism to allow for integration of new receptor inputs during directional changes. This provides potential in how signaling pathways can be redirected when engineering the HL60 cell line for improved efficiency during chemotaxis.

Activation of these G-protein-coupled receptors also leads to another signaling pathway involved in HL60 cell motility during chemotaxis, known as calcium signaling. HL60 cells move in a manner of rolling and adhering to the affected/inflammatory site with the assistance of adhesion molecules. This is regulated by the binding of P-selectin to the P-selectin glycoprotein ligand-1, which is then followed by a process known as intracellular calcium bursting of the cells [15]. Huang et al. discovered that this calcium signaling is dependent on mechanical force and concentration of immobilized P-selectin. In addition, an influx and efflux of calcium ions occur through specific calcium channels. The increase of calcium flow through the cytoplasm contributes to other factors that enhance cell motility, including modified gene expression associated with gene regulation of calcium signaling and cytoskeleton rearrangement [16].

Differentiated HL60 cells and engineered HL60 cell lines have been compared throughout the literature to assess the impact such cell signaling mechanisms and extracellular environment factors have on their migration speed compared to primary neutrophils. In a study conducted by Babatunde et al., researchers compared primary neutrophils, HL60 cells differentiated with DMSO, and HL60 cells differentiated with DMSO and supplemented with nutridoma in terms of chemotaxis and swarming abilities [17]. They assessed the speed of each using fMLP and LTB4 gradients. They noted that the differentiated HL60 cells moved at an

approximate rate of 24 and 15 μm/min, differentiated and supplemented HL60 cells moved at an approximate rate of 15 and 12 μm/min, and primary neutrophils moved the fastest at an approximate rate of 35 and 42 μm/min in fMLP and LTB4 gradients, respectively. From the official Dicty World Race, they compared the speeds of all HL60 and Dicty cell teams. It was noted that the average velocity of the HL60 cells, 18 μm/min, was approximately two times faster than that of the Dicty cells with an average velocity of 8 μm/min [6].

2.2.1.4. HL60 Cell Biological Markers

To gain a deeper understanding of key characteristics that define the HL60 cell phenotype, it is important to assess the biological markers that are associated with this cell line. According to the literature, HL60 cells have been differentiated into mature neutrophil-like cells using various conditions, including dimethyl sulfoxide (DMSO), dimethylformamide (DMF), all-trans retinoic acid (ATRA), 1α, 25-dihydroxyvitamin D_3 ($VitD_3$), and dibutyryl cyclic adenosine monophosphate (dbcAMP).

Various experiments have reported distinct patterns in marker expression associated with the differentiated HL60 cell line. In a study conducted by Boss et al., HL60 cells were differentiated using DMSO, and they were reported to express antigens related to the granulocyte and leukocyte phenotypes, a smaller abundance of beta 2-microglobulin and HLA-A,B,C markers that could have been related to a smaller population of mature cells, and an increased activation of C3d receptors [18].

Another study differentiated HL60 cells for vaccine development using DMF, ATRA, and $VitD_3$. The cells were observed to express an increase in CD11c, CD14, CD64, CD18, and CD32 markers with an emphasis placed on CD11c as a useful differentiation marker for HL60 cells [19]. In a study conducted by Rincón et al., researchers wanted to investigate the impact of differentiation efficiency of HL60 cells and PLB-985 cells (a sub-line of HL60 cells used to study chemotaxis) into mature neutrophils among different differentiation protocols and create a comparison in transcriptomes against primary neutrophils [20]. They determined that DMSO provided the best outcome in terms of differentiation marker expression and cell viability among the differentiation inducers. Key biological markers the researchers were examining for were FPR1 (formyl-peptide-receptor 1) and CD11b (Integrin alpha M). CD11b was considered because it is an early marker of differentiation since it appears in a 3-day time frame after differentiation with DMSO. FPR1 was considered because it is identified as a late neutrophil differentiation marker since it is only observed within a 5 to 6-day time frame after differentiation. While these markers were expressed in the HL60 cells after exposure to a differentiation inducer, they were not expressed in the undifferentiated cells.

In addition to these biological markers, HL60 cells are characterized by specific cell surface receptors and G-proteins (guanine nucleotide-binding proteins). G-protein-coupled receptors are essential in signaling processes that guide HL60 cells during directional sensing cell migration toward a chemoattractant. A thorough review conducted by Klinker et al. outlines the G-protein coupled receptors that have been commonly expressed in HL60 cells, which include complement C5a receptors, leukotriene B4 (LTB4) receptors, histamine H1- and H2-receptors, and beta 2-adrenoceptors [21]. Additionally, they have found that the following effector systems regulated by G-proteins are commonly expressed among HL60 cells:

phospholipase D, nonselective cation channels, NADPH oxidase, and adenylyl cyclase. They also mentioned that common G-proteins that have been found to be expressed in HL60 cells are G-proteins and Gs-proteins of the Gq-family as well as pertussis-toxin-sensitive Gi-proteins. The extent to which these markers and signaling pathways are expressed vary by differentiation conditions.

2.2.2. HL60 Culture Techniques

HL60 cells grow primarily in suspension culture which makes them easier to passage but also requires daily viability measurements to ensure proper growth patterns are followed[22]. Since the limiting factor for growth is the concentration of cells in the media as opposed to the surface area of the adhesive surface in adhesion culture, HL60 cell growth can be scaled up relatively fast[22].

There are two main morphologies that HL60 cells can be differentiated into: granulocytes and monocyte-macrophages. Granulocytes are the most common class of white blood cells and are characterized by the release of enzyme granules in the event of an infection. Granulocytes themselves can be divided into three subgroups: neutrophils, eosinophils, and basophils. Neutrophils are the most common type of granulocytes and are often considered the first line of defense in the body against infection. They are circular at rest but can change shape in the event of an infection in order to optimize movement around the body to target said infection. When neutrophil contractility is impaired, the body's immune response is severely impacted as the neutrophils can no longer navigate as efficiently through the various molecular roadblocks in the blood vessels of the body[23]. Eosinophils and basophils are also important parts of the immune system which primarily respond to allergens by secreting histamine and heparin in the presence of unwanted allergens. Monocytes are physically the largest white blood cells with a two-bodied, or bilobed, nuclei being a distinct characteristic. Monocytes can either become macrophages or dendritic cells with macrophages being responsible for removing dead cells and ingesting foreign material from the site of an infection and dendritic cells being responsible for initiating antigen-specific immune responses[22]

HL60 cells have an extensive history in the literature of being able to be differentiated into many different forms depending on the compounds used in the culture media. Compounds such as dimethyl sulfoxide(DMSO), retinoic acid, and actinomycin D have been shown to induce granulocyte differentiation[22]. Sodium butyrate, phorbol esters, and cholecalciferol(Vitamin D3) are commonly used to induce monocyte-macrophage differentiation[24]. Differentiation under DMSO and retinoic acid protocols leads to condensation of nuclei which take on a lobed appearance that matches the nuclei of segmented neutrophils. The cytoplasm also becomes more diffuse as the overall cell size decreases[23]. Histochemical changes include decreased myeloperoxidase activity and reduction of the granulocyte marker nitroblue tetrazolium[23]. The addition of phorbol esters and Vitamin D3 in the macrophage differentiation protocols lead to spindle-shaped cells and nuclear condensation to a lesser extent compared to DMSO differentiation[24].

2.2.3. Genetic Engineering

2.2.3.1. Overview

Genetic engineering is defined as the use of laboratory technology to change the genetic makeup of an organism. This can come in many forms, such as changing individual base pairs and adding or deleting sections of DNA. This can fundamentally change how the organism in question functions on levels ranging from molecular to behavioral. Genetic engineering is a very diverse field and has been applied to things like modifying crop species to provide better yield and in the development of human medical treatments [25].

2.2.3.1.1. DNA

DNA is a type of large nucleic acid. It is composed of nucleotides, which organize into pairs and then into a larger double helix structure. These nucleotides include four types of nitrogenous base: adenine (A), thymine (T), cytosine (C), and guanine (G). They bind exclusively in pairs of AT and CG. The sequence of these bases in a DNA molecule encodes instructions for the production of proteins by a cell.

2.2.3.2. Transfection

Transfection refers to a lab technique that introduces new nucleic acids into a target cell or cells. This may include DNA as well as various forms of RNA depending on the goal of the transfection. Transfections are typically classified by the time over which the introduced genetic material will remain functional in the target cell. A transient transfection does not introduce the nucleic acids into the genome of the target and therefore the effects are only seen over a limited amount of time. This is most useful when the short-term causes and effects of gene expression are being studied. A stable transfection aims to introduce nucleic acids into the genome of the target. When successful, this allows the effects of the transfection to be studied over the long term. This is usually done with DNA but can also be done with certain types of engineered RNA molecules [26]. The effects of stable transfection can even be seen passed down to the offspring of the original target in some cases. These RNAs would not be inserted into the genome but would still show long term effects . There are many techniques for transfecting cells. They are very diverse and are mainly derived from biological, chemical, and physical processes. Each cell type may be more susceptible to a different method of transfection and therefore the type of nucleic acid and the method used must be tailored to the needs of the individual experiment.

2.2.3.2.1. Viral Vectors

Biological methods used to induce transduction typically use viral vectors to do so. This takes advantage of the predeveloped machinery for introducing new genetic material into a cell already present in many viruses. Many varieties of virus have been used for this purpose, each being applicable in different situations and types of cells. Some of the most common of these are adenoviruses, adeno-associated viruses (AAV), and retroviruses. Adenoviruses and AAVs are widely used for transfection of many types of cells because of their versatility and stability. Their main drawbacks are inducing a strong immune response if employed in a living organism and the time of gene expression [26]. The DNA is not inserted directly into the chromosome of the target and therefore the time of gene expression is limited to a transient scale. Retroviruses, and most commonly lentiviruses, are RNA viruses that transcribe their genetic material directly into the

genome. This makes them a better choice for longer term studies and cell lines that will be used for multiple experiments over a longer period [26].

2.2.3.2.2. Physical Transfection Strategies

These transfection methods generally involve the physical placement of the genetic material into the cell by some means. Microinjection is likely the simplest of these techniques. A small needle is used to inject genetic material into a single cell. When done correctly, the success rate of this technique is very high. It can approach 100%, much higher than most other transfection methods. The obvious drawback is that it can only be done on a relatively small scale because it requires one cell to be transfected at a time. This is most commonly done on cells that are difficult to work with using other platforms or that are only available in very small numbers. Additionally, the injection process can be physically stressful to the cell, which can cause viability issues in some cases [26].

Another approach has been named “biolistics”, a combination of “ballistics” and “biology”. It is so named because it involves shooting particles containing genetic material into the cell at high speeds. It was first developed for use in plant cells because it can overcome physical boundaries like the cell wall. Research has since expanded into use *in vivo* because it can penetrate the outer layer of skin and potentially into deeper tissue, which is valuable in such genetic engineering attempts. This process can also be combined with another, called electroporation, for increased effectiveness. Electroporation is the use of an electric field to induce inc[26]reased permeability of the cell membrane by disrupting its electrical properties [26]. The properties of the electrical impulse generated modifies the specifics of the permeability induced on the target cells. In general use, this allows certain molecules to diffuse across the membrane where they normally would not be able to. A similar effect can also be achieved through the use of lasers. Cellular membranes are permeabilized using laser light and molecules that are usually excluded are allowed to diffuse inside [26]. It is not yet clear exactly what mechanism allows this to happen yet.

2.2.3.2.3. Chemical Transfection Strategies

Many diverse chemical methods have been characterized for transfection purposes. One such approach employs magnetic fields to introduce the DNA into the cell. This is done by binding the genetic material to a small magnetic particle and then exposing the mixture of cells and these particles to a magnetic field . This causes the genetic material to move into the cells via processes like endocytosis. Some other methods use positively charged molecules to interrupt the structure of the cellular membrane to allow for diffusion of normally excluded particles. Finally, nanoparticles made of various substances are also a prevalent method of nucleic acid delivery. These can be comprised of lipids, polymers, and some inorganic compounds. They encase the genetic material in order to move it across the membrane and deliver it to the nucleus [26].

2.2.3.3. CRISPR

2.2.3.3.1. Function

Clustered regularly interspaced short palindromic repeats (CRISPR) is a genetic engineering technology that is based on a mechanism for immunity against viruses observed in

bacteria. The basis of the technology is CRISPR RNA (crRNA) which is RNA with a customizable sequence used to target a specific location in the DNA. crRNA guides a nuclease, often Cas9 in this case, to the sequence the treatment is aiming to target [27]. This method of targeting often proves to be much easier to design than methods that use protein dependent specificity. The targets of crRNA are limited by its dependence on protospacer adjacent motifs (PAMs). These are short sequences of base pairs that need to be located immediately downstream of a target region for the CRISPR/Cas system to function correctly. This means that only sequences with the appropriate proximity to PAMs can be targeted by the technology but also serves to increase specificity. The Cas protein serves to cleave the strand of DNA at the specified location. This activates the cellular DNA repair mechanisms, and this can be exploited in various ways to achieve the desired modifications, additions, or deletions to the genetic sequence [27].

2.2.4. Induced Pluripotent Stem Cells

Induced pluripotent stem cells (iPSCs) are a variety of stem cells that can be produced from the somatic cells of an organism. They are reprogrammed using a mix of transcription factors to allow them to regain their ability to differentiate into any other cell type that is desired. The transcription factors, referred to as Yamanaka factors, include OCT4, SOX2, KLF4, and MYC. Once the cells are returned to a stem-like state, researchers can expose them to molecular regimens to cause them to differentiate into the desired cell type. iPSCs have opened many doors to research that was difficult or impossible to conduct before. They are especially useful for studying cell types that are difficult to obtain. Neurons and cardiomyocytes are examples of these which have been easier to research with iPSC technology. iPSCs are commonly used to produce models for diseases and in screening drug candidates. Another reason for the popularity of iPSCs is that they allow researchers to bypass any ethical issues raised about the use of embryonic stem cells [28].

2.2.5. N-Formylmethionine-leucyl-phenylalanine (fMLP or fMLF) Molecular Pathways

2.2.5.1. Background

fMLP was the chemoattractant utilized in the 2014 Dicty World race and will be the chemoattractant at the end of the maze for this project [6]. Therefore, it is paramount to understand everything regarding fMLP's structure, binding, and cellular effects. fMLP is a short three-residue-long peptide, consisting of a formylated amino-terminus followed by a methionine, leucine, and a carboxy-terminal phenylalanine residue [29]. Short, formylated peptides are common, pattern-associated molecular markers of bacteria and, when released in the human body, can trigger a wide range of inflammatory responses [29], [30]. In addition to its proinflammatory function, fMLP is known to be a potent chemoattractant, providing a molecular address and chemical pathway for immune cells to locate and fight an invading pathogen[2], [10], [11], [29], [30]. Many immune cells, principally neutrophils, have receptors for formylated peptides that allow them to find invaders and activate their effector functions to quash infection.

2.2.5.2. Formyl-Peptide-Receptor 1 (FPR1)

To sense invading pathogens, cells need receptors to bind to pathogen-associated molecules and subsequently signal internal changes to the cell. To sense the formylated peptides of bacteria, immune cells contain formyl peptide receptors (FPRs) [29]. Counterintuitively, the formyl moiety on the amino-terminus is not a requisite for peptide binding, however, the presence of the formyl group increases binding affinity 100-fold or more [29]. While there are three well characterized FPRs (1-3), FPR1 is the principal receptor for fMLP and promoting intracellular signaling events and changes including chemotaxis and immune effector functions such as degranulation, phagocytosis, and cytokine production[4], [7], [8], [29].

Single nucleotide polymorphisms (SNPs) and polymorphisms in FPRs are associated with accumulation of bacteria around the body and poor pathogen clearance by the immune system [29]. The importance of FPR1 in pathogen locating and identification has been underscored with myriad experiments. One experiment exposed wild-type and Fpr1-/- mice to cigarette smoke, which is known to contain bacterial products including formylated peptides. After exposure to cigarette smoke, the immune cells of the wildtype mice migrated to the lungs, guided by the FPR interactions, and mounted an immune response causing severe pulmonary inflammation, whereas the Fpr1-/- mice exhibited no lung pathology as their immune cells could not migrate to the lungs without FPR1 [29]. Moreover, bacteria S. aureus has been shown to produce an inhibitor of FPR1 and thus evade the immune system[29].

The FPRs are G-protein coupled receptors (GPCRs) which consists of a seven transmembrane membered protein, providing the N-terminus and three loops to the cell's exterior for binding events and a C-terminus and three loops intracellularly for signaling events[1], [2], [10], [11], [29]–[35]. The structural biology of the receptor explains its preference for short, formylate peptides, specifically fMLP. The binding pocket contains the following: a residue that favorable hydrogen bonds with the formyl group, a hydrophobic pocket with a hidden basic residue to stabilize the methionine and provide the sulfur atom a favorable electrostatic interaction, and more hydrophobic pockets to support the leucine and phenylalanine residues of fMLP [29].

2.2.5.3. Signaling events following binding of fMLP to FPR1

The signaling events that follow binding of fMLP to FPR1 are numerous and complex [15-23]. Adhering to the classical events that follow ligand binding of GPCR, the effector alpha and beta-gamma subunits dislodge from the receptor following the phosphorylation of GDP to GTP [34]. Of importance to neutrophil chemotaxis is the beta-gamma subunit activating a family of enzymes phosphatidylinositol-3-OH-kinases (PI3Ks) [31], [34], [36]]. One direct result of active PI3K is the phosphorylation of the membrane inositol phospholipid phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P2) to phosphatidylinositol-3,4,5-triphosphate (PtdIns(3,4,5)P3. The abundance of chemoattractant at the front of the cell thus coincides with elevated binding of fMLP to FPR1 at the front of the cell, leading to an abundance of PtdIns(3,4,5)P3 at the front of the cell. This both recruits and activates Rho GTPases, namely Rac and Cdc42. These in turn activate the Arp2/3 complex, promoting the nucleation of actin and its subsequent polymerization at the front of the cell which becomes a pseudopodia extension forward[1], [2],

[10], [11], [29]–[31], [33]–[35]. In this way, fMLP at the front of the cell can initiate local signaling events to produce extensions at the front of the cell to provide the protrusion and force needed to propel the cell forward.

Actin polymerization only occurs at the leading edge of the cell due to the presence of another protein PTEN (phosphatase and tensin homolog) which dephosphorylates PtdIns(3,4,5)P3 back to PtdIns4,5)P2 [31], [36]. Binding of fMLP at the front of the cell dislocates PTEN to the rear and sides of the cell where it degrades PtdIns(3,4,5)P3 and thus no pseudopodia are formed in these faces. This provides a mechanism to ensure that the cell moves only in one direction: up the concentration gradient. Concurrent with the actin polymerization at the leading edge of the cell is the accumulation of myosin II filaments at the rear and lateral edges of the cell. This is thought to be regulated by a wide variety of factors including cGMP, MLC, MLCK, and more [37]. In effect, this provides a contractile molecule at the rear and sides of the cell to constrict the cell body and propel the cell forward.

In addition to promoting chemotaxis, binding of fMLP to FPR1 also induces production of reactive-oxygen-species (ROS) (a common effector mechanism for neutrophils), degranulation of the neutrophil's toxic granules and agents, cytokine expression to signal to neighboring immune cells, phagocytosis of complement-tagged pathogens or other non-self entities, and changes in the cell surface proteins [5], [7], [8], [11], [29], [35].

As important a role that FPRs play in neutrophil chemotaxis and these other mechanisms, they do not tell the full story of cell migration. Experiments that knockout or knockdown FPR severely inhibit immune cell migration but do not abrogate it completely, speaking to alternative mechanisms in place to help the cells move [11]. These experiments support the notion that cell motility is a complicated mechanism with certainly more than one pathway involved.

2.3. Patent Review

Due to the more abstract nature of our designed product (i.e. an engineered HL60 cell line), relevant patents for this aspect of the project are extremely limited. Rather, relevant patents are more in the scope of the microfluidic maze device component of the project. Even though the maze device is not the central focus of the design efforts, relevant patents are still included to demonstrate a thorough search of existing intellectual property and provide an argument against potential infringement for any aspect of the project.

2.3.1. Search Criteria

In order to conduct a sufficiently thorough patent search the team conducted a search through the Google Patents database using the following terms:

"*HL60 cells*", "*cell chemotaxis*", "*neutrophil chemotaxis*", "*microfluidic maze*", "*motility assay*"

Using multiple combinations of these terms allowed the team to narrow down the pool of relevant patents to those that were most applicable to the planned features of the maze and were not expired or abandoned.

2.3.2. *US8970891B2-Microfluidic Cell Motility Assay*

This patent has several claims but the three main ones are the ability to isolate a singular cell from a tissue sample, allow the cell to enter a microcapillary channel where the cross-sectional area of the channel is less than that of the maximum cell diameter, and detecting unidirectional movement of the cell along the channel in the absence of a chemical gradient[38]. Figure 1 shows a schematic of the device with two circular reservoirs connected by several of the microcapillary channels in parallel[38]. The device within this patent poses similarities to the maze device eventually developed for the first race, however the lack of a chemical gradient and the detection of only unidirectional movement make this product distinctly different than the race maze.

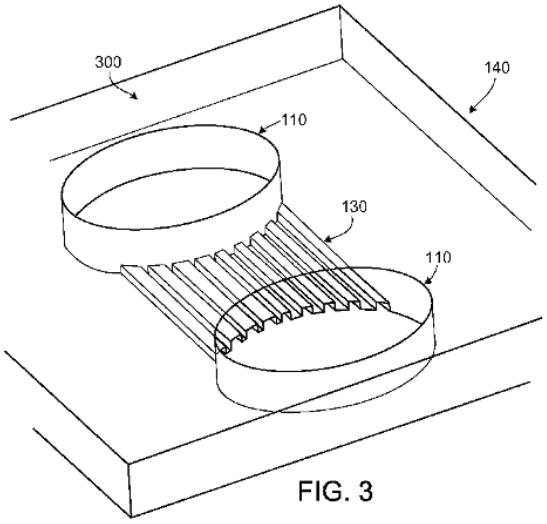


Figure 1: Schematic of the microfluidic reservoirs and capillaries in the motility assay device

2.3.3. *US8268614B2-Method for Assaying Cell Movement*

This patent describes a method to quantify cellular movement by constraining cell movement within a well to a subsection of that well. The main claims of the patent involve providing a multiwell plate along with an elongated member which can contact the surface of the bottom of the well and constrain cells along that surface[39]. Cells are then to be incubated and cultured in the well containing the elongated member before conducting an assay from one of the following classifications to characterize cell movement: colorimetric, fluorometric, optical density, or light scattering[39]. Figure 2 below provides a drawing of the proposed well and cell seeding system[39]. For the purposes of our project, this patent will not pose an issue for infringement because the proposed maze will not adopt the strategy of using an

elongated member to constrain cells in order to measure movement. The eventual maze for our race will also employ the use of a chemoattractant to promote cell movement which this patent does not cover in its claims.

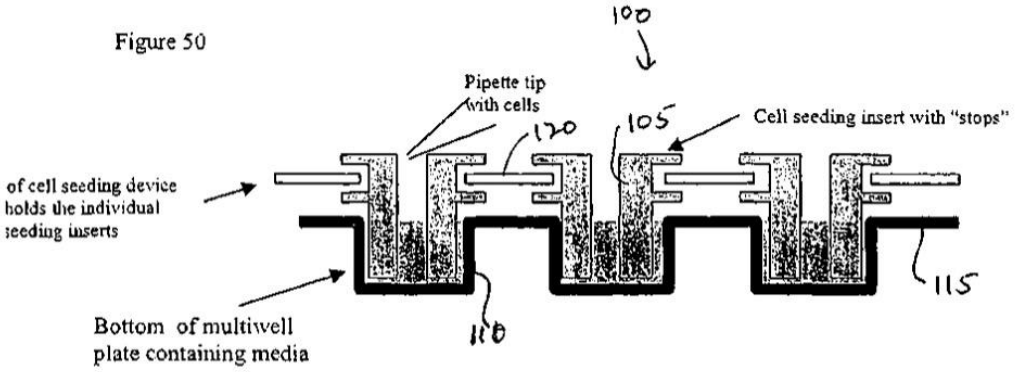


Figure 2: Depiction of multiwell plate containing cell media and elongated member(cell seeding device) to constrain said cells

2.4. Existing Product Review

Similar to the patent review, the team was able to find several products on the market that laid a strong foundation for the basis of the microfluidic maze but none that related to the engineered cell line. These products provide an essential foundation for state-of-the-art assays that will inform the future testing decisions of our project without infringing on copyrights or IP protections.

2.4.1. Millicell™ μ-Migration Assay Kit

While the current product market was limited in producing results for the target product of an HL60 engineered cell line, there were some products that were relevant for the maze portion of the project. One such product is the Millicell™ μ- Migration assay kit, which provides a novel way of observing and quantifying cell chemotaxis over linear and stable concentration gradients[40]. Other assays on the market do not produce consistent linear concentration gradients for chemoattractants or provide the ability to image the cells as they migrate. The migration slide itself is made of a plastic with optical qualities similar to glass to ensure clear tracking footage can be recorded[40]. While this assay is certainly interesting in its ability to characterize cell chemotaxis in newly efficient ways, it required that the cells being tested are adherent, which the HL60 cell line is not as they grow in suspension. In Figure 3, a cross-

section of one of the chambers is shown with two reservoirs on either side of the observation window where the cells would be recorded during migration[40].

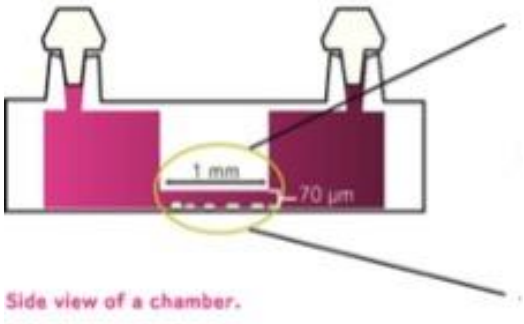


Figure 3: Profile view of µ-Migration Assay chambers with accompanying chemoattractant reservoirs and observation window

2.4.2. Cytoselect™ 96-Well Cell Migration Assay, 3m

Another similar product on the market comes from Cell BioLabs with the Cytoselect™ 96-Well Cell Migration Assay. The assay features a suspension of cells within a polycarbonate membrane chamber in each of the wells above a solution of media and chemoattractant[41]. The cells then migrate through the polycarbonate membrane towards the chemoattractant where they are dissociated from the membrane using a Cell Detachment Buffer solution[41], [41]. The migratory cells are then quantified using a CyQuant™ GR Fluorescent dye while being analyzed with a fluorescent plate reader[41]. This process is summarized below in Figure 4.

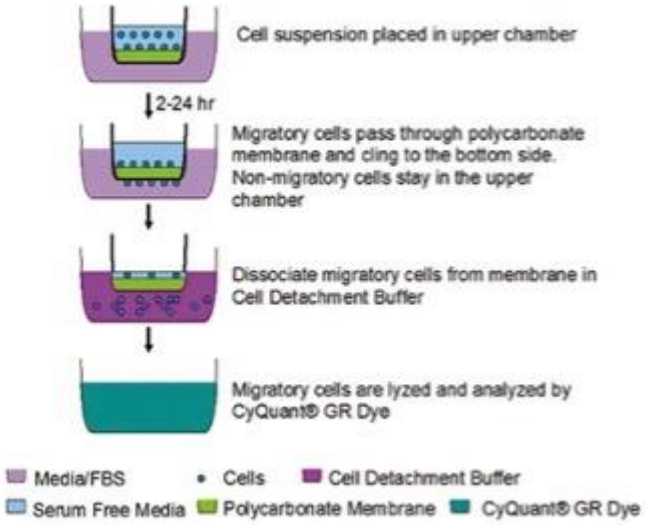


Figure 4: Cytoselect™ Migration Assay Principle

2.4.3. Previous Dicty Race Strategies

Another key aspect of the team's investigation into prior solutions was researching the methods of the past teams in the original Dicty world race. All fourteen teams were able to engineer their cell lines to be faster than their respective control(wild type HL60 or Dicty cells) while traveling throughout the maze[6]. The approaches taken by each team are summarized below in Figure 4. Out of all participants, only three of the teams chose to work with HL60 cells and their approaches included enhancing calcium signaling through the IL8/PLC-β/IP3 pathway, overexpressing light chain myosin through the RhoA/p160ROCK/myosin II pathway to increase contractility, and enhancing speed through Boyden chamber selection[6]. In order to ensure our project adheres to novelty requirements, the past strategies of the other HL60 cell teams will be off-limits for any design solutions used for our project. An important takeaway from the results of the first race can be seen in Figure 6 with the graph of average cell speed plotted against the navigational efficiency metric for each team[6]. As the cells moved through the maze with faster speeds, they did not perform as well in navigating the maze. Also an important note is that the first and second place teams ended up on opposite ends of this tradeoff, which shows how even though the teams opted to enhance different cell characteristics, they were still able to achieve strong results. In the context of cellular chemotaxis this can be interpreted as not taking the path that displayed the largest concentration of chemoattractant at any given turn. Optimizing this tradeoff will be an essential component of the design process for this project.

Team #	Team members	Cell type	Strategy	Cell tracks and full description
1	David Queller, Joan Strassman, Debbie Brock, Tracy Douglas, Susanne DiSalvo, and Suegene Noh, Washington University, St. Louis, US	Dicty	Wild Dicty cells.	https://figshare.com/s/ebf97b9cf877696dc20a
4	Guillaume Charras, University College London, UK	HL60	Increase contractility and speed by overexpression of the regulatory light chain of myosin II [60].	https://figshare.com/s/b64e04751618eb05a621
5	Natacha Steinckwich-Besancon, National Institutes of Heatlh NIH/NIEHS, US	HL60	Enhance calcium signaling.	
7	Terri Bruce, Clemson University, US	Dicty	Increase actin polarization at the leading edge by overexpression of constitutively active Rab8.	https://figshare.com/s/5c60547ee8b6a9757f89
9	Robert Insall, Jason King, Peter Thomason, Beatson Institute, UK	Dicty	Eliminate the negative effects of axenic mutations and the associated mutations introduced during axenisation.	https://figshare.com/s/a349669707049b8fef33
10	Carsten Beta and Oliver Nagel, U. Potsdam, Germany	Dicty	Decrease cell-substratum adhesion with talin null cells.	
11	Jan Faix, Alexander Junemann, Christof Franke and Stefan Bruehmann, Hanover Medical School, Germany	Dicty	Enhanced actin polymerization by overexpression of Rac1A [57].	https://figshare.com/s/7cda43a267f18c65abc3
12	Peter van Haastert, Arjan Kortholt, Rama Kataria and Ineke Keizer-Gunnink, U. Groningen, Netherlands	Dicty	Enhance gradient sensing by overexpressing Ric8, a non-receptor GEF for Gα2 [24].	https://figshare.com/s/a1c4630a0f45bfd91192
14	Annette Müller-Taubenberger and Matthias Samereier, LMU Munich, Germany	Dicty	Decrease cell-substratum adhesion.	
15	Michael Myre, Robert Huber and Susan Cotman, Harvard Medical School, US	Dicty	Precocious development and expression of the chemotactic machinery with CLN3 null cells. The Cln3 gene is involved in Batten disease, a severe childhood neurodegenerative disorder [64].	https://figshare.com/s/d0f92fdd972a3f13a006
17	Alan R. Kimmel and Netrapal Meena, National Institutes of Health NIH/NIDDK, US	Dicty	Enhance directionality of chemotaxis by knocking out Gα9 [65, 66].	https://figshare.com/s/e481e604c546c2bb0930
18	Robert Kay, Douwe Veltman, MRC Cambridge, UK	Dicty	Enhance the actomyosin cortex in the back of the cell by overexpression of RacGEF in NC4.	https://figshare.com/s/7b70c54846075640e078
19	Eric Tschirhart, Sébastien Plancon, University of Luxembourg	HL60	Enhance speed by selection using a Boyden chamber with reference line CCL-240 from ATCC.	
20	Peter Devreotes, Kristen Swaney, Thomas Lampert, Johns Hopkins University, US	Dicty	Increase speed by reducing the number of lateral pseudopods by overexpression of CynA [67].	https://figshare.com/s/f4f6c9ab79e85c7ac8f9
Ctrl 1		Dicty	Control Dicty (wildtype AX3 strain)	https://figshare.com/s/cedc88200fda4f2cdd84
Ctrl 2		HL60	Control HL60 (ATCC CCL-240)	https://figshare.com/s/54f82e81e91018e9fddb

doi:10.1371/journal.pone.0154491.t001

Figure 5: Summary of team methods from the 2014 Dicty World Race

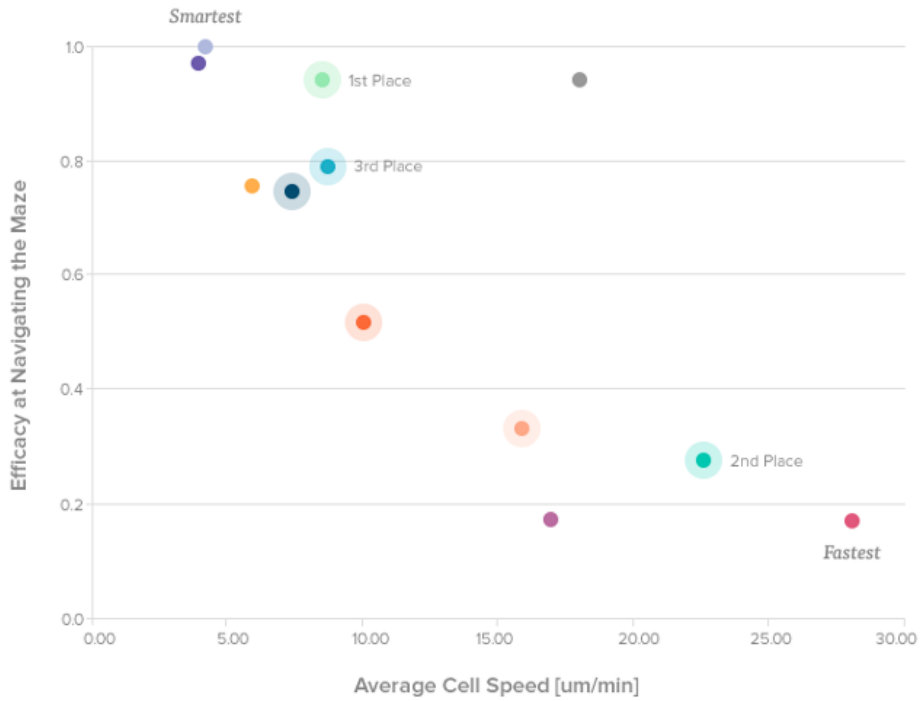


Figure 6: Graph of average cell speed vs navigational efficiency metric

3. Intellectual Property Considerations, Agreements and Disclosures

The team has no Intellectual Property agreements with any outside parties. Any potential patents or other intellectual property will be handled according to established procedures for Bioengineering Capstone projects at Northeastern University.

4. Design Requirements

Project Goals: The goal of this project is to produce an NIH 3T3 cell line that is capable of migrating through membrane of the Boyden chamber in the chemotaxis assay by following a chemical gradient of the chosen chemoattractant (PDGF). The cell line produced should be able to migrate further than the wild type NIH 3T3 cells in the chemotaxis assay.

4.1. The NIH 3T3 cells that undergo lipofection shall achieve a transfection rate of at least 23%.

Rationale: Studies done with similar cell types reported average transfection efficiencies of ~23% when using the Lipofectamine LTX protocol [1].

Method of Verification: We will use the count tool to quantify transfected cells pre-selection from Thermo Scientific EVOS FL Digital Inverted Fluorescence Microscope based on GFP-tagged fluorescence.

4.2. Modified NIH 3T3 cells shall achieve an invasion percentage deemed significantly higher than the unmodified cells at a significance level of $p \leq 0.05$.

Rationale: Modified cells should be able to exhibit improved migratory properties based on the overexpression of our target proteins.

Method of Verification: We will construct a standard curve based on fluorescence readout from the chemotaxis assay and calculate the percent invasion for both groups of cells before running a t-test for statistical significance.

4.3. The solution shall not require any protocols that cannot be conducted or equipment that cannot be used in the Northeastern BSL-2 Bioengineering teaching lab.

Rationale: The team does not have access to any wet lab facilities outside of the Northeastern BSL-2 Bioengineering teaching lab so any protocols used in the project must be compliant with the equipment and regulations of the lab.

Method of Verification: Solutions will be discussed with our advisor Narges Yazdani or other Bioengineering faculty to ensure any proposed techniques can be feasibly and safely conducted with the resources available. All experiments done in the BSL-2 teaching lab will be documented with date and time to ensure proper safety protocols are followed.

4.4. The total cost of the project shall not exceed $1500.
Rationale: The sponsor has a limited budget and will not provide more money than this allotted amount to complete the project.
Method of Verification: A bill of materials (BOM) for the project will be tabulated.

5. Design Solutions

5.1. Definitions of Criteria

In order to effectively assess potential design solutions across multiple trade studies, the team first had to come up with a defined set of criteria with their own subsequent weights to score each solution. After consulting the literature and discussing together, the team came up with a list of essential criteria that would each range from a score of 1-5, with 1 being the least desirable for the solution and 5 being the most desirable for the solution. The rationale for the criteria and their translated 1-5 scores are given below.

Criteria:

Feasibility

We define feasibility as the ability of our group to learn and carry out a solution and any laboratory techniques it would require. This score is based on the requirements of each potential design solution as well as the knowledge and experience of each group member. This is important to consider because we will be the ones designing and performing the experiments for the project and so we must be confident that we are capable of doing so to ensure we will perform well. More complicated design solutions or ones that members of the group do not have relevant experience for are being considered but such solutions would have a higher chance of setbacks and so would need to have extra time budgeted for. The scores 1-5 are defined as follows:

1: This solution is exceedingly complex for the scope of Capstone and our collective skill set.

2: This solution is extremely complex for the scope of Capstone and our collective skill set but could still be attainable.

3: This solution is moderately complex for the scope of Capstone and our collective skill set.

4: This solution is minimally complex for the scope of Capstone and our collective skill set.

5: This solution is well within the scope of Capstone and our collective skill set.

Effectiveness

We define effectiveness as how much better a given solution would make our HL60 cell line at migrating through the maze quickly. The goal of the capstone project is to produce the fastest cell line possible so this is a particularly relevant concern. This criterion suggests that solutions which have a higher potential to create faster cells should be picked over others which have lower potential to do so. Because many of the design solutions we are considering may not have been studied directly for similar purposes to ours some of these rankings are partially subjective out of necessity. Extra weight is given to solutions which are backed by relevant literature.

1: This solution will most definitely not be effective at improving HL60 cell speed through the maze.

2: This solution has a strong likelihood of not being effective at improving HL60 cell speed through the maze.

3. This solution has a questionable likelihood of being effective at improving HL60 cell speed through the maze.

4: This solution has a reasonably high likelihood of being effective at improving HL60 cell speed through the maze.

5: This solution has an extremely high likelihood of being effective at improving HL60 cell speed through the maze.

Cost

We define cost as how much of the project budget will be required to purchase the necessary materials for the design solution. The project has a fixed budget of $1500 which cannot be exceeded. After purchasing required baseline materials(i.e. HL60 cell line, RDMI media, DMSO, etc…), we estimate the remaining usable budget to be around $1000, which was used to scale this criterion in equal intervals described below.

1: This solution will cost between $800-$1000 to execute.

2: This solution will cost between $600-$800 to execute.

3: This solution will cost between $400-$600 to execute.

4: This solution will cost between $200-$400 to execute.

5: This solution will cost between $0-$200 to execute.

Time

We define time as the time we believe it would take to execute a certain solution. Our project is subject to two deadlines. The first is the end of the Spring 2024 semester. We will not be able to continue work on the project after the semester ends so all work must be completed before the relevant dates at the end of the class. The second deadline will be the date decided on to run the HL60 cell race with the other capstone team. The cells must be prepared to compete in the race before this date. We have been advised that lab work in a capstone project often takes much longer than originally expected and so this is something we need to take into consideration when choosing a design solution.

1: This solution will exceed the length of time given in Capstone 2 to work on the project **or** there is no available information to gauge an approximation for the duration of the solution.

2: This solution will take most available time to work on the project in Capstone 2 and will have minimal to no room for error built into the time estimate.

3: This solution will take a significant amount of time to complete during Capstone 2.

4: This solution will take a moderate amount of time to complete during Capstone 2.

5: This solution will take a negligible amount of time to complete during Capstone 2.

Cell Safety

We define cell safety as the likelihood that the design solution will have detrimental effects on the cells of our HL60 cell line or their ability to navigate the maze. The cells that compete in the maze must be healthy and viable, therefore any potential for negative effects on their health must be taken into consideration.

1: This solution poses an extreme amount of risk to cell safety and/or viability.

2: This solution poses a significant amount of risk to cell safety and/or viability.

3: This solution poses a moderate amount of risk to cell safety and/or viability.

4: This solution poses a small amount of risk to cell safety and/or viability.

5: This solution poses negligible risk to cell safety and/or viability.

In the trade studies, the criteria were weighted according to the values seen in Column 8 of Table 1. These values were determined by having all group members rank each criterion for importance on a scale of 1-10. These rankings were then added and a weight calculated as a percentage of total ranking points issued.

Criteria						Averag	Standard

						e	Average(Weight)
Feasibility	8	8	8	9	9	8.4	0.22
Effectiveness	5	4	5	8	8	6	0.26
Cost	7	10	8	6	3	6.8	0.16
Time	6	9	9	8	9	8.2	0.22
Cell Safety	4	4	8	8	5	5.8	0.14

Table 1: Weights for Selected Criteria

5.2. Trade Studies

In order to make informed and reasonable decisions regarding the future direction of the project, the team conducted several trade studies to effectively compare a series of preliminary design solutions against each other using our determined criteria. The team grouped potential solutions into three categories for ease of running the trade studies: polarization, chemotaxis, and physical change. Every solution is given a score of 1-5 in each criteria, and the weighted scores for each solution are added up in each column to give a total final score for each solution. Each trade study uses the same criteria with the same weights to ensure fairness across each study.

5.2.1. Polarization Trade Study

Show in Table 2 below are the results of the polarization trade study, which consists of the following solutions: PTEN overexpression, calpain inhibition, TDM/AKT overexpression, actin polymerization via Jasplakinolide, and rho kinase overexpression.

Criteria	**Weight**	**PTEN overexpression**	**Calpain Inhibition**	**TDM/AKT**	**Actin Polymerization (Jasplakinolide)**	**Rho Kinase Overexpression**
Feasibility	0.22	3	5	3	4	3
Effectiveness	0.26	5	3	3	3	3
Cost	0.16	2	5	3	4	2
Time	0.22	2	5	4	5	3
Cell Safety	0.14	4	5	2	2	4
Total	1	3.28	4.48	3.00	3.68	2.98

Table 2: Polarization Trade Study Results

5.2.1.1 PTEN Overexpression

PTEN is a protein that is heavily involved in the regulation of chemotaxis within HL60 cells. Its main role is to dephosphorylate PtdIns(3,4,5)P3 into PtdIns(4,5)P2 which prevents the formation of pseudopodia on the faces where PTEN is delocalized to as mentioned in Section 2.2.5.3. PTEN is chiefly responsible for how HL60 cells are able to navigate in response to chemoattractants by regulating polarization at the leading and trailing edges of the cell, thus why it was grouped in the polarization trade study. One study notably tested the effects of PTEN knockdown on cell directionality in the presence of fMLP chemoattractant and intermediate chemoattractants arranged in a perpendicular fashion[1]. The cells that experienced PTEN knockdown diverged from the path of the fMLP chemoattractant at the junction between the fMLP chemoattractant and the intermediate chemoattractants, seemingly becoming "distracted" by the intermediate chemoattractants[1]. Conversely, cells that did not experience PTEN knockdown were able to remain "focused" on the fMLP chemoattractant pathway and displayed significantly less deviation compared to the knockdown group[1].

In terms of feasibility, the team opted to give the PTEN overexpression solution a score of 3 because of the complex transfection procedures that would be involved in overexpressing a protein such as PTEN. Although the team has not decided on a particular strategy to apply to genetically engineered solutions, all potential techniques in this category, such as CRISPR-Cas9 and lentiviruses, carry with them a certain level of complexity that warrants a score which reflects such complexity. Additionally, no team members have extensive experience in applying these strategies, so a learning curve will also be present for most members of the team. Based on the study done on the effect of PTEN knockdown on neutrophil directionality, the team feels quite strongly that increasing the expression of PTEN in the HL60 cells will improve navigational efficiency, and thus opted to give the PTEN overexpression solution a score of 5 for effectiveness[1]. Navigational efficiency and speed are the two main parameters in determining success for the cells in the maze as previously discussed in section 2.4.3, so significantly contributing to cell navigational efficiency should lead to strong positive results for our cells in the maze. PTEN overexpression was given a score of 2 for both cost and time, which ties back into the uncertainty regarding which transfection strategy the team would employ if this solution is chosen. Select kits found on the market related to lentiviral transfection ranged between $500-$800, and involved protocols that are estimated to take up a large percentage of available lab time in Capstone 2. PTEN overexpression was given a score of 4 for cell safety because the prior research done with PTEN did not indicate any inherent danger would present for the cells in manipulating PTEN levels, however the team cannot say with complete certainty that the cells would face no danger at all with this strategy.

5.2.1.2. Calpain Inhibition

Calpain is a calcium activated protease and in many cell types actually induce apoptosis. However, for neutrophils calpain plays a role in chemotaxis and cellular speed. For the trade study conducted above, calpain inhibition scored a 4.32, which led the design solutions for this

trade study. Calpain inhibition scored a five for feasibility. Methods for calpain inhibition were mostly comprised of treating the neutrophils with a specific inhibitor and incubating the cells for no longer than 30 minutes [12]. Considering the ease of this process, calpain inhibition scored a perfect score on feasibility. The effectiveness score for this design solution was determined to be a 3 out of 5. This score could have been a 2 or even a 4, considering the effect of calpain inhibition on neutrophils is a double edged sword. On one hand, calpain inhibition has been proven to increase neutrophil speed by a conservative estimate of 4 microns per minute faster than regularly differentiated HL60 cells with DMSO [12]. This is promising data and alone could have been reason to score either a 4 or a 5 on the effectiveness scale. However, the inhibition of calpain has also been shown to reduce HL60 cell chemotaxis and actually reduced its migratory ability towards a chemoattractant gradient [43]. This is certainly a cause for concern, and these findings caused the effectiveness score of calpain inhibition to reasonably become a 3. This design solution scored a perfect 5 for the cost criteria. This is because the commonly used calpain inhibitors used in studies are no more than $200 and are often much closer to $100. The most commonly used calpain inhibitors ALLN, ALLM, and PD 150606 [12], [43]. Similarly to feasibility and cost, time and safety to the cells scored a perfect 5 for calpain inhibition. As previously mentioned, the most amount of time HL60 cells were incubated across the used studies was 30 minutes of treatment. Along with no reported cell viability drops or significant cell death [12], [43]. The design solution of calpain inhibition scored very highly mostly due to its ease of execution and cost. However, there are real concerns surrounding the effectiveness, as there is no way to concretely know whether the gain of speed is worth the drop in chemotactic ability when in a microfluidic maze. This will be a method our team attempts simply because of the feasibility, safety, and cost, but there will need to be further testing whether or not this is a positively effective method.

5.2.1.3. TDM/AKT Overexpression

TDM, or trehalose 6,6'-dimycolate, is a cord factor found in the cell wall of the *Mycobacterium tuberculosis* species. It is involved in immune responses such as macrophage activation, which signals the events that initiate granuloma formation [44] . In a study conducted by Lee et al., they reported that TDM was involved in enhancing F-actin polymerization, which neutrophils rely on for extending pseudopodia toward high concentrations of chemotaxis-related molecules [45]. In addition, they reported that a TDM receptor, Mincle (macrophage-inducible C-type lectin), is involved in neutrophil signaling for cytoskeletal reorganization, integrin expression, and cell adhesion, which are significant for facilitating cell movement. According to their study, they aimed to investigate the impact and mechanisms of enhanced TDM expression on neutrophil migration. This was conducted by analyzing chemotaxis patterns of differentiated HL60 cells treated with TDM. Key results showed that TDM enhanced expression of the receptor Mincle, enhanced secretion of pro-inflammatory cytokines, and enhanced the number and distance of HL60 cells migrating toward the concentration gradient of the chemoattractant fMLP. Furthermore, stimulation with fMLP showed that prolonged AKT (protein kinase B) phosphorylation at serine residue 473 occurred as well, indicating that TDM plays a role in enhancing neutrophil migration through AKT phosphorylation.

For feasibility, this method was ranked with a 3 because some of the team members have not previously performed procedures that are listed in the paper. Room for error may be needed to learn how to run specific assays and quantitative real-time PCR. Optimal temperature conditions and timing before application of the TDM treatment would need to be assessed. From the paper, specific temperature and timing conditions were set for the context of analyzing the cell movement in a 12-well Transwell chamber. Effectiveness of this method was ranked with a 3 because this paper displayed multiple promising results for enhancing neutrophil migration toward a chemoattractant. However, there is not much information in the literature regarding TDM when specifically studying patterns in neutrophil migration. Researchers in this study addressed how the literature reports the roles TDM plays in neutrophil migration, but there is much unknown about the mechanisms for how TDM this process and the downstream signaling molecules that are involved [45]. Cost for this method was ranked with a 3 because this would require much money with the qPCR and various assays. The 12-well plates alone used for the chemotaxis assay would cost between $94-$365, viewing the product from companies such as Corning-Costar and ThermoFisher. Costs are approaching around the $400-600 range considering reagents as well. The ranking for time was 4 as the methods for utilizing this method were not as time intensive overall compared to those of the other design solution methods considered. The ranking for cell safety was 2 because according to the literature, TDM is not widely studied due to its toxicity to cells. In a study conducted by Sarkar et al., the cell viability of different tumor cell lines appeared to decrease as the concentration of TDM was increased [46].

5.2.1.4. Actin polymerization via Jasplakinolide

Jasplakinolide is a toxin produced by *Jaspis johnstoni,* a species of marine sponge. It was originally isolated from this sponge but is now able to be produced using bacteria and recombinant DNA. It is known for stabilizing actin polymerization. The mechanism by which it does this is binding to a site on a trimer of three actin monomers. It competes with another protein called phalloidin to bind at this site. Jasplakinolide binding in this way stabilizes the trimer enough to allow it to polymerize instead of dissociating. Under typical conditions, actin requires four monomers to associate rather than three like with jasplakinolide. This means that the critical concentration of actin required to begin polymerizing is lowered significantly. It is also believed that the toxin increases the speed of actin polymerization moderately but this has much less of an effect than its inhibition of depolymerization [47]. The jasplakinolide would be spiked in with the cells in an attempt to cause them to have enhanced actin polymerization. This would allow them to migrate faster by forming membrane protrusions faster.

This solution received a 4 in Feasibility. The solution only requires the toxin to be introduced to the cells at an appropriate time before the race. The only complexities we would need to deal with would be determining the concentration of toxin to apply to the cells and at what amount of time prior to the race. This would be minimally complex and should be easily achievable for our team. The design solution received a 3 for Effectiveness. The reason that it

was scored a 3 is that there is some debate over the effects of the toxin in living systems. It promotes and stabilizes actin polymerization but may also disrupt the typical way that this process functions and have a negative overall effect on the cell. Therefore, it is possible but not ensured that it would have the effect of increasing cell migration speed. It received a 4 for Cost. Jasplakinolide is available for a cost of $232.85 for 50 µg from Fisher Scientific [48]. This falls within the $200-400 range for a 4. It received a 5 for Time. The time scale for use of jasplakinolide is generally on the order of hours [49]. While there should be some initial testing to determine the best way to apply it, this design solution itself would only require minimal time on the day of the race. It received a 2 for cell safety. This is because Jasplakinolide is originally a toxin and can have detrimental effects on cells. At too high a concentration the toxin would kill the HL60 cells. At lower concentrations, there is some belief that it can still cause apoptosis in cells of the immune system in certain environments. It has been theorized that this is because of the role of the actin cytoskeleton in cellular signaling that is disrupted by jasplakinolide. The specifics of this effect have not yet been fully characterized but it is important to consider nonetheless [49].

5.2.1.5. Rho Kinase Overexpression

Rho kinase is a downstream effector of Rho GTPase which plays a significant role in many cytoskeletal activities such as cell contraction, cell polarization, and focal adhesion formation[50]. Structurally speaking, rho kinase is a serine/threonine kinase with an N-terminal catalytic domain, a coiled central domain, and a C-terminal PH domain[50]. In the context of neutrophil chemotaxis, rho kinase plays an essential role in the phosphorylation of PTEN, a protein that is a central to a previously discussed design solution, and the phosphorylation of PAR-3, a domain of the PAR-6/aPKC complex[50], [51]. PAR-3 dissociates from this complex after phosphorylation which indirectly disrupts Rac activation by inhibiting aPKC stimulation of Rac pathways[51]. This phosphorylation activity is observed at both the leading and trailing edges of the cell, which signifies the role of Rho-kinase in regulating cell protrusions through Rac inhibition[50].

Rho kinase overexpression was given a score of 3 for feasibility due to team concerns over the complexity required to execute genetic engineering techniques required for the expression of this effector. Rho kinase overexpression was given a score of 3 for effectiveness because although previous literature has shown promising results for the role of rho kinase in pathways related to cell polarization and protrusion, there are still concerns over the exact downstream effects on navigational efficiency on rho kinase overexpression. In contrast, the overexpression of PTEN was more directly linked to the formation of localized protrusions and was further downstream of the end result compared to rho kinase[51]. Rho kinase overexpression was given a score of 2 for cost because relevant materials on the market related to the inhibition and expression of rho kinase as well as the transfection kits that would potentially be involved were in the upper end of our price range at around $800. Rho kinase overexpression was given a score of 3 for time because the team would likely have to devote significant time in and out of the lab for learning and executing the correct protocols related to transfection. Rho kinase

overexpression was given a score of 4 for cell safety because the expression of rho kinase does not interfere with pathways which could pose a strong risk of cell death.

5.2.2. Chemotaxis Trade Study

Show in Table 3 below are the results of the chemotaxis trade study, which consists of the following solutions: Increase FPR expression, P13K overexpression, and MF2 overexpression.

Criteria	Weight	Increase FPR Expression	P13K Overexpression	MF2 Overexpression
Feasibility	0.22	3	4	4
Effectiveness	0.26	4	5	3
Cost to implement	0.16	3	2	3
Time to run in lab	0.22	2	2	3
Safety(to cells)	0.14	4	3	4
Total	1	3.18	3.36	3.36

Table 3: Chemotaxis Trade Study Results

5.2.2.1. Increase FPR1 expression

FPRs sense formylated peptides, and specifically FPR1 sensed fMLP [29]. The binding of fMLP to FPR1 is the first event necessary for successful chemotaxis, and without this initial binding there is no signaling cascade that ultimately rearranges the cytoskeleton to propel the cell forward [10], [11], [29]–[33], [35]. Thus, we can enhance the ability of FPR1 to bind fMLP by upregulating the amount of receptors on the surface to ensure more are free to bind the chemoattractant and move along the gradient.

The feasibility of this solution scored 3 due to the complexity of transfection procedures that will be required to upregulate FPR1. Once again, the nature of which transfection protocol we will use has yet to be determined, but nonetheless this will require some advanced skills and knowledge to execute. The effectiveness of this solution scored 4 because the binding event of fMLP to FPR1 is of principal importance in ensuring the cell moves efficiently. If we can enhance this interaction the cell will have much better overall speed in the maze. This did not score a 5 on effectiveness because there is a lack of literature defining how many FPR1s are already on the cell surface, so we lack concrete evidence as to whether or not upregulating the receptor will have a significant effect. This solution received a 3 for cost because it will fall in the middle range of cost. This solution scored a 2 for time to run in the lab because it will take a majority of our time to transfect the cells. This solution scored a score of 4 to the cells because

upregulating the FPR should not have any effects on important cellular processes but nonetheless upregulating any protein can cause off-target side effects that we might want to consider.

5.2.2.2. P13K overexpression

Phosphoinositide 3-kinase (PI3K) has a vital role in cell mobility and chemotaxis by controlling the cell's actin cytoskeleton. It regulates levels of PtdInds(3,4,5) and its activity, in combination with PTEN, forms localized intracellular gradients which are required for chemotaxis. The main mechanism it controls in neutrophils is PtdInDs(3,4,5)P3 accumulate at the leading ends of the cell in a dependent manner. In a study conducted involving mammalian expression, neutrophils that were lacking in P13K moved much slower when compared to wild type cells [52].

The feasibility of P13K scored a score of 4 in the feasibility of this solution because the paper regarding cell motility was very clear that P13K inhibitor has a stark impact on cell mobility, but does not go into overexpression which we will be examining. P13K received a score of 5 for effectiveness because there has been research that directly links the correlation between cell navigability and movement when compared to P13K expression. This solution was given a 2 for the cost factor due to being slightly above the middle range for cost. This solution will also be fairly time intensive due to the amount of time required to be spent in the lab to learn and implement the overexpression of the gene. The safety of the cells was then given a three because it was found in a study that hyper activation of the P13K and AKT pathways result in cell death in lymphocytes [55].

5.2.2.3. MF2 overexpression

Mitofusin 2(MF2) is a mitochondrial fusion protein associated with regulating mitochondrial morphology and oxidative phosphorylation during neutrophil chemotaxis[53]. Under typical circumstances, neutrophils utilize glycolysis for energy; however during chemotaxis, neutrophils utilize mitochondrial oxidative phosphorylation to extract ATP[53]. The silencing of MF2 was shown to inhibit oxidative phosphorylation activity and subsequently chemotaxis in the presence of fMLP chemoattractant[53]. With the overexpression of MF2, there is the potential for an increase in oxidative phosphorylation which could give the cells a speed advantage from an energetic perspective.

MF2 overexpression received a score of 4 for feasibility because the key paper on MF2 silencing was relatively simple in its methods compared to previous papers on protein silencing/overexpression[53]. MF2 overexpression received a score of 3 for effectiveness because there was not overwhelming evidence found in the literature for direct impacts on cell speed through microfluidic channels with the expression of MF2. MF2 overexpression received a score of 3 for cost because the reagents used in the MF2 silencing study were fairly expensive(in particular some of the antibody reagents were priced at $600 per unit)[53]. However, the more expensive antibodies used in the previous study would not be needed for our solution, so the cost barrier is slightly more reasonable and the total cost for implementing this solution would be closer to $450[53]. MF2 overexpression received a score of 3 for time because the imaging

required for mitochondrial work would add significantly to the lab time required for this solution. MF2 overexpression received a score of 4 for cell safety because there is minimal risk to the cells in the addition of MF2 protein to their existing energetic pathways.

5.2.3. Physical Change Trade Study

Show in Table 4 below are the results of the physical change trade study, which consists of the following solutions: Heating the cells, shrinking the cells, and natural selection.

Criteria	Weight	Heat Cells	Shrink Cells	Natural Selection
Feasibility	0.22	5	5	5
Effectiveness	0.26	3	2	2
Cost to implement	0.16	5	5	3
Time to run in lab	0.22	5	5	1
Safety(to cells)	0.14	4	2	5
Total	1	4.34	3.8	3.02

Table 4: Physical Change Trade Study Results

5.2.3.1. Heating the cells

Neutrophils exposed to temperatures from 30 degrees Celsius to 42 degrees Celsius have a direct, positive correlation between the temperature and their velocity [13]. This increase in temperature decreases the amount of membrane proteins that anchor the cells to surfaces and increasing the temperature has been shown to increase the amount of actin in the cell [13]. Increasing the temperature of neutrophils is a natural response in the body when a fever is induced or vessels at inflammation sites are dilated to increase local temperature and help the immune cells fight infections. In this way, providing the cells with more kinetic energy can give them the boost they need to race through the maze.

This solution received a score of 5 for feasibility as heating the cells is not complex. This solution received a score of 3 for effectiveness because although the overall speed of cells increases with temperature, the existing studies have only shown this to be true of cells in large culture flasks, not in cells placed in narrow channels [13]. Thus, heating the cells will certainly increase speed but may negatively affect their ability to flow through the maze channels. Heating the cells will cost 0-200$ so it received a score of 5. This will take almost no time at all to heat the cells so it scored 5 for time. It scores 4 for cell safety because cell viability should be completely conserved assuming we do not pass a certain threshold for temperature which we will

be conscientious of when it comes to deciding the optimal temperature to increase speed and minimize cell death.

5.2.3.2. Shrinking the cells

It has been reported in the literature that HL60 cells differentiated with DMSO were shown to increase in size and length. This makes it more difficult for the cells to move through narrow microfluidic channels. In fact, the previous race reported that multiple teams' cells clogged a channel within the maze [6]. With this information, we reasoned that decreasing the cell size might surpass these hurdles and give the cells a better chance of reaching the end of the maze.

This solution received a score of 5 for feasibility as a reduction of the cell size is not a complex process nor does it require advanced knowledge. The effectiveness of this solution scored a 2 because although this intervention will help the cells fit through the channels better, it will not address any other aspect of chemotaxis or its associated pathways. The cost for this solution would be 0-200$ as cell size can be decreased by incubating them with a hypertonic solution. This scored a 5 for time as it would take virtually no time, and this scored a 2 for cell safety because shrinking and dehydrating the cells beyond their limit can cause them to die.

5.2.3.3 Natural selection

In the previous Dicty World Race, never did all of the cells for any team complete the maze. Only a subset of these cells were able to complete the maze. We reasoned that we could run our cells through the maze and isolate this sub-population of cells that were the "most fit" and amplify them. We could then repeat this process many times to slowly enrich a cell population that has evolved with the only purpose of traversing mazes.

This solution scored a 5 for feasibility because we are completely capable of executing this solution and will not require any advanced skills or knowledge. This solution received a score of 2 for effectiveness because an exhaustive literature review has yielded no supporting evidence that this solution would actually work. The cost of this solution scores 3 because we would need to culture the cells for a long time which in the end we believe would be within the 400-600$ range. This solution scored 1 for time because we have no idea as to how many times we would need to complete the maze; this could be 100 times or even 1000. This solution received a 5 for cell safety because the solution has no foreseen negative effects on cell viability.

5.2.3.4. New Trade Study Criteria

Biosafety Regulation

Biosafety Regulation refers to the perceived likelihood of a given item, cell line, or protocol being accepted by the Northeastern IBC. This was given a weight of 0.5 because it was the top priority when making these decisions. The IBC meets once per month and if our paperwork was not accepted in the February meeting it would be likely that we would be

extremely limited in our opportunity to use the lab after being accepted at the March meeting. Rankings for this criterion were based on out knowledge of the biosafety regulations at Northeastern as well as conversations with the Bioengineering Safety Manager Kathryn Lasseter and other Bioengineering faculty.

5: A 5 is awarded when there is either preexisting biosafety approval for the item or there is no biological or chemical element to the item at all. These items will cause no delays to the project for safety reasons.

4: A 4 is awarded when the item is very likely to be accepted on the first try for registration with the IBC. The item may be RG1 or similarly categorized and should pose minimal to no safety risk to a reasonable lab operator. These items are viewed as very unlikely to delay the project further.

3: A 3 is awarded when there is some uncertainty about whether the item will be accepted on the first attempt and without revisions to the biosafety paperwork. These items will possibly delay the project and proposing the use of them should be considered carefully.

2: A 2 is awarded when there is significant doubt that the item will be accepted by the IBC. These items may have been previously discussed with Kathryn Lasseter as being unlikely to be accepted or likely to require extra safety protocols (such as requiring a PI to be present during all work). These items will almost certainly delay the project further if they are accepted at all and should be avoided.

1: A 1 is awarded to materials that will not be accepted by the Northeastern IBC for use in an Undergraduate Capstone Project under the current circumstances. Attempting to use these materials will delay the project significantly and will almost certainly never be accepted. These materials will not be selected for use for our project.

Cost/Shipping

Cost and shipping takes into account the total cost and shipping time that it would take as this project is working with a set timeline and budget. This was given a weight of 0.25 because it was seen as the second most important criteria in our considerations. Approval for usage of materials was seen as the most important, with second being the cost and time considering we are working with a budget and short timeline. The rankings were decided based largely on the percentage of budget that the material or item consumed.

5: A 5 is awarded when the item is either free and immediate, or less than 10% of the total project budget and has shipping within one week. These materials' cost and shipping would be far from the limiting factor for execution of lab work.

4: Within 10-20% of our budget and/or shipping more than 8 days.

3: Within 20-30% of our budge and/or shipping more than 10 days.

2: Within 30-40% of our budget and/or shipping more than 12 days

1: More than 40% of our budget and/or shipping more than 14 days.

Time in Lab

Time in Lab refers to the expected length of time the group would need to successfully use an item or protocol in the Northeastern Bioengineering Teaching Lab. This includes the time required to set up and maintain any relevant lab materials as well as the readout of data if relevant. Protocols that take longer, both in terms of lab time per day and total days in the lab will score lower. Also considered in this section is the expected training and troubleshooting time required. Items and protocols that are simple and/or members of the group have significant experience with will score higher in this section.

5.2.4 Cell Line Selection Trade Study

Criteria/Solution	Weight	NIH3T3	Dicty	J774A.1	P388D1
Biosafety Regulation	0.50	5	4	4	4
Cost/Shipping	0.25	5	2	3	1
Effectiveness	0.25	4	3	4	4
Weighted Total	1.00	4.75	3.25	3.5	3.25

Table 5: Cell Line Selection Trade Study Results

5.2.4.1 NIH3T3 Cells

The NIH3T3 cells scored a 5 for the biosafety regulation criteria. This is because these cells are currently in use within the teaching lab, meaning there is a precedent set for this cell line's use in the ISEC teaching lab. This cell line scored a 5 in the cost and shipping criteria as well. This score is because the cells would not need to be purchased or shipped for this project, there are vials available for use in the teaching lab that can be thawed and cultured for free. This cell line scored a 4 for effectiveness because based on literature this cell line is able to be somewhat easily adapted and edited [60].

5.2.4.2 Dictyostelium Cells

The dictyostelium cells scored a 4 for biosafety regulation criteria. Through discussions with Kathryn and precedent set by previous capstone teams, it is likely these cells would have been approved for teaching lab use. This was not deemed a 5 however considering there is not current use and approval of these cells in the teaching lab. These cells scored a 2 for cost and

shipping. This is because these cells cost $620 on ATCC and would require 1-3 business days to ship [61], considering this cost is just over 40% of the total budget these cells scored a 2 for this criteria. This cell line scored a 3 for effectiveness because this cell line according to literature and the dicty race is able to be modified but it slightly trickier than NIH3T3s [62].

5.2.4.3 J774A.1 Cells

The J774A.1 cells scored a 4 for the biosafety regulation criteria. This is because once again there is precedent set by previous capstone projects of the use of rodent derived cell lines and after speaking with Kathryn they were confident this cell line would get approved. This cell line scored a 3 for cost and shipping criteria. This is because the cost of this cell line is $555 [63] which would be less than 40% of the total budget, however still absorbing a large chunk of the $1500 total budget. This cell line scored a 4 for effectiveness because similarly to the NIH cell line, this mouse cell line would be able ot be modified and manipulated somewhat easily [64].

5.2.4.4 P388D1

The P388D1 cell line scored a 4 in the biosafety regulation criteria. Once again, there is a precedent set by the biosafety committee of approving rodent derived cell lines and Kathryn though they would be highly likely to approve this cell line. For the cost and shipping criteria this cell line scored the lowest in the group at a 1. This cell line would cost $708 [65], making it the most expensive potential cell line and absorbing roughly 47% of the total project budget. This cell line scored a 4 for effectiveness because this rodent derived cell line also has literature supporting the idea that it is somewhat easy to modify and adapt [67].

5.2.5 Chemotaxis Assay Selection Trade Study

Criteria/Solution	**Weight**	**Abcam**	**Millicell µ-Migration**	**Darwin Microfluidics**
Biosafety Regulation	0.50	4	4	4
Cost/Shipping	0.25	4	2	5
Time in Lab	0.25	5	3	3
Weighted Total	1.00	4.25	3.25	4

5.2.5.1. Abcam

The Abcam Cell Migration/Chemotaxis assay kit uses a Boyden Chamber design to evaluate cells' ability to migrate towards a chemoattractant of the experimenter's choice. The Abcam assay kit was given a 4 in the Biosafety Regulation category because, though it still needs to be approved by the IBC, it should fall well within the bounds for acceptance for our project and we expect no issues based on our conversations with Kathryn Lasseter. This assay also received a 4 in the Cost/Shipping category. The manufacturer offers same day delivery and has very short lead times otherwise to locations in Boston. The kit is somewhat expensive at

$640 but, given that the Bioengineering Department has offered to help the team with the cost of the chemotaxis assay given the difficult circumstances, this should not prove prohibitively expensive at all. Finally, the assay kit received a 5 in the Time in Lab category. The assay comes with a recommended protocol that is very simple and the team believes that little to no troubleshooting should be necessary for the assay itself. A plate reader that fulfills all the requirements for the assay readout is already available in the Bioengineering Teaching Lab.

5.2.5.2. Millicell µ-Migration

The Millicell µ-Migration kit scored a 4 for the biosafety regulation criteria. This is because this would be highly likely to get approved by the biosafety board as there are no major safety risks involved with this assay. This kit scored a 2 for the cost and shipping criteria. This is mostly because this product would require contact with the vendor and a quote. In terms of time to ship and purchase this scored low because of the level of effort to simply acquire the product. This kit scored a 3 for the time in the lab.

5.2.5.3. Darwin Microfluidics

The Darwin Microfluidics chip scored a 4 for Biosafety Regulation. This is because, similarly to the other assays, the chip would still need to be approved by the IBC but there would be no expected issues with gaining approval for it because it does not contain any biological or chemical material that we have been told will be difficult to gain approval for. This chip costs less than $50 which is comparatively very inexpensive which is why it was given a 5 for Cost/Shipping. The shipping times are also reasonable, so no points were docked for that reason. Finally, this chip was given a 3 in Time in the Lab. This is because, unlike the other options, this is not a validated assay with a protocol provided. The only thing included is the chip itself which means that we would have to develop the means to measure cell migration or chemotaxis ourselves. While possible, we believe that this would take significantly longer and require far more troubleshooting than the other options and therefore receive a lower score [69].

5.2.6 Transfection Method Trade Study

Criteria/Solution	Weight	Lipofection	Lentivirus	AAV	CRISPR
Biosafety Regulation	0.50	4	0	0	3
Cost/Shipping	0.25	4	N/A	N/A	1
Time in Lab	0.25	5	N/A	N/A	3
Weighted Total	1.00	4.25	0	0	2.50

Table 6: Transfection Method Trade Study Results

5.2.6.1. Lipofection

Lipofection was the highest scoring transfection method for this trade study by a significant margin. Lipofection scored a 4 for the biosafety regulation criteria, in large part because through conversations with Kathryn and coordination with the teaching lab, it is

expected for this transfection method to be approved. This method scored a 4 on cost and shipping. The Lipofectamine LTX with plus reagent could be split with the other cell team, meaning this halved the cost of this method, making it the cheapest by a significant margin. The only reason this did not score a 5 is concern around shipping time. This method scored a 5 for time in the lab, this is because this would be a 2-day process of transient transfection method as per the Thermofisher protocol [66].

5.2.6.2. Lentivirus vector

Lentiviral vectors scored a 0 for biosafety regulation criteria. This is because our project was informed that we would not be cleared for using viral based delivery methods. With this in mind, we did not bother assigning or further researching this method since we knew it would be impossible for us to select this transfection method.

5.2.6.3. AAV vector

Similarly to the lentiviral vectors, AAV scored a 0 on biosafety regulation, nullifying it entirely from the rest of our trade study. Once again, this is because we were informed by Kathryn that we would certainly be denied if attempting to use a viral vector. We did not follow through or properly grade this method for this trade study as it was going to be impossible to use this transfection method.

5.2.6.4. CRISPR

Using a CRISPR based transfection method to deliver the genetic modification CRISPR received a 3 in the Biosafety Regulation category. A CRISPR kit can create stable gene expression which has been mentioned as something that may make gaining approval more difficult. The additional nucleic acids required may also require a more complicated review process depending on their content. CRISPR received a 1 in the Cost/Shipping category. Most commercially available sources of CRISPR kits were prohibitively expensive for our project [68]. We would be unable to afford other necessary items for our project and remain within budget if we were to purchase a CRISPR kit. CRISPR was given a 3 for the Time in Lab category. We have been advised that working with CRISPR can be difficult and may take more time to develop a protocol that works well and consistently. We will likely be on a tight schedule once in the lab and will not be able to suffer setbacks like this and still finish on time.

5.2.7. Genetic Modification Targets Trade Studies

Defining New Trade Study Criteria

Risk of adverse effects

This criterion was taken into consideration for this trade study because when editing a cell there is always risk for inducing apoptosis or other unwanted effects. This criterion was given a weight of 0.2 for this trade study. The justification for this weight is adverse effects are

not greatly anticipated regardless, and even so we have time in the lab to correct the mistake or try again. For this trade study we gave the least weight to this category as adverse effects are always a risk and we felt the other two criteria to be more important for this trade study.

5: No anticipated negative adverse effects in the cells based on literature.

4: <50% chance of negative adverse effects that do not include cell death or compromising of major performance.

3: ~50% chance of negative adverse effects seen in the cell, whether it be intended performance drop or drop in viability.

2: >50% chance of negative adverse effects that would inhibit cell performance or cause cell death.

1: >80% chance of negative adverse effects that would likely result in cell death.

Effectiveness

This criterion focuses on the impact each modification would likely have on chemotaxis in the cells. Effectiveness got a 0.4 weight for this trade study, tied for most important category. This criterion received this weight because we want the method of modification we select to be effective at increasing chemotaxis. Our group felt the effectiveness of the modification we selected was the most important goal for our project.

5: Anticipated to be an extremely effective modification that results in a statistically significant increase to chemotaxis in the cell line.

4: Anticipated to be an effective modification that results in a clear increase in chemotactic response in the cells.

3: Anticipated to be a positive modification that results in any form of chemotactic advantage over the wild type cells.

2: Unsure whether the modification would be positive or have any effect on chemotaxis.

1: Most likely would result in a negative effect when implemented and see a drop in chemotaxis in the cells.

Cost of Plasmid

This criterion looks at the actual cost of each plasmid required to execute this modification. This criterion was weighted 0.4. This is because our team is working within a strict project budget, and anything that absorbed an extremely large portion of our budget would take away materials for the rest of our project. This is the most important logistic to consider when assessing our modifications.

5: The cost of this plasmid is <$100

4: The cost of this plasmid is $100-$175

3: The cost of this plasmid is $175-$250

2: The cost of this plasmid is $250-$325

1: The cost of this plasmid is >$325

Criteria/Solution	Weight	PDGFR Alpha	PDGFR Beta	EGFR	TIAM1
Risk of Adverse Effects	0.20	4	4	4	3
Cost of Plasmid	0.40	5	5	1	5
Effectiveness	0.40	4	3	2	2
Weighted Total	1.00	4.60	4.00	2.00	3.80

5.2.7.2. PDGFr-alpha

The PDGFr-alpha pathway is the receptor responsible for sensitivity to PDGF-AA chemoattractant [56]. PDGF is a common chemical the body secretes at wound sites, thus overexpressing the receptor for this chemoattractant should in theory increase chemotaxis in the cells. The PDGFr-alpha pathway scored a 4 on risk of adverse effects. In the literature search there was evidence of some uncommon minor adverse effects that researchers ran into, however nothing deterimental to the cells [56]. This modification scored a 5 for cost of plasmid because the cost of this plasmid is $95 on AddGene [57]. This modification scored a 4 for effectiveness, as there was evidence in the literature search that suggested it is possible to increase the chemotaxis of NIH3T3 cells with PDGF as the chemoattractant [56].

5.2.7.3. PDGFr-beta

The PDGFr-beta pathway is the receptor responsible for sensitivity to PDGF-BB chemoattractant [58]. There is some evidence in literature that this receptor can also respond to PDGF-AA, although there is less evidence that overexpressing this receptor leads to increased chemotaxis when AA is the attractant rather than BB [58]. PDGFr-beta overexpression scored a 4 on risk of adverse effects. Similarly to alpha, there were only uncommon and minor adverse effects seen in some cell lines when using this modification method [58]. This modification scored a 5 for cost of plasmid, since this plasmid also costs $95 on AddGene [59]. The effectiveness of this modification scored a 3, and this is in large part due to the specificity of the PDGF we chose. If we had chosen PDGF-BB then beta would have likely won this trade study, but considering we moved forward with AA, the beta scored one point lower than the alpha pathway.

5.2.7.4. EGFR

Epidermal growth factor receptor (EGFR) is a cell membrane receptor that binds to epidermal growth factor (EGF). There was limited evidence found that increasing EGFR expression would have a high chance of adverse effects on the cell. We feel reasonably comfortable assigning it a 4 in the Risk of Adverse Effects category because of this. EGFR plasmid was significantly more expensive than the other options because it was not available on Addgene. Its price exceeded $325 and was therefore assigned a 1 in the Cost/Shipping criterion because of how it would limit our purchasing ability for the rest of the project[70]. An expected effectiveness score of 2 was given to this proposed genetic modification. EGFR has been shown to localize to the leading edge of the cell during certain types of chemotactic response. Unfortunately, EGFR is not sensitive to PDGF (our chosen chemoattractant) and this means it would have limited potential in the context of our project[71].

5.2.7.5. TIAM1

TIAM1 is a gene that has multiple functions, the most relevant of which for our purposes is regulation of GTPase activity as well as microtubule binding related to polarization of the cell during chemotaxis[72][73]. TIAM1 was given a score of 3 for the risk of adverse effects when introduced to the cell. TIAM1 is known to be implicated in many functions but the exact mechanism of action is not very well understood at this point [74]. The potential for changing the expression of the gene to affect an unknown number of cellular functions led us to assign a lower score in this category. TIAM1 is also often reported to be highly expressed in cancer cells. A score of 5 was given in the Cost/Shipping category because the plasmid is available on Addgene for $85 [75]. This fits well within our allotted project budget. Finally, TIAM1 was given an effectiveness score of 2. While the TIAM1 gene is involved in some relevant cell functions for our project there was limited evidence that increasing the amount of TIAM1 function would lead to an increased chemotactic response. We did not feel confident predicting the exact effects of this genetic modification and therefore gave it a lower expected effectiveness score.

5.3. Design Solution Impacts

During the development of the myriad potential design solutions presented in this report, the team also did extensive research into the downstream effects of these potential solutions across several categories. These categories include, but are not limited to, environmental, socioeconomic, public health, and ethical impacts. The impacts, both positive and negative, are discussed below.

5.3.1. Environmental Impacts

Ultimately, the implementation of our solution will require extensive use of lab materials. Cell culture and transfections are two activities in the lab that require many consumables and lots of plastic including flasks, tubes, pipettes, media, and more. We want to be conscientious

of minimizing our use of consumables as they will ultimately end up in a landfill. In addition, we will be conscientious of the proper disposal protocols for reagents since some reagents may be more hazardous than others and need further precautions to be taken. We want to prevent any hazardous materials from entering wastewater and sewage systems. We also want to keep in mind that any biological waste we produce will need to eventually be incinerated which can produce smoke and release combustion products into the atmosphere. If our eventual cell therapy is upscaled to be used for the treatment and studying of diseases, we will want to be aware of the power and water demands of the lab procedures that will be performed.

5.3.2. Socioeconomic Impacts

We want to consistently be mindful of how our solution might impact various social and economic groups. With the hopes of creating a therapy that is not only available to wealthier classes, it is our duty to minimize the cost of our solution as much as possible to ensure equitable accessibility for all. In addition we want to be mindful of drawing conclusions. It is well known that many reference genomes consist exclusively of data from individuals of European descent and do not accurately treat groups of African descent[54]. The HL60 cell line utilized for our research was derived from a white female of European descent. If we do find a cell therapy to improve chemotaxis, we will want to explore how the therapy applies to immune cells from individuals with diverse ethnicities and genetic backgrounds.

5.3.3. Public Health/Welfare Impacts

Our solution, whether it improves NIH 3T3 migration efficiency or not, will add to the large bank of knowledge and research that helps scientists better understand the immune response and how to treat diseases and promote wound healing. Our work will supplement research while also in the long run improving the lives of any individual susceptible or already affected by diseases, burns, or traumatic injuries that require an improved immune response. In addition, our findings of how to enhance cellular migration may indirectly hint at ways to impede cellular migration, which can help individuals suffering from auto-immune disorders. Overall, this study has various implications in immunology and the welfare of the public. Furthermore, we will want to be considerate of genetic engineering as our approach to improving chemotaxis. This topic may be seen as controversial by various cultural, political, and religious groups, and should be executed with care to ensure that no harm is done and that the science is not used for any nefarious purposes.

6. Design Implementation

This section will detail the planned implementation protocols pending biosafety approval.

6.1. NIH 3T3 Culture Protocol

NIH3T3 cells will be sourced from the Bioengineering Teaching Lab as they are already grown and stored there for another course. We will take one vial/flask and scale up the cells for our project. Cells will be grown in T75 cell culture flasks. To prepare media for the NIH 3T3 cells, Dulbecco's Modified Eagle's Medium (DMEM) will be supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin. When passaging, spent cell culture media will be removed, cells will be washed with 1X PBS, and then a 0.25% Trypsin-EDTA solution will be added to dissociate the cells from the flask. Cells will be counted, centrifuged and resuspended in fresh complete culture media before being introduced into a new culture flask. Passaging would take place every 3-4 days. Cells will be grown until reaching the desired 70-90% confluency needed for the lipofection protocol. A maximum of eight T75 flasks will be maintained simultaneously with a maximum volume of 20 mL of media per flask.

6.2. Plasmid Isolation Protocol

Our team will be ordering our plasmids for PDGFRalpha and PDGFRbeta from Addgene in the form of a bacterial agar stab. To isolate our plasmid of interest from the agar stab we will use the QiAPrep Spin MiniPrep Kit from Qiagen. The following protocol will outline the selection of our plasmids from their initial bacterial agar stab form. The first step is to obtain a Luria Broth (LB) agar plate and use a sterile pipette tip to transfer bacteria from the stab culture to the surface of the plate by creating a streak. Two more pipette tips will be used to create subsequent streaks from the initial streak. The plate will then be incubated at 37°C for at least 12 hours to recover individual colonies of the bacteria. The next phase will involve inoculating the bacteria colonies from the plate into a liquid LB culture. LB will be purchased premade at an appropriate volume (e.g. 500mL). Antibiotics will be added at an appropriate concentration for the plasmid being purified (PDGFRalpha and PDGFRbeta require Hygromycin at 200ug/mL). Using a sterile pipette tip, a single colony will be selected from the agar plate and placed in the tube with the liquid culture and antibiotic. This culture will be covered in foil and incubated at 37°C for at least 12 hours to allow the bacteria to grow. Once growth is observed the next day, the bacteria will be stored as a glycerol stock if work is not continuing the next day. If work continues, we will begin with the protocol described in the mini-prep kit in order to isolate the plasmid of interest. All work with bacteria will be performed inside Biosafety Cabinets to avoid contamination and exposure. Proper waste disposal and cleanup procedures for RG1 material will be followed during and after any procedures.

6.2.1 Mini-Prep Kit Protocol

The QiAPrep Spin MiniPrep Kit will be used in order to isolate and purify our plasmid DNA from our bacterial cultures[76]. Prior to beginning the protocol, the following steps must be taken: LyseBlue is added to Buffer P1 at a ratio of 1:1000, RNase A is also added to Buffer P1, and 100% ethanol is added to Buffer PE before use. The first step is to pellet 5mL of the previously incubated bacterial culture via centrifugation at 8000 rpm for 3 minutes at room temperature. Pelleted cells should then be resuspended into 250uL of P1 Buffer in a

microcentrifuge tube. 250uL of P2 Buffer will be added and the solution will be mixed until it turns blue. This marks the beginning of the lysis step which should proceed for no more than 5 minutes. After this, 350uL of Buffer N3 will be added and the solution will be shaken until it turns colorless. The colorless solution will be centrifuged for 10 minutes at 13,000 rpm. This supernatant will be added to a QiAPrep spin column and spun for 1 minute at 13,000 rpm. The spin column will be washed with 500uL of Buffer PB and 750uL Buffer PE with centrifuge steps of 1 min at 13,000 rpm in between. The spin column will then be placed into a 1.5mL microcentrifuge tube. To elute the DNA, 60uL of Buffer EB will be added to the column. The buffer will sit for 1 minute and centrifuged for 1 minute at 13,000 rpm. The DNA will then be extracted from the column and used in the proceeding lipofection step.

6.3. Lipofection Protocol

Our team will be proceeding with lipofection as our chosen method of transfection. Lipofection delivers the gene of interest into the cell via delivery by a lipid complex. The lipid includes particles that carry the nucleic acids into the nucleus where they can be expressed by the cell. Genes of interest that are being considered for the lipofection protocol are PDGFRalpha, PDGFRbeta, TIAM1, and EGFR, which correspond to our potential cell targets for enhancing chemotaxis. After a review of the literature and evaluating how each potential target will influence cellular response during migration and how it will respond to our selected chemoattractant, we decided that we will first be proceeding with PDGFRalpha and PDGFRbeta as our chosen targets. These genes of interest will be knocked in using lipofection to upregulate their expression for stimulating chemotactic pathways.

Procedure:

This procedure is derived from the Lipofectamine LTX Protocol with PLUS Reagent by ThermoFisher [78].

1) NIH 3T3 cells will be seeded at about 70%-90% confluency in a 96-well plate before transfection.
2) At the time of lipofection in a 96-well plate, the Lipofectamine LTX reagent will be diluted in Opti-MEM medium (0.15uL in 5uL x 2).
3) A master mix of DNA will be prepared by diluting 0.2ug of our plasmid of interest in 10uL of Opti-MEM along with 0.4uL of PLUS reagent.
4) The diluted DNA master mix will then be added to the diluted Lipofectamine LTX in a 1:1 ratio (5uL:5uL).
5) This mixture will be incubated for 15 minutes at room temperature.
6) 10uL of the DNA-lipid complex will be added to each well of cells and then incubated for 3 days at 37 °C.
7) For the last step, the transfected cells will be analyzed using the EVOS FL Digital Inverted Fluorescence Microscope in the Bioengineering Teaching Lab.

6.3.1. Fluorescence Selection

ImageJ analysis will be performed using Thermo Fisher's EVOS FL Digital Inverted Fluorescence Microscope in the Bioengineering Teaching Lab. This software has an integrated fluorescent channel for green fluorescent protein (GFP), which is the fluorescent marker that is tagged in our purchased plasmids. This will be used to distinguish successfully transfected cells. Higher levels of GFP fluorescence will be detected for cells that have been successfully transfected with the gene of interest. The software also contains a count tool, which provides quantitative outputs for the distinguished categories of cells entered into the software (i.e., it will provide both the number and percentage of cells for transfected and non-expressing groups). This will assist with verifying our design requirement for having a minimum of a 23% transfection efficiency.

6.3.2. Antibiotic Selection

When cells are transfected with the plasmid containing the antibiotic resistance gene, the culture medium will be supplemented with the appropriate amount of the relevant antibiotic. The antibiotics being considered for use are Hygromycin at 200 ug/mL and Neomycin at 400 ug/mL respective to the specific antibiotic selection marker that the plasmid contains. The purpose of the antibiotic supplemented media is to select for cells transfected with the gene of interest and antibiotic resistance gene by killing the cells without antibiotic resistance. A maximum of eight T75 flasks will be maintained simultaneously with a maximum volume of 20 mL of media per flask.

6.4. Chemotaxis Assay Protocol

The team will be using the Cell Migration/Chemotaxis Assay Kit (96 well, 8uM) from Abcam in order to test the effect of our protein modifications on cell migration[77]. The following protocol sections were derived from the recommended protocol provided by Abcam[77].

<u>Loading the Cells:</u>

1. Grow enough cells to perform a Cell Migration Assay and a Standard Curve in desired media and culture conditions.
2. Adherent cells should be cultured to ~80% confluence. Prior to the assay, starve cells for 18-24 h in serum-free media (0.5% serum can be used if needed).
3. After starvation, harvest cells and centrifuge at 1,000 x g, for 5 minutes to pellet them.
4. Resuspend the cell pellet in serum-free media and count the number of cells using a hemocytometer or an automated cell counter.
5. Resuspend cells at 1 x 10^6 cells/mL in a serum-free media.

6. Under sterile conditions, disassemble the Cell Chamber (96 x 8µm)/Cell Migration Chamber and carefully remove the plate cover and the top chamber.
7. Bottom Chamber: Add 150 µL of serum-free media per well containing desired chemoattractant to the bottom chamber.
8. Omit the chemoattractant in the control wells.
9. For positive control, add 15 µL of Control Migration Inducer to 135 µL of media in the bottom chamber.
10. Place the top chamber back into the bottom chamber.
11. Ensure no air bubbles are trapped between the top and the bottom chamber.
12. Top Chamber: Add 50 µL (50,000 cells) of cell suspension to each well of the top chamber.
13. Add desired stimulator or inhibitor to the top well, and gently mix.
14. Make up the volume to 100 µL by cell media. Carefully place the plate cover and incubate the Cell Chamber (96 x 8µm)/Cell Migration Chamber at 37°C in CO2 incubator for 2-48 hours.
Note: Migratory cells pass through the polyester membrane and/or cling to the outer side of the top chamber. Nonmigratory cells stay in the upper chamber.
Note: If required, media with 0.1% serum can be used in top chamber.

Standard Curve:

1. Each cell type requires a separate Standard Curve. Prepare a Standard Curve by adding 50 µL cell suspension (1 x 10^6 cells/mL, 50,000 cells) per well in a 96-well white plate with clear bottom.
2. Serially dilute the cells 1:1 in Wash Buffer II/Wash Buffer and generate a Standard Curve of cells (50,000, 25,000, 12,500, 6,250, 3,125, 1,562 and 781) in 100 µL total volume.
3. As blank, use 100 µL of Wash Buffer II/Wash Buffer.
4. Add 10 µL of Cell Dye I/Cell Dye to each well.
5. Incubate at 37°C for 1 hour.
6. Read the fluorescence at Ex/Em = 530/590 nm.
7. Plot the Standard Curve of Number of Cells Vs RFU obtained.
8. Fit the data points using a linear trendline with zero intercept.
9. The equation for the straight line and R-square value are used for data analysis of samples.
Note: The Cell Migration RFU reading should fall in the linear range of the Standard Curve. We recommend using triplicates for Standard Curve.

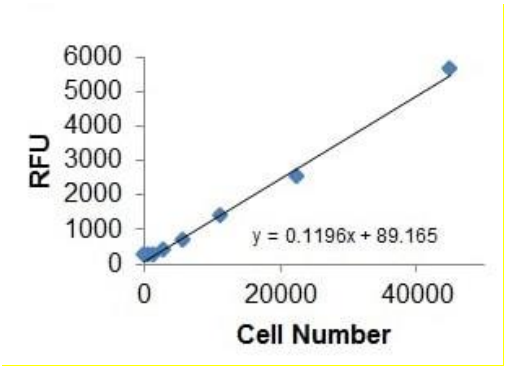


Figure 6: Example Standard Curve for Cell Counting[77]

Separation of Invasive Cells:

1.After the desired incubation with cell invasion inducers/inhibitors, carefully remove the plate cover and aspirate media from the top chamber without puncturing the membrane and matrix.
2. Remove cells from the top chamber using a cotton swab. Disassemble the Cell Invasion Chamber by removing the top chamber. Invert the top chamber and set it aside.
3. Place the plate cover on top of bottom chamber and centrifuge the plate at 1,000 x g for 5 minutes at room temperature.
4. Carefully aspirate the media from the bottom chamber and wash the chamber with 200 µL Wash Buffer II/Wash Buffer.
5. Centrifuge the plate at 1,000 x g for 5 min. at room temperature and aspirate the Wash Buffer II/Wash Buffer from the bottom chamber.

Count Invasive Cells:

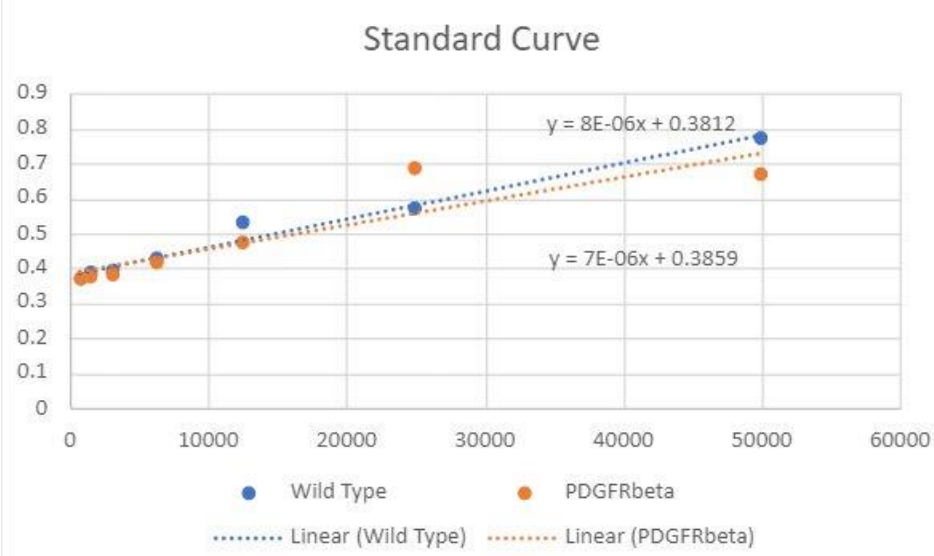
1. Prepare a mix of 100 µl of Cell Dye I/Cell Dye in 1 mL of Cell Dissociation Solution I/Cell Dissociation Solution. Mix well. Make the Cell Dye I/Cell Dye solution as desired depending on the number of wells.
2. Add 110 µL of the mix to each well of the bottom chamber. Reassemble the Cell Invasion Chamber by placing the top chamber into the bottom chamber. Incubate at 37°C in CO2 incubator for 60 minutes.
3. After incubation, disassemble the Cell Invasion Chamber, remove the top chamber and read the plate at Ex/Em = 530/590 nm.
4.Calculate the number of cells invaded using the equation of the straight line obtained from Standard Curve.
5. Percentage Invasion can be calculated as follows:

$$\%\ Invasion = \frac{\#\ of\ cells\ in\ lower\ chamber}{Total\ \#\ of\ cells\ added\ to\ top\ chamber} * 100$$

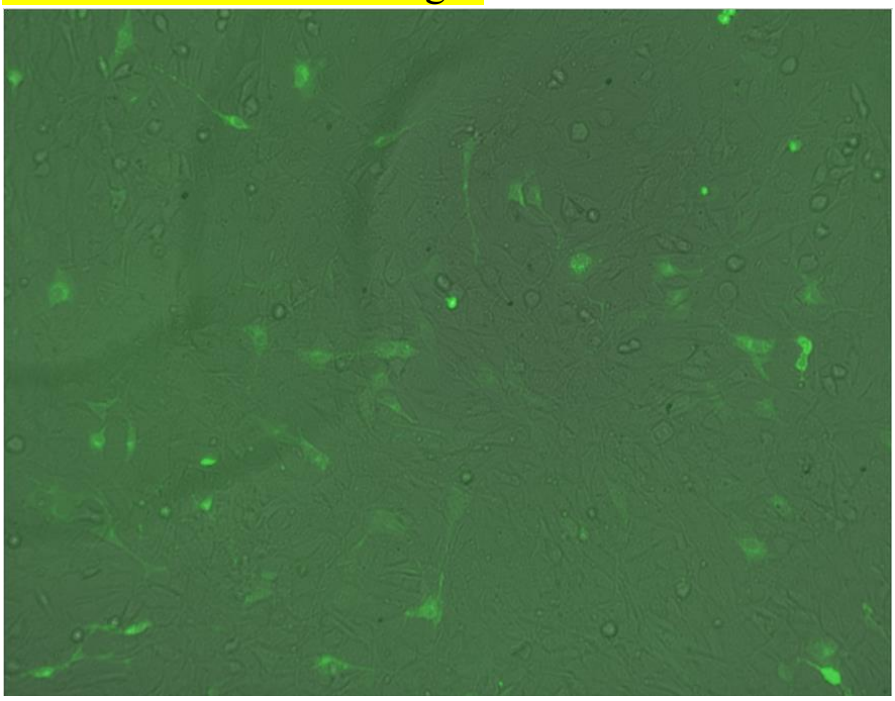
7. Testing and Verification

7.1 Standard Curve

To determine the cell count in each well based on the fluorescence reading, we constructed a standard curve for each of our cell populations (WT and beta). Section 6.4 details the experimental protocol used to prepare and read the 96-well plate to create the standard curve. Appendix F contains the raw fluorescence data from the BioTek plate reader along with the calculations used to generate the curve for both conditions in Excel.

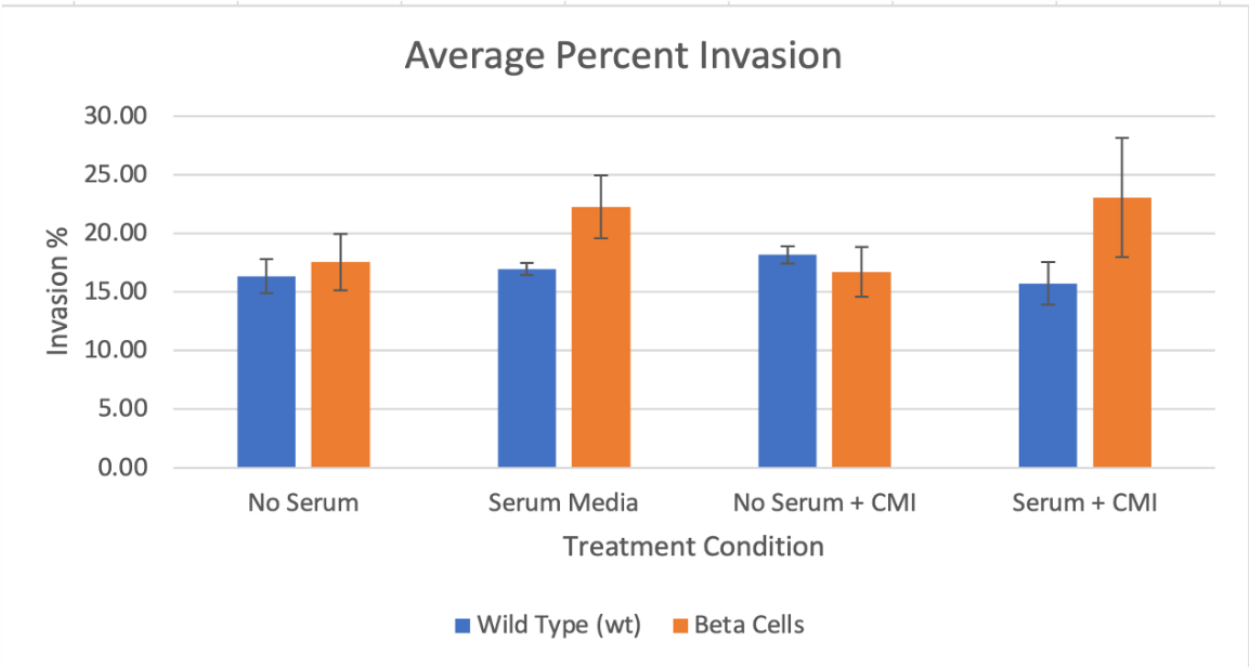


7.2 Transfected Cell Images



This is an EVOS fluorescent microscope image of PDGFR-beta transfected cells. In this image we see the successful transfection of this plasmid into our NIH3T3 cells where the edited cells are fluoresced green.

7.3 Percent Invasion Results



This plot shows the data generated by the chemotaxis assay. The full table of raw data can be found in Appendix F. The assay was run on wild type NIH-3T3 cells and NIH-3T3 cells transfected with the PDGFRbeta plasmid. Each cell type was plated according to the following conditions: FBS free media, 10% FBS media, FBS free media + CMI (control migration inducer), and 10% FBS media + CMI. The data is presented as a percent invasion which is calculated by using the linear regression of the standard curve for the relevant cell type to convert fluorescence reading into number of cells and dividing this number by the total number of cells initially seeded in each well (50,000). Error bars represent standard error and were calculated in Microsoft Excel. Two-Sample T-tests were used to determine statistical significance between samples with a cut off for significance of $p \leq 0.05$. Three tests were run on the following groups:

$p=0.127$: WT vs PDGFRbeta cells in serum media

$p=0.359$: WT in non-serum vs serum media

$p=0.263$: PDGFRbeta in non-serum vs serum media

No statistical significance was found between these experimental conditions. However, we believe that a trend of increased migration was shown in PDGFRbeta conditions that included serum. With a higher sample size and other improvements to the process, which are discussed in more detail in Section 10. Future Considerations, there may have been significance in the results.

8. Final Design

8.1 Changes Made

Several changes were made between the initial and final design for this project. As previously discussed, the team pivoted cell lines from HL60 to NIH 3T3 and pivoted

endpoint assays from a microfluidic maze to a trans-well chemotaxis assay. During implementation, we had planned on selecting our bacteria with hygromycin antibiotic however we realized after administering this that the lack of a promoter only found in mammalian cells prevented the bacteria with the plasmid from conferring resistance to the hygromycin. We also did not see enough PDGFRalpha plasmid (3.5µg total) when we measured the plasmid concentration using the Nanodrop 2000 based on our initial calculations for transfection. There was enough of the PDGFRbeta plasmid (7µg total) however this caused an issue over whether to transfect the same amount of plasmid or transfect the same amount of wells. We decided to transfect each well with the same amount of respective plasmid summarized in the figure below, with one leftover well of PDGFRbeta receiving slightly more plasmid. Also attached is a sample of the final design implementation we made with our transfection reagent volumes to transfect our cells with the plasmid PDGFRbeta.

1.8µg PDGFRalpha	1.8µg PDGFRalpha	0µg (empty well)
1.8µg PDGFRbeta	1.8µg PDGFRbeta	3.4µg PDGFRbeta

Sample Transfection Volumes with PDGFRbeta

Reagents for DNA Dilution Treatment	Volume
PDGFRbeta plasmid	49 µL (3.5 ug)
PLUS Reagent	2.5 µL (Calculated from manufacturer's recommended volume of 14 µL total in a 6-well plate and adjusted to factor higher plasmid concentration)
Opti-MEM media (for diluted DNA)	73.5 µL
Total:	125 µL

Reagents for LTX Dilution Treatment	Volume
LTX	12.5 µL (Selected from recommended manufacturer range)
Opti-MEM media (for diluted LTX)	112.5 µL
Total:	125 µL

DNA-Lipid Complex Total:	250 µL (Recommended total from manufacturer of Lipofectamine LTX Kit)

Visualization of the cells 48 hours post transfection revealed that the PDGFRbeta cells had much higher percent transfection compared to the PDGFRalpha cells to the point where we decided to not move forward with the alpha cells since there would not be enough cells to conduct the chemotaxis assay. The transfection efficiency of the PDGFRbeta cells was also not very high on its own and we decided to skip antibiotic selection with hygromycin at this stage as well due to concerns over killing the cells before the chemotaxis assay. We also could not stain the cells with DAPI so we were unable to verify transfection efficiency quantitatively.

8.2 Design Requirements Met

The team was able to meet some of our major design requirements despite setbacks. We were able to transfect a relatively sufficient amount of PDGFRbeta plasmid however we were not able to quantify transfection accurately. We also did not transfect enough PDGFRalpha into our cells. We did not achieve statistically significant differences between our modified and unmodified cells in terms of percent invasion. We kept our project budget under the $1500 limit and ensured all required protocols were approved for safety and logistics.

9. Conclusions

Our project focused on increasing cellular migration. We started as the HL60 Cell Race team and planned to simulate the Dicty World Race with another capstone team. The original race was a competition between teams of researchers who engineered HL60 cells to complete a microfluidic maze as fast as possible. In Capstone 1, we conducted thorough background research, developed design requirements, evaluated our initial solutions with trade studies, and developed a lab plan for Capstone 2 in the spring.

Beginning Capstone 2, we learned of university regulations preventing the handling of human cell lines by undergraduate capstone students, so we could no longer use HL60 cells. Thus, the team spent considerable time over the past few months revamping the project into something that could still provide meaningful scientific results while also adhering to all required safety regulations. We shifted the focus of our project to utilize NIH 3T3 murine fibroblasts as a model for wound healing. Instead of validating our cells in a microfluidic maze race, we assessed migration using a chemotaxis assay kit.

After deciding on using lipofection to transfect our gene of interest and identifying our targets as PDGFRalpha and PDGFRbeta, we finalized our budget and ordered our materials. We developed lab protocols for our experiments and ensured their compliance with Northeastern biosafety regulations. By isolating a plasmid containing PDGFRalpha and PDFGRbeta, transfecting them into NIH3T3s, and measuring migration with an assay, we demonstrated a subtle increase in migration in our engineered cells compared to the wildtype, but this difference was nonsignificant. There was a visual trend in the data that favored the engineered cells in terms of percent invasion, but a concrete conclusion cannot be made without further experiments. With more time and improvements, our project has the potential to uncover a pathway that could be exploited to increase cell migration and benefit those with poor wound healing. The improvements we would make in our future work are described in the following section.

10. Future Work and Considerations

There are many improvements our team could make to this project if we were to repeat this experiment in the future. It is important to note that if we had the time, these are all improvements that we could have made this semester to enhance our project results. First, we could have made our experimental workflow easier by directly ordering a plasmid from IDT instead of ordering it in the form of a bacteria stab. This would have saved us approximately three days in the lab and also reduced any error we introduced while isolating our plasmid. In addition, we would have liked to send off our isolated DNA sample for sequencing to verify that the chosen colony for inoculation was not a mutant.

With extra time, we could have optimized the bacterial inoculation to ensure greater bacterial expansion, because we failed to get the maximum amount of DNA from our DNA isolation kit. Although we obtained sufficient PDGFRbeta plasmid, we did not for the PDGFRalpha plasmid. This meant we had to reduce the amount of DNA that we transfected into the cells. As for the transfection, there are many improvements to be made. The ratio of optimem to LTX to PLUS to DNA for an optimal transfection is different for every cell line. Finding the perfect ratios of all reagents could have been determined in an assay using a 96 well plate and varying the concentrations of each, then reading fluorescence to see which ratio of reagents was best for transfection.

Since we could not optimize the transfection, the number of transfected cells was extremely low. For this reason, we could not perform an antibiotic selection. This was a major setback for our project, as we planned for the antibiotic selection to make our cell population purer. Without performing the antibiotic selection, our experimental cells (PDGFRbeta) were a mixed population of WT and transfected cells. If we had the time, we could have conducted a killing curve to test different concentrations of Hygromycin to determine the ideal concentration to maximize the transfected cell population. This would have dramatically enhanced the success of our project. Alternatively, if we had access to a cell sorting machine such as flow cytometry, we could have used this to separate our transfected cells from the WT. Ultimately, what would have been better than lipofection would have been to do a stable transfection using lentivirus. In this way, our gene would have been incorporated into the NIH3T3 genomes instead of just transiently existing in the nucleus. This would have enabled longer-term experiments to be performed and been better for overall expression levels. This was not possible with our time and budget but could be a significant improvement in the future.

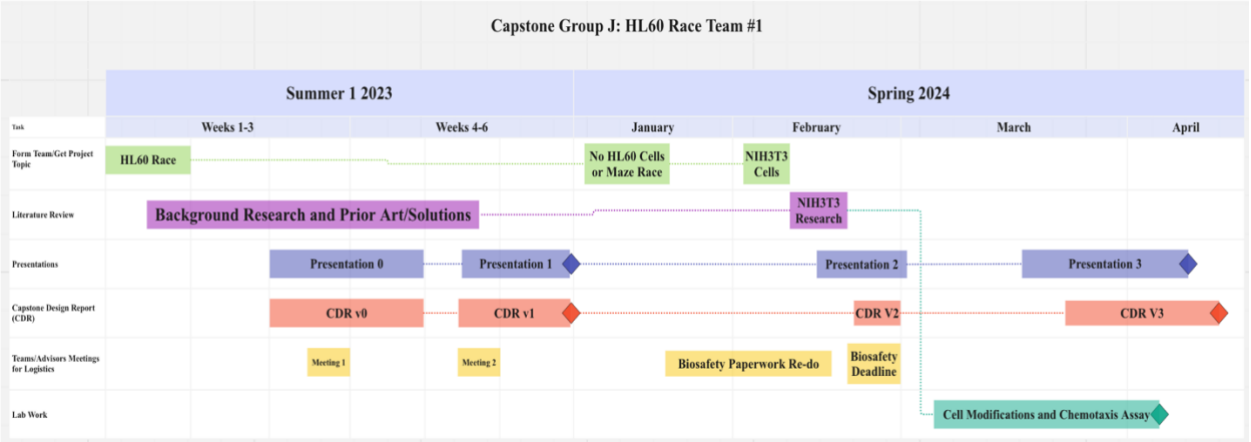
In addition, we could have conducted more assays to verify the presence of our gene of interest. Performing a western blot on our wildtype cells as well as transfected cells could have told us the relative expression levels of our gene of interest. This would have cost more money since we would need the equipment and reagents for the assay as well as an antibody against PDGFR. Performing a western blot pre-chemotaxis assay and post-chemotaxis assay would have told us if the transient transfection we performed lasted long enough to demonstrate an effect in the assay. Again, this could have been avoided with a lentiviral stable transduction instead of lipofection.

Another improvement we could have made was using our intended chemoattractant of PDGF. Since this did not arrive in time, we had to use FBS as the chemoattractant, even though the gene we chose to upregulate was the receptor for PDGF. We could have observed significant results had we used this chemoattractant. Our final improvement would be to transfect an empty vector plasmid alongside our plasmid containing our gene. In the end, we had no empty vector control for the transfection, so it is possible that any differences in results we observed could be due to the presence of our gene of interest or because of the transfection process and plasmid backbone. If we had included an empty vector control, we could have ruled out this possibility and confidently claimed that any observed differences were because of our gene of interest, not the transfection process.

All in all, for the time we had to conduct our experimental process, we achieved a great feat. Had the time in lab begun in February instead of the end of March, it is possible that we could have executed all these improvements this semester. Nonetheless, these improvements lay the groundwork for a future team or group to better conduct this experiment and hopefully observe a significant difference in migration with the assay.

We would like to thank our Capstone 1 advisor Mike Jaeggli for guiding us through the beginning steps of capstone and helping us lay the groundwork for our project. We would also like to thank our Capstone 2 advisor Dr. Narges Yazdani. She gave us unconditional support throughout the semester and made sure that we were going to be successful no matter what went wrong. She was balancing her final semester of her PhD work while also advising us, which was no easy feat. We thank her for her time and guidance throughout this project. We also would like to thank Saige McGinnis and Kathryn Lasseter for their contributions in developing our lab protocols. These were quite complicated to complete but with their recommendations we were able to successfully develop protocols and execute our experimental plan to the best of our ability.

10.1. Gantt Chart



11. References

[1] B. Heit *et al.*, “PTEN functions to ‘prioritize’ chemotactic cues and prevent ‘distraction’ in migrating neutrophils,” *Nat Immunol*, vol. 9, no. 7, pp. 743–52, Jul. 2008, doi: 10.1038/ni.1623.

[2] P. J. Van Haastert and P. N. Devreotes, “Chemotaxis: signalling the way forward,” *Nat Rev Mol Cell Biol*, vol. 5, no. 8. pp. 626–34, 2004. doi: 10.1038/nrm1435.

[3] C. N. Jones *et al.*, “Spontaneous neutrophil migration patterns during sepsis after major burns,” *PLoS One*, vol. 9, no. 12. p. e114509, 2014. doi: 10.1371/journal.pone.0114509.

[4] C. Nathan, “Neutrophils and immunity: challenges and opportunities,” *Nat Rev Immunol*, vol. 6, no. 3. pp. 173–82, 2006. doi: 10.1038/nri1785.

[5] J. Hazeldine, P. Hampson, and J. M. Lord, “The impact of trauma on neutrophil function,” *Injury*, vol. 45, no. 12, pp. 1824–1833, Dec. 2014, doi: 10.1016/j.injury.2014.06.021.

[6] M. Skoge *et al.*, “A Worldwide Competition to Compare the Speed and Chemotactic Accuracy of Neutrophil-Like Cells,” *PLOS ONE*, vol. 11, no. 6, p. e0154491, 2016, doi: 10.1371/journal.pone.0154491.

[7] A. E. Williams and R. C. Chambers, “The mercurial nature of neutrophils: still an enigma in ARDS?,” *Am J Physiol Lung Cell Mol Physiol*, vol. 306, no. 3. pp. L217-30, 2014. doi: 10.1152/ajplung.00311.2013.

[8] H. L. Wright, R. J. Moots, and S. W. Edwards, “The multifactorial role of neutrophils in rheumatoid arthritis,” *Nat Rev Rheumatol*, vol. 10, no. 10. pp. 593–601, 2014. doi: 10.1038/nrrheum.2014.80.

[9] L. K. Fritz-Laylin, “Actin-based protrusions of migrating neutrophils are intrinsically lamellar and facilitate direction changes.” 2017.

[10] G. Servant, O. D. Weiner, P. Herzmark, T. Balla, J. W. Sedat, and H. R. Bourne, “Polarization of chemoattractant receptor signaling during neutrophil chemotaxis,” *Science*, vol. 287, no. 5455. pp. 1037–40, 2000. doi: 10.1126/science.287.5455.1037.

[11] P. A. Nuzzi, M. A. Lokuta, and A. Huttenlocher, “Analysis of neutrophil chemotaxis,” *Methods Mol Biol*, vol. 370. pp. 23–36, 2007. doi: 10.1007/978-1-59745-353-0_3._

[12] M. A. Lokuta, P. A. Nuzzi, and A. Huttenlocher, “Calpain regulates neutrophil chemotaxis,” *Proc Natl Acad Sci U S A*, vol. 100, no. 7. pp. 4006–11, 2003. doi: 10.1073/pnas.0636533100.

[13] G. Khachaturyan *et al.*, “Temperature-sensitive migration dynamics in neutrophil-differentiated HL-60 cells,” *Sci Rep*, vol. 12, no. 1. p. 7053, 2022. doi: 10.1038/s41598-022-10858-w.

[14] Amalia Hadjitheodorou, George R. R. Bell, Felix Ellett, Shashank Shastry, Daniel Irimia, Sean R. Collins, Julie A. Theriot, “Directional reorientation of migrating neutrophils is limited by suppression of receptor input signaling at the cell rear through myosin II activity,” *Nature Communications*, vol. 12, 2021, doi: 10.1038/s41467-021-26622-z.

[15] Bing Huang, Yingchen Ling, Jiangguo Lin, Ying Fang, Jianhua Wu, “Mechanical regulation of calcium signaling of HL-60 on P-selectin under flow,” *BioMedical Engineering OnLine*, vol. 15, no. 2, 2016, doi: 10.1186/s12938-016-0271-1.

[16] Lieke A. Golbach, John G.M. Philippi, Jan J.M. Cuppen, Huub F.J. Savelkoul, B.M. Lidy Verburg-van Kemenade, “Calcium signalling in human neutrophil cell lines is not affected by low-frequency electromagnetic fields,” *Bio Electro Magnetics*, vol. 36, no. 6, pp. 430–443, 2015, doi: 10.1002/bem.21924.

[17] Kehinde Adebayo Babatunde, Xiao Wang, Alex Hopke, Nils Lannes, Pierre-Yves Mantel,

Daniel Irimia, “Chemotaxis and swarming in differentiated HL-60 neutrophil-like cells,” *Scientific Reports*, vol. 11, 2021, doi: https://doi.org/10.1038/s41598-020-78854-6.
[18] MA Boss, D Delia, JB Robinson, MF Greaves, “Differentiation-linked expression of cell surface markers on HL-60 leukemic cells,” *Blood*, vol. 56, no. 5, pp. 910–916, 1980, doi: https://doi.org/10.1182/blood.V56.5.910.910.
[19] Kyung-Hyo Kim, Ju Young Seoh, Su Jin Cho, “Phenotypic and Functional Analysis of HL-60 Cells Used in Opsonophagocytic-Killing Assay for Streptococcus pneumoniae,” *Journal of Korean Medical Science*, vol. 30, no. 2, pp. 145–150, 2015, doi: https://doi.org/10.3346%2Fjkms.2015.30.2.145.
[20] Esther Rincón, Briana L. Rocha-Gregg, Sean R. Collins, “A map of gene expression in neutrophil-like cell lines,” *BMC Genomics*, vol. 19, 2018, doi: https://doi.org/10.1186/s12864-018-4957-6.
[21] Jan F. Klinker, Katharina Wenzel-Seifert, Roland Seifert, “G-Protein-coupled receptors in HL-60 human leukemia cells,” *Elsevier*, vol. 27, no. 1, pp. 33–54, 1996, doi: https://doi.org/10.1016/0306-3623(95)00107-7.
[22] G. D. Birnie, “The HL60 cell line: a model system for studying human myeloid cell differentiation,” *Br J Cancer Suppl*, vol. 9, pp. 41–45, Dec. 1988.
[23] S. J. Collins, “The HL-60 promyelocytic leukemia cell line: proliferation, differentiation, and cellular oncogene expression,” *Blood*, vol. 70, no. 5, pp. 1233–1244, 1987, doi: 10.1182/blood.V70.5.1233.1233.
[24] A. B. Hauert, S. Martinelli, C. Marone, and V. Niggli, “Differentiated HL-60 cells are a valid model system for the analysis of human neutrophil migration and chemotaxis,” *Cell Motil.*, vol. 34, no. 7, pp. 838–854, Jul. 2002, doi: 10.1016/S1357-2725(02)00010-9.
[25] M. Smith, “Genetic Engineering,” vol. 2023, no. June 2, 2023. National Institutes of Health, Jun. 01, 2023. [Online]. Available: https://www.genome.gov/genetics-glossary/Genetic-Engineering#:~:text=Definition&text=Genetic%20engineering%20(also%20called%20genetic,a%20new%20segment%20of%20DNA.
[26] A. Fus-Kajawa *et al.*, “An overview of methods and tools for transfection of eukaryotic cells in vitro,” vol. 9. Frontiers in Bioengineering and Biotechnology, Jul. 20, 2021.
[27] F. Zhang, Y. Wen, and X. Guo, “CRISPR/Cas9 for genome editing: progress, implications and challenges,” *Human Molecular Genetics*, vol. 23, no. R1. pp. 40–46, 2014.
[28] Y. Shi, H. Inoue, J. C. Wu, and S. Yamanaka, “Induced pluripotent stem cell technology: a decade of progress,” *Nature Reviews Drug Discovery*, vol. 16, no. 2. pp. 115–130, 2017. doi: 10.1038/nrd.2016.245.
[29] T. Sato, T. Hongu, M. Sakamoto, Y. Funakoshi, and Y. Kanaho, “Molecular mechanisms of N-formyl-methionyl-leucyl-phenylalanine-induced superoxide generation and degranulation in mouse neutrophils: phospholipase D is dispensable,” *Mol Cell Biol*, vol. 33, no. 1. pp. 136–45, 2013. doi: 10.1128/MCB.00869-12.
[30] J. A. Nick *et al.*, “Common and distinct intracellular signaling pathways in human neutrophils utilized by platelet activating factor and FMLP,” *J Clin Invest*, vol. 99, no. 5. pp. 975–86, 1997. doi: 10.1172/JCI119263.
[31] O. D. Weiner, P. O. Neilsen, G. D. Prestwich, M. W. Kirschner, L. C. Cantley, and H. R. Bourne, “A PtdInsP(3)- and Rho GTPase-mediated positive feedback loop regulates neutrophil polarity,” *Nat Cell Biol*, vol. 4, no. 7. pp. 509–13, 2002. doi: 10.1038/ncb811.
[32] A. Millius and O. D. Weiner, “Chemotaxis in neutrophil-like HL-60 cells,” *Methods Mol Biol*, vol. 571. pp. 167–77, 2009. doi: 10.1007/978-1-60761-198-1_11.
[33] L. K. Fritz-Laylin, “Actin-based protrusions of migrating neutrophils are intrinsically lamellar and facilitate direction changes.” 2017.
[34] R. H. Insall and O. D. Weiner, “PIP3, PIP2, and cell movement–similar messages, different meanings?,” *Dev Cell*, vol. 1, no. 6. pp. 743–7, 2001. doi: 10.1016/s1534-5807(01)00086-7.

[35] D. A. Dorward, C. D. Lucas, G. B. Chapman, C. Haslett, K. Dhaliwal, and A. G. Rossi, “The role of formylated peptides and formyl peptide receptor 1 in governing neutrophil function during acute inflammation,” *Am J Pathol*, vol. 185, no. 5. pp. 1172–84, 2015. doi: 10.1016/j.ajpath.2015.01.020.
[36] B. Heit *et al.*, “PTEN functions to ‘prioritize’ chemotactic cues and prevent ‘distraction’ in migrating neutrophils,” *Nat Immunol*, vol. 9, no. 7. pp. 743–52, 2008. doi: 10.1038/ni.1623.
[37] D. Vesperini, G. Montalvo, B. Qu, and F. Lautenschl□ger, “Characterization of immune cell migration using microfabrication,” *Biophys Rev*, vol. 13, no. 2. pp. 185–202, 2021. doi: 10.1007/s12551-021-00787-9.
[38] Daniel Irimia and USPTO, “Microfluidic cell motility assay,” US8790891B2, Jul. 29, 2014
[39] B. I. Christopher Murphy and USPTO, “Method for assaying cell movement,” US8268614B2, Sep. 18, 2012
[40] Sigma Aldrich, “Millicell micro-Migration Assay Kit,” May 25, 2023.
[41] Cell BioLabs, “CytoSelect 96-Well Cell Migration Assay,” 2023.
[42] Svetlana O. Carrigan, Amy L. Weppler, Andrew C. Issekutz, Andrew W. Stadnyk, “Neutrophil differentiated HL-60 cells model Mac-1 (CD11b/CD18)-independent neutrophil transepithelial migration,” *Immunology*, vol. 115, no. 1, pp. 108–117, 2005, doi: https://doi.org/10.1111%2Fj.1365-2567.2005.02131.x.
[43] A. Zainol, M. Rohani Jais, E. Nur Ismail, and R. Ishak, “The role of calpain enzyme on neutrophils chemotaxis ability,” *Pharm. Sci. Asia*, vol. 48, no. 1, pp. 46–51, 2021, doi: 10.29090/psa.2021.01.19.064.
[44] Jessica Indrigo, Robert L. Hunter Jr, Jeffrey K. Actor, “Cord factor trehalose 6,6′-dimycolate (TDM) mediates trafficking events during mycobacterial infection of murine macrophages,” *Microbiology Society*, vol. 149, no. 8, 2003, doi: https://doi.org/10.1099/mic.0.26226-0.
[45] Wook-Bin Lee, Ji-Jing Yan, Ji-Seon Kang, Seok Chung, Lark Kyun Kim, “Mycobacterial cord factor enhances migration of neutrophil-like HL-60 cells by prolonging AKT phosphorylation,” *Microbiology and Immunology*, vol. 61, no. 12, 2017, doi: https://doi.org/10.1111/1348-0421.12544.
[46] Sampa Sarkar Sampa Sarkar School of Science, STEM College, RMIT University, Melbourne 3001, Victoria, Australia More by Sampa Sarkar Orcidhttps://orcid.org/0000-0003-3265-4546 , Abhishek Mishra, Selvakannan Periasamy, Brendan Dyett, Prashant Dogra, Andrew S. Ball, Leslie Y. Yeo, Jacinta F. White, Zhihui Wang, Vittorio Cristini, Chinnaswamy Jagannath, Arshad Khan, Sarvesh K. Soni, Calum J. Drummond, and Charlotte E. Conn, “Prospective Subunit Nanovaccine against Mycobacterium tuberculosis Infection─Cubosome Lipid Nanocarriers of Cord Factor, Trehalose 6,6′ Dimycolate,” *ACS Applied Materials and Interfaces*, vol. 15, no. 23, 2023, doi: https://doi.org/10.1021/acsami.3c04063.
[47] M. Bubb, I. Spector, B. Beyer, and K. Fosen, “Effects of Jasplakinolide on the Kinetics of Actin Polymerization: AN EXPLANATION FOR CERTAIN IN VIVO OBSERVATIONS,” *J. Biol. Chem.*, vol. 275, no. 7, pp. 5163–5170, Feb. 2000.
[48] Fisher Scientific, “Cayman Chemical Jasplakinolide,” Jun. 23, 2023. https://www.fishersci.com/shop/products/jasplakinolide-50-ug/NC0502405
[49] S. C. Posey and B. Bierer, “Actin Stabilization by Jasplakinolide Enhances Apoptosis Induced by Cytokine Deprivation,” *J. Biol. Chem.*, vol. 274, no. 7, pp. 4259–4265, Feb. 1999.
[50] M. Amano, M. Nakayama, and K. Kaibuchi, “Rho-kinase/ROCK: A key regulator of the cytoskeleton and cell polarity,” *Cytoskeleton*, vol. 67, no. 9, pp. 545–554, Sep. 2010, doi: 10.1002/cm.20472.
[51] Z. Li *et al.*, “Regulation of PTEN by Rho small GTPases,” *Nat. Cell Biol.*, vol. 7, no. 4, pp. 399–404, Apr. 2005, doi: 10.1038/ncb1236.

[52] V. Kolsch and P. Charest, “The regulation of cell motility and chemotaxis by phospholipid signaling,” *J Cell Sci*, 2009, doi: 10.1242/jcs.023333.
[53] Y. Mazaki *et al.*, “Mitofusin 2 is involved in chemotaxis of neutrophil-like differentiated HL-60 cells,” *Biochem. Biophys. Res. Commun.*, vol. 513, no. 3, pp. 708–713, Jun. 2019, doi: 10.1016/j.bbrc.2019.04.037.
[54] S. A. Misek *et al.*, “Germline variation contributes to false negatives in CRISPR-based experiments with varying burden across ancestries,” Cancer Biology, preprint, Nov. 2022. doi: 10.1101/2022.11.18.517155.
[55]L. Pixu, H. Cheng, and T. Roberts, “Targeting the phosphoinositide 3-kinase (PI3K) pathway in caner.” Nat Rev Drug Discov, 2011. Accessed: Jun. 23, 2023. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3142564/
[56] Yu, Jiuhong, et al. “Both Platelet-Derived Growth Factor Receptor (PDGFR)-α and PDGFR-β Promote Murine Fibroblast Cell Migration.” Biochemical and Biophysical Research Communications, vol. 282, no. 3, 6 Apr. 2001, pp. 697–700, www.sciencedirect.com/science/article/pii/S0006291X01946224 https://doi.org/10.1006/bbrc.2001.4622. Accessed 26 Feb. 2024.
[57] “Addgene: pcDNA5FRT-EF-Pdgfralpha-EGFPN,” *www.addgene.org*. https://www.addgene.org/66787/ (accessed Mar. 04, 2024).
[58] Kundra, V., et al. “Regulation of Chemotaxis by the Platelet-Derived Growth Factor Receptor-Beta.” Nature, vol. 367, no. 6462, 3 Feb. 1994, pp. 474–476, pubmed.ncbi.nlm.nih.gov/8107807/, https://doi.org/10.1038/367474a0. Accessed 9 Dec. 2021.
[59] “Addgene: pcDNA5FRT-EF-Pdgfrbeta-EGFPN,” *www.addgene.org*. https://www.addgene.org/66790/ (accessed Mar. 04, 2024).
[60] Urciuolo F, Passariello R, Imparato G, Casale C, Netti PA. Bioengineered Wound Healing Skin Models: The Role of Immune Response and Endogenous ECM to Fully Replicate the Dynamic of Scar Tissue Formation In Vitro. Bioengineering (Basel). 2022;9(6):233. Published 2022 May 27. doi:10.3390/bioengineering9060233
[61] “Dictyostelium discoideum Raper - 201386 | ATCC,” *www.atcc.org*. https://www.atcc.org/products/201386 (accessed Mar. 04, 2024).
[62] Willard SS, Devreotes PN. Signaling pathways mediating chemotaxis in the social amoeba, Dictyostelium discoideum. Eur J Cell Biol. 2006;85(9-10):897-904. doi:10.1016/j.ejcb.2006.06.003
[63] “J774A.1 | ATCC,” *Atcc.org*, 2022. https://www.atcc.org/products/tib-67
[64] Green TD, Park J, Yin Q, et al. Directed migration of mouse macrophages in vitro involves myristoylated alanine-rich C-kinase substrate (MARCKS) protein. J Leukoc Biol. 2012;92(3):633-639. doi:10.1189/jlb.1211604
[65] “P388D1 [P388 D1] - CCL-46 | ATCC,” *www.atcc.org*. https://www.atcc.org/products/ccl-46
[66] Thermofisher, “LIPOFECTAMINE® LTX & PlusTM reagent,” Lipofectamine® LTX & PLUSTM Reagent, https://assets.thermofisher.com/TFS-Assets/LSG/manuals/LipofectamineLTX_PLUS_Reag_protocol.pdf (accessed Mar. 4, 2024).
[67] Terheggen P, Van Loveren H, Den Otter W. Macrophage-like tumor cells as tools to study chemoattractive activity. J Leukoc Biol. 1985;38(6):747-752. doi:10.1002/jlb.38.6.747
[68] O. T. Inc., “Mouse Xist activation kit by crispra,” OriGene Technologies Inc., https://www.origene.com/catalog/gene-expression/crispra-kits/ga213173/mouse-xist-activation-kit-by-crispra (accessed Mar. 3, 2024).

[69] “4 straight channels Chip - Mini luer,” Darwin Microfluidics, https://darwin-microfluidics.com/collections/microfluidic-flow-cells/products/4-straight-channels-chip-mini-luer?variant=8105942515757 (accessed Mar. 3, 2024).
[70] “Mouse EGFR cdna ORF clone, c-myc tag, MG51091-cm | sino biological,” Mouse EGFR cDNA Clones, https://www.sinobiological.com/cdna-clone/mouse-egfr-mg51091-cm (accessed Mar. 4, 2024).
[71] Bailly, M et al. “Epidermal growth factor receptor distribution during chemotactic responses.” Molecular biology of the cell vol. 11,11 (2000): 3873-83. doi:10.1091/mbc.11.11.3873
[72] J. Chernoff, “Faculty opinions recommendation of the par-TIAM1 complex controls persistent migration by stabilizing microtubule-dependent front-rear polarity.,” Faculty Opinions – Post-Publication Peer Review of the Biomedical Literature, Dec. 2007. doi:10.3410/f.1095909.553614
[73] Minard, M.E., Kim, LS., Price, J.E. et al. The Role of the Guanine Nucleotide Exchange Factor Tiam1 in Cellular Migration, Invasion, Adhesion and Tumor Progression. Breast Cancer Res Treat 84, 21–32 (2004). https://doi.org/10.1023/B:BREA.0000018421.31632.e6
[74] Zhu, Guang et al. “The prognostic value of Tiam1 correlates with its roles in epithelial-mesenchymal transition progression and angiogenesis in lung adenocarcinoma.” Cancer management and research vol. 11 1741-1752. 21 Feb. 2019, doi:10.2147/CMAR.S195093
[75] Addgene, “Tiam1 scfv [L130/1] (plasmid #190510),” Tiam1 scFv [L130/1], https://www.addgene.org/190510/ (accessed Mar. 3, 2024).
[76] Qiagen MiniPrep Spin Kit, https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/plasmid-dna/qiaprep-spin-miniprep-kit
[77] Abcam Chemotaxis Assay Kit, https://www.abcam.com/products/chip-kits/cell-migrationchemotaxis-assay-kit-96-well-8-m-ab235673.html
[78] Thermofisher Lipofectamine LTX with PLUS reagent, https://www.thermofisher.com/us/en/home/life-science/cell-culture/transfection/transfection-reagents/lipofectamine-ltx-plus-reagent.html

Appendix A-List of Acronyms

AAV - adeno-associated virus
ARDS - acute respiratory distress syndrome
ATRA - all-trans retinoic acid
ATP- adenosine triphosphate
CD - Cluster of Differentiation
cGMP - Cyclic guanosine monophosphate
CRISPR - clustered regularly interspaced short palindromic repeats
crRNA - CRISPR RNA
dbcAMP - bibutryl cyclic adenosine monophosphate
DMF - dimethylformamide

DMSO - dimethyl sulfoxide
DNA - deoxyribonucleic Acid
fMLP - N-formylmethionine-leucyl-phenylalanine
FPR - formyl peptide receptor
FPR1 - formyl-peptide-receptor
GDP - Guanosine diphosphate
GPCR - G-protein coupled receptors
GTP - Guanosine triphosphate
GTPase - guanine triphophatases
HL - human leukemia
HLA - human leukocyte antigen
iPSCs - induced pluripotent stem cells
IP- intellectual property
LTB4 - leukotriene B4
MF2-Mitofusin 2
MLC - myosin light chain
MLCK - myosin light chain kinase
PAM - protospacers adjacents motif
PI3K - phosphatidlylinositol 3-kinaxe
PtdIns(3,4,5)P3 - phosphatidylinositol-3,4,5-triphosphate
PtdIns(4,5)P2 - phosphatidylinositol-4,5-bisphosphate
PTEN - phosphatase and tensin homolog
RNA - ribonucleic Acid
ROS- reactive oxygen species
SNP - single nucleotide polymorphism
VitD3 - 25-diohydroxyvitamin D3
ALLN - N-acetyl-Leu-Leu-Nle-CHO
ALLM - N-acetyl-Leu-Leu-methioninal
PD 150606 - (*Z*)-3-(4-Iodophenyl)-2-mercapto-2-propenoic acid

Appendix B – Advisor Meeting Logs

Date of meeting: May 16, 2023
Location of meeting: Snell Engineering 342
Attendees: All of Team J + Prof. Jaeggli
Designated Secretary: Jon Kim

Minutes:

Jaeggli checked that:

- We have a sharing platform and communication method à Teams/SharePoint and group message
- We have citations manager à Endnote with Word

Going over Team Contract:

- Add to goals that we want measurements and data to support the conclusions we make
- Add section to address what happens when someone doesn't do their part of assignment
- Do not social loaf! LOL

Presentation Time—see Capstone Advisor Meeting #1 slides for in-depth information

Notes from introduction presentation:

- Think about what we want chemoattractant to be…probably going to use cAMP like the previous races did but need to contact other teams
- Need to consider how the cells fluoresce in the maze to be tracked during race
- Can we manipulate the HL60s to become IPSCs? How long can we culture them? Need to come up with rules

CDR Tips:

- Literature review
  - Whatever is important that a reader needs to know, enough information that a technically literate non-expert can understand the report
  - Will be iterative but start working on it now
- Some sections will be different than traditional groups
  - Current products + solutions should be discussing previous races
  - Products should focus on each previous team's cell line and what they specifically did to alter it
  - Try to find the most similar patents (3-4) to what we are trying to do
    - Essentially show that we will not have an issue with patent violations because we do not have an actual product
- Common mistakes
  - Running out of time! We all know working with cells can be time consuming and unpredictable
  - Need to be first group in the lab! We cannot wait to start wet lab work half-way through capstone. Get in lab ASAP
  - If we have high attention to detail we will make the project much easier
  - Plan wet lab work. Lab work needs scheduling with TA, ordering ALL materials. Try to think many steps ahead

Design requirements:

- CDR V0 doesn't require ANY design requirements!! But we should work on them anyway
- Need to be demonstrable, cannot be vague,
- Try to quantify whenever possible
  - If we don't know what number to use, put in X and continue knowing that we need to figure out this value (ex. cells should be X viability, the cells shall complete the maze in X amount of time)
- Need clear and concise method verification section to ensure requirement isn't so vague that it can't be verified.
  - Ex) chair for space shuttle
  - Ask ourselves "Can this clearly be verified"
- Be liberal with these requirements at first! The more the better and Jaeggli will shoot them down if they're dumb
- Budget is $1500!!! This is a requirement. Need to think about (or ask someone) what we already have/what we need to order (media, FBS, PBS, etc)

*DO NOT start thinking about solutions yet! We need to focus on requirements and background stuff
*Best way to reach Jaeggli: Teamsà use chat for the recurring meetings

Action items:

- Need to contact other HL60 team and write up some rules! Send them an email asking (what is chemoattractant, what maze should we order, what are any rules, discuss WT cell line for control group in the race)
- Need to pick a Maze because there is no Maze team! Talk to Prof. Markwich she has some ideas
- Start literature review!
- Think about design requirements (these are not written in stone!)—we cannot start making decisions without these

BY NEXT WEEK FOR JAEGGLI: agreed upon rules, maze, preliminary design requirements

Capstone Meeting Minutes Approval Received from each attendee:

Name	Approved (e.g. email, in person)	Approval Date
Jon Kim	In person	May 16, 2023

Jacob Miller	In person	May 16, 2023
Chris Schmidt	In person	May 16, 2023
Victoria Rivera	In person	May 16, 2023
Harris Goodwin	In person	May 16, 2023
Keshav Kotteswaran	Virtual	May 16, 2023

Date of meeting: May 23, 2023
Location of meeting: Snell Engineering 342
Attendees: All of Team J + Prof. Jaeggli
Designated Secretary: Harris Goodwin

Minutes

- keep up with excel sheet of to do list
- Get to choose chemoattractant that we want, same maze for both teams though
- Have agenda for Thursday meeting with the other team
- Need to do a lot of background research about HL60 cells
    - Growth rate
    - Characteristics
    - High density vs low density properties
    - What HL60 cells express to make them distinctly HL60
- Look into contacting Mass General to make mazes for us
    - If not, just buy a maze
- 4-5 design requirements are there for this just edit and specify them
- ****For background research, we must know signaling pathways and receptors and how they move and mechanisms and everything about these cells****
- An enormous amount of background to write, tons of research
- How the race works

- How cells move
- How they respond to the chemoattractant
- Knockout could be easier than a full-on modification of the genome
- In CDR, “products section” refers to the previous solutions by the teams of the Dicty race
- Work with SILS, basement of ISEC has microscopy lab, tons of microscopes
- For lit review, summaries should be in writing, but tables and figures are helpful as well

Design Requirements

- Visible/trackable are two different things, pick only one thing for design requirements (either break into 2 design requirements, or be more specific)
    - Visible is too vague, trackable could be clearer (position/coordinate system x-y-z)
    - “the cells” is too vague, specific cells or clusters of cells
- Viability/passaging/concentration are expected not a design requirement
    - This is part of protocol for culturing cells
- Produce 20,000 cells for the maze run (instead of viability)
- Don’t need a lot of design requirements
    - Quality over quantity
    - Add design requirements as we learn more about the cells and decide what we do with them
- Speed and correct turns for chemoattractant is 90% of our design requirements
    - For speed requirement, faster than the wild type (specific number)
    - “Correct turn” design requirement is way too vague and the point of our Capstone
- “Traveling through the maze faster than wild type” is too vague, no units or demonstrable number to verify
- Chemoattractant is not a part of design decision (a bit of a grey area)

- Don’t delete, but not a requirement for solution

To do:

- Tons of background research
  - How the race works
  - How cells move
  - How they respond to the chemoattractant
  - What their mechanisms are
  - Methods of modifying cells (different viral vectors and CRISPR techniques)
- Would be funny to reprogram into pluripotent stem cells or the “Usain bolt of cells” lol
- Viral vectors are a reasonable pathway most likely
- Have a section of common gene editing techniques
- Section on commonly targeted proteins
- Agree on rules with other team
- Write a VERY detailed lit review
- Send out CDR draft Monday night in teams meeting not email

BY NEXT WEEK FOR JAEGGLI: Practice presentation, need a really strong background section of lit review (CDR v0 draft), we don’t need whole CDR draft but need a strong lit review section to show to Jaeggli on May 30th

Capstone Meeting Minutes Approval Received from each attendee:

Name	Approved (e.g. email, in person)	Approval Date
Jon Kim	In person	May 24, 2023
Jacob Miller	In person	May 24, 2023
Chris Schmidt	In person	May 24, 2023

Victoria Rivera	In person	May 24, 2023
Harris Goodwin	In person	May 23, 2023
Keshav Kotteswaran	Virtual	May 24, 2023

Date of meeting: May 30, 2023
Location of meeting: Snell Engineering 342
Attendees: All of Team J + Prof. Jaeggli
Designated Secretary: Harris Goodwin

Notes

- Look into YAP/TAZ pathway
- Clear up title, focus on made aspect as well since that is the graphic
- IF we must cut something, instead of axing something, take a little part of this or that out
    - start by simplifying slides before nuking a slide
- Maybe add supplemental slides?
    - if we did not have time to include something
    - if we are anticipating a specific question that a material would help us answer
- Edits of title slide must make both beginning and end
- Answer questions by making a two-way communication
- Leave sections empty for CDR that we are not doing for CDR v0, empty sections won't bother Jaeggli
- Common mistakes for answering questions
    - give mini presentation on area the question was asked
    - not directly answering the question
    - NEVER ask "did that answer your question"
    - if we get a question that blindsides us, we can say "that is a great question, and we did not look into that properly so we will for our next presentation!"
        - we get to use this ONCE, no more

Presentation feedback by slide

1st slide

- little wordy but not bad, capitalize the first word of the bullets
- List 1-2 most important findings that came from HL60 research, or HOW many people use these
    - some metrics that this is not a niche cell line

2nd slide

- picture of cellular migration or chemotaxis, visual for these properties
- maybe split this into 3 slides, first 2 bullets, then slide on cellular migration, then slide on chemotaxis (ALL WITH VISUAL AIDS)

3rd slide

- be more specific
- maybe two examples of why cell migration is essential for survival
- add direct evidence that you could cite a journal reference

4th slide

no changes (good job Chris!)

5th slide

- Do we need to get into decisions we made with the other group?

6th slide

- video is short and frame rate is bad, YouTube pops up video recommendation, but idea of doing a video is good
- try getting to a format that can imbed directly into PowerPoint
- have video loop a few times
- figure on right is too small to read, edit to pull out the important stuff
- perhaps the results chart from the website?

7th slide

- make sure to address the figures, talk about the xy coordinate system and what this means
- make sure to have a very clear idea of why the figures are there and talk through them
- work on how to communicate what figures are showing

8th slide

- good slide, go through faster maybe (good job Victoria!)

- maybe cut text a bit
- YAP/TAZ pathway (response to intracellular stresses)

9th slide

- Describe the photo or figure, directly reference the image
- quantify the cells (x % of cells)
- talk about chemoattractant in higher level of understanding terms (more technical info/terms)

10th slide

- method of verification needs work
- do not “attempt” we must verify
- use similar methodology to what they did to track and quote what they did
- requirement is too vague, more clarification
- could divide maze into grid to say which zone each cell is in? Or pixels?

11th slide

good Gantt chart

12th slide

- could be nice to know what came up the Dicty race
  - have definitive takeaways from the Dicty race
- don't bother mentioning Dirty chemotactic advantage
- add something that is more specific to our project
- looking to find a novel way to complete the maze
- reduce text and add visual aid

BY NEXT WEEK FOR JAEGGLI: maybe come up with 4-5 VERY preliminary targets/modifications to the cells we can discuss / no bad ideas for brainstorming / Just generate ideas, be positive and don’t really shoot down ideas / results of a brainstorming session for cell modifications that could be useful

Capstone Meeting Minutes Approval Received from each attendee:

Name	Approved (e.g. email, in person)	Approval Date

Jon Kim	In person	June 2, 2023
Jacob Miller	In person	May 30, 2023
Chris Schmidt	In person	May 30, 2023
Victoria Rivera	In person	May 30, 2023
Harris Goodwin	In person	May 30, 2023
Keshav Kotteswaran	Virtual	

Date of meeting: June 6, 2023

Location of meeting: Snell Engineering 342

Attendees: All of Team J + Prof. Jaeggli

Designated Secretary: Keshav Kotteswaran

Notes:

Focused on talking about trade studies: Parameters, metrics and scales

Hierarchy of decisions: TBD

Main decisions: what we're targeting in the cell, how the modification is delivered

- Yap Taz:
  - High stiffness ---> Yap-Taz: how would it affect motion: would it make it slower?
  - Do we want to increase or decrease focal adhesions?
  - Yap-Taz---> need more information on impact
- Calpain inhibition:
  - Promising due to increased polarity and more pseudopod
  - CID-TAT: How can we get it?
    - § Does it use HIV as a viral vector?
- Promote Actin Polymerization
  - Use different proteins from previous dicty world race
  - General idea of promoting F actin

- Increase expresssion of formyl peptide receptor
  - Good idea to combine with another idea
  - Is there a significant depletion?
  - Is it a cytosolic target or does it translocate into the nucleus?
    - § How fast is the process?
  - Recruits actin toward where it binds chemoattractant
    - § More available FPR would make it have a higher spacial awareness?
      - Better directionality so it can be more responsive from wherever
- TDT and AKT
  - Try to bin brainstorms into broad categories and proceed with trade study
- PI3K
  - Involved in the FPR cascade
    - § Could be one of the target solutions
- PTEN
  - Helps in directional motion could possibly help with overexpression
  - How does it localize to the trailing edge?
- LPS
  - For macrophages?
    - § Is there a cell phenotype that is more sensitive to chemoattract and if so why?
  - Neutrophils: different phenotypes of location based or epigenetic changes?
    - § Does signaling in certain parts of the body affect plastic or more permanent change?
- Mitofusion 2
  - If we overexpress, do we get an energetic advantage?
  - Promising idea
  - Targeting cells metabolism --> new mechanism
- Rho family GTPases
  - Promising
- IL8 stimulation
  - Solutions to avoid
- Reprogram to IPSC
  - Go to ipsc or naïve ipsc but what would be a better phenotype?
  - What is a better lineage than HL-60
- Shrinking cells
  - Risky but easy to try
- Warm the cells
  - Heat shock proteins
  - How do they respond and can we down regulate some heat shock proteins
- Train the cells

- Epigenetic changes to cells are heritable
- Produce a lineage
  - § Natural selection?
  - § Enrich culture for successful cells

· Trade study spreadsheet
- Add something that incorporates risk/uncertainty (feasibility)

Good groundwork

For next week we have to put all these ideas into general boxes of solutions. Train across metrics and pick some of the most promising ones. Rate all of these and see if there are any possible combinations. Consider practical solutions and risk/time/reliability. Must have trade studies done on these ideas next week.

Capstone Meeting Minutes Approval Received from each attendee:

Name	Approved (e.g. email, in person)	Approval Date
Jon Kim		
Jacob Miller	In Person	June 6, 2023
Chris Schmidt	In person	June 6, 2023
Victoria Rivera	In person	June 6, 2023
Harris Goodwin	In person	June 6, 2023
Keshav Kotteswaran	In person	June 6, 2023
Mike Jaeggli		

Date of meeting: June 13, 2023

Location of meeting: Snell Engineering 342

Attendees: All of Team J + Prof. Jaeggli

Designated Secretary: Keshav Kotteswaran

Notes:

Trade Studies:

Weighting determined by averaged rankings

Feasibility vs time to run in lab? What is the difference?

Feasibility: Can you do it?

- Should take into account time and cost

Efficiency should be higher

- Might force us into an easier thing that is less effective

Safety(to people) may not need to be on the trade

- If its not safe we can't do it anyways
- Reallocate into other categories (like effectiveness)

Safety how confident are we in getting protocol improved

Time and feasibility good to have highly rated

Estimated time for wet lab work should be multiplied by 3

Want to be very sure we can get it done in the time we have

Heating the cells

- Why is effectiveness a 3?
- Heated neutrophils showed higher migration speeds
  - Heat whole maze if possible?

Natural selection

- Error bars on trade study numbers
- Time required pushing the score down
  - How long does it take to run maze, grow winners up, etc.

In CDR:
describe each criterion and what each level of 1-5 means

Explain why each score was given to solution with references.

Which scores/solutions are we most confident in?

Explanation of each solution

Easy way to test methods without maze?

- Assays are expensive

Original maze potentially available from MGH

- How many could/should we ask for?
- At least 2 for final
- How many for testing?
- Compare to data from original races

Zoom call with Dr. Irimia

- Very well planned, thought out questions
- Don't waste their time
- Ask about HL60 cells

Gentleman's agreement with other team about presentations and solutions?

- Don't look at other team for inspiration

Makes sense that heat cells won

- Not mutually exclusive with other solutions

Chemotaxis trade

- Price, time, effectiveness up in the air
- Have potential to be very good but are probably less feasible in the time we have
- Look for a quote for cost of transfection kits

5 should be accompanied by reference with strong support

Polarization trade study

- Actin and calpain solutions possible without genetic modification
- Calpain seems very feasible, safe for cells
- PTEN has good source for 5 in effectiveness
    - Requires genetic modification

Genetic modification is feasible

- More difficult but has been done before

Could be smart to do 1 or 2 safe options and one more ambitious.

Combination of different solutions may be a good option too

Come up with 1 or 2 easy options and 1 or 2 hard options to focus on.

Easy options: short to implement, can work on any cell line, available to fall back on if needed

Hard options: high potential but not guaranteed to work

What solution are we most confident would work well if it worked?

- Gold star

Go for the gold star option and a few highly feasible options

Lentivirus is standard protocol

- Good chance at success if we learn protocols

Add into CDR

- Including background information on each solution/pathway

Presentation practice for next meeting

Capstone Meeting Minutes Approval Received from each attendee:

Name	Approved (e.g. email, in person)	Approval Date
Jon Kim		
Jacob Miller	In person	June 13, 2023
Chris Schmidt	In person	June 13, 2023
Victoria Rivera	In person	June 13, 2023
Harris Goodwin	In person	June 13, 2023
Keshav Kotteswaran	In person	June 13, 2023
Mike Jaeggli		

Date of meeting: 6/20/23

Location of meeting: 342 Snell Engineering
Attendees: Harris Goodwin, Jacob Miller, Jonathan Kim, Victoria Rivera, Keshav Kotteswaran, Christopher Schmidt
Designated Secretary: Christopher Schmidt, Victoria Rivera

Notes:

Presentation Suggestions

Trade study chart slide

- Tell more about the proposed solutions
- What is shown right now is more of a set of trade study summaries
- Provide a slide where information is put forward to show that there is more in the trade studies and that the ones we provided were the winners We had trades on the physical
- Strategies looked at for physical – lsit them with references, don't jhave to trade them
- Before getting into numbers, explain what PTEN is ,how would we overexpress it, what would calpain inhibition do
- Several more slides are needed to give more information on the numbers in the trade study
- Do not have to talk about some criterion like cell safety
- Idea: Here are the candidates we will talk about from here this should lead into the trade study

- Provide background on what is going on with the methods listed in the trade study table, provide high level knowledge on the mechanisms, do not have to thoroughly explain a signaling pathway

- Go into a slide that briefly tells the winners that we have selected for engineering methods, then go into the trade study, then put in a slide for all methods we are considering (the supplemental slide)

- What we are trying to target with this presentation: provide the audience with the sense that we have done a lot of research, thought about many solutions, and have decided to proceed with the one that is going to be the most successful, did we give enough information when discussing our design requirements and solutions – cut more of the earlier stuff and less of the design solution stuff

Impacts slide

- Move through this slide faster
- Take 2 or 3 bullet points off for positive and negative and talk about them in more detail

Background

- Don't cut, but talk about in less detail

Prior art

- Go quicker through prior art
- Do not remove information, but do not talk about it in quite as much detail
- Reflection to think about when modifying this slide: what information does this slide have to convey anything that doesn't make the cut does not have to be talked about as much

Slide beginning with "Approaches from teams in past races…"

- Talk more about the figure, axes can't be seen too well
- Ex: 2nd place is here, 3rd place is here highlight the cell teams that did well

Knowledge Gap Slides

- Retain what's being communicated and do it more quickly
- More emphasis needs to be placed on the second half of the presentation

Design Requirement Slides

- Get the information across in a quicker amount of time
- More clarity on the images provided, explain the color scheme
- Tell more about the picture, reference it more when explaining this slide

Gantt chart slide

- This was good

Presentation Time: 16 minutes

CDR Suggestions

CDR – Trade Study

- For each trade study – provide a background/mini abstract of the decision making
- Since the same criteria are used for each trade study have one section that will tell what the criteria are, what the scale is (this is a 1 this is a 3), then go into the trade studies
- Mention why a solution got X score for every criteria

Impacts

- Unlimited room for impacts in the CDR, narrow them down in the presentation

Approval Received from each attendee:
Name: Approved: (**e.g. by email**) Approval Date:

Capstone Meeting Minutes Approval Received from each attendee:

Name	Approved (e.g. email, in person)	Approval Date
Jon Kim	In person	June 20, 2023
Jacob Miller	In person	June 20, 2023
Chris Schmidt	In person	June 20, 2023
Victoria Rivera	In person	June 20, 2023
Harris Goodwin		
Keshav Kotteswaran		
Mike Jaeggli		

Date of meeting: January 17, 2024
Location of meeting: Microsoft Teams
Attendees: All of Team J + Narges
Designated Secretary: Harris Goodwin

- Biosafety paperwork
  - Plan for changing/submitting
- Potential alternate endings for our project
  - Collaborating with a lab on campus that specializes in microfluidic mazes
  - Conducted a simpler chemotaxis/mobility test
  - Freezing down the cells to be tested by a future capstone group

- How should we be approaching the CDR and changing to using a mouse model?
    - Adding sections to discuss the transition to a mouse model but not redoing research from the beginning
- Which cell line should we use going forwards?
    - J774A.1 suggested
        - Concerned with adherent nature
- Overall timeline for our project given setbacks
    - Go over what was talked about with Lannin and Kathryn

Date of meeting: January 24, 2024
Location of meeting: Microsoft Teams
Attendees: All of Team J + Narges
Designated Secretary: Jon Kim

Minutes

Summary of state of the project:

- We were emulating the Dicty World Race, planning to modify HL60 cells in some way to navigate through a maze
- We did background research on HL60 cell motility and we came up with potential solutions to increasing cell speed and navigational efficiency
- We had a plan to work with a researcher at MGH who ran the initial race to obtain mazes from him and learn how to measure the cell speed through the maze, BUT this fell through and now we have no selected maze for a motility assay
- Come January 2024, biosafety paperwork never accepted, told we need to switch cell lines to something less biohazardous
- New mouse cell line proposed by BioE dept. is adherent which would not work with maze so we need to define new end goals for our Capstone project

The Maze

- We need to look at commercial kits, find a lab working with similar devices, look into different assays
- Narges under the impression that the maze is a 3-D printed device made of biomaterials containing fMLP bound to the maze for the cell to react with
- The original maze however used a soluble fMLP gradient and had the molecules diffuse from the end of the maze to the beginning of the maze

Presentation

- We need to present to the other groups and advisors that we have researched a specific pathway and how we intend to modify it and what the subsequent effect will be

- For presentation 2 tell everyone: we want to do this, we have researched it with preliminary data, this is how were gonna do it, this is what it's going to show

CDR

- It's totally ok in our CDR to say we planned to do X,Y,Z BUT now we have to change our plans and do something different. Narges is super flexible with this

Next week let's meet in person! Try BCC but if not look at another room Narges might be able to book

"No one be stressed at all we are all just learning." – Narges

ACTION ITEMS:

- Narges to ask labmate about migration assays? And if they worked?
- BY FRIDAY Team J to explore potential mazes/assays we could buy commercially to run our cells through after modification and share findings with Narges
- BY FRIDAY Team J to explore new cell lines and share findings with Narges
  - Try to see if mouse neutrophils are an option or a similarly differentiated cell type
  - Look for cells with similar properties to HL60, even though initial research by Narges and team J found no similar mouse cell
  - Look at other species too!
  - See what cells other studies are using and find out if we can order them commercially to use
  - Once we find a cell line TRY TO NARROW down to **3 factors** we can modify
- BY NEXT MEETING Team J: WHAT QUESTION DO WE WANT TO ANSWER
  - Create ~ 1 page description of the question we want to answer with our project with ~2 subaims
  - What is our goal? What do we expect to see? How do we want to do it?
- Team J to start a Teams chat with Narges to share our findings more informally and quickly

Date of meeting: January 31, 2024

Location of meeting: BCC

Attendees: All of Team J + Narges

Designated Secretary: Harris Goodwin

Minutes

- Contact technical services of the vendors and describe what we are doing to get them to help
    - "We were going to use HL60 cells but cannot and are looking for a very similar cell line as a replacement"
    - Thermo and ATCC
- Ask the 'leader?' Of the project about cell line?
- Check the chat, Narges sent information about different cell lines and reagents necessary for it
- Better to pitch the budget to BioE, and then ask about them buying the maze/chemotaxis assay
- Be sure to be in lots of contact with Team E to split costs on things (cells, chemotaxis assay, reagents)
    - Maybe set up regular meeting with them until we decide crucial details like cell line, main aim of capstone, reagents, and assays
- Narges asked other team to have weekly plan of key decisions and progress
- Have a map of weeks and due dates for the semester (rough feb plan below)
    - Helps Narges keep us on schedule and gives us a chance to check in with her before due dates
- February
    - Exact protocol in excruciating detail
        - Give folder access to Narges so she can review protocols
    - Presentation
    - Biosafety paperwork submitted
    - Ordering reagents, cells, assays
- Include cells, reagents, media, assays, into budget
- What type of consumables are we provided?
    - Shake flasks
    - Pipettes
- Ask ChatGPT about cells
- BioE was supposed to buy the maze, so if we are not using a maze, they should provide the chemotaxis assay or whatever key assay
- Abcam migration kit seems to be good, teaching lab has fluorescence plate reader
- **We can ALWAYS change our aims, not set in stone**
- Fibroblasts as cell type?
    - Propose the fibroblast idea to team E and see what they think
    - Based on growth factors, adherent cell line
- **An aim that is easier for us an achievable within the time and scope of capstone is most important**
    - It is okay if we do something differently than Team E
    - Pick something that is achievable within capstone
    - We have some freedom to choose what cell line we want to do, what the aim of it is, and how we will prove it
    - Just be transparent to Narges about changes in aims and processes
- **A new proposal**

        - Fibroblast cell type --> research proteins to overexpress or knockout --> buy fibroblast migration assay --> compare our edited cells to wild type as result
        - Just make sure to have something new or innovative that shows we did not just copy another study
        - Have a rough idea or draft of this proposal of Fibroblasts for Team E Friday
    - Protocols
        - Buy reagents or assays with somewhat established protocols (Thermo is great)
        - Our protocol should just be a combination and refined/detailed versions of protocols written by vendors

Main Aims document

- What protein specifically?
    - Even without cell line chosen, have exact proteins chosen
- Specific aim of expression of protein, how we will prove specifically?
    - What assays or techniques to prove it? (western blot, flow etc.)
- Just the expression of protein? Or the genetic improvement of the whole system?
- Developing code or getting code online of how to measure speed of cells (chatgpt)
    - Do we have the capability to execute this in the first place?
    - Requires long block of time for timelapse images
- Main aim doc is a good first draft, just needs to be more specific
    - What are the proteins? How do we effectively intergrate these proteins into the cells?
    - How do we specifically want to measure this expression or genetic changes?
    - How do we execute chemotaxis assay? Other assays?

Date of meeting: February 14, 2024
Location of meeting: BCC
Attendees: All of Team J + Narges
Designated Secretary: Keshav Kotteswaran

Minutes

Summary of state of the project:

- Should get feedback on safety form submission around or before the 28th
- Can't buy any materials until approval but can create cards before hand
- Same applies to protocols until paperwork approval
- Main goals for current state of project:
    - Making sure we're on the same page as the other team (assays etc.)
    - Choosing target gene based on research
- Budget updated based on current goals
    - Adjust based on materials we can use in teaching lab ex: shake flasks
    - Many plasmids listed but will select one or two for experiment

    - Chemotaxis Assay: Discussed that this would be an acceptable overflow part of the budget
- Focused on using plate reader for determining cell movement (teaching lab)
    - Can we use chemotaxis plate on plate reader, or do we need to take cells out? (would need to use secondary plate incase)
        - After assay is run need to take cells out and dye cells for plate reader
            - No antibodies
    - Materials required but not supplied?
        - Double check with Sage material compatibility: (GFP based)
            - Do we need black plates?
    - Goal to finalize small details / which factor we want to focus on
- Better to stick to the budget as much as we can due to possible delays with going over
    - Preset budget so more signatures/etc. Needed
- For timeline look for plasmid to not buy bacteria prep kits
    - Needs to be an approved seller and same targets we have
- Check on if we need to start before or after spring break with the other team

<u>Presentation:</u>

- How do we go about talking about deviation/other obstacles
- Explain capstone 1/ how we changed and why
- Presentation 2 still requires all previous aspects of previous presentations
    - Proof of work/concept
        - Describe why we can't start
        - Why lab work isn't underway
- "By the way" slide at beginning of presentation for context.
- Title slide
    - Old title with a cross through
- Plan to be ready with a draft for next week
- Review and go over first draft at next week's meeting
- Presentation 2 split into 6 sections
    - Title Intro: Jacob
    - Motivation: Jon
    - Prior Art: Keshav
    - Design Req: Chris
    - Design considered: Harris
        - New trade studies
            - Need to edit trade studies for new targets
    - Testing: Victoria

<u>Main Goals for next meeting:</u>

- Finalized targets & budget
- Draft of Presentation 2
- Draft protocols for:
    - Cell culture

    - Bacterial culture
    - Plasmid Isolation
- Talk about how we update CDR
    - Start the preliminarily work
- Meet Monday evening (around 5pm) to go over where we are at

Date of meeting: February 21, 2024
Location of meeting: BCC
Attendees: All of Team J + Narges
Designated Secretary: Christopher Schmidt

Minutes

-Protocols started
-Budget finalized with agreed upon chemotaxis assay
-Need to add methods trade study to supplementary slides

Review current state of presentation- each discuss the slides we contributed to
-have to keep sentence titles
-project shifted to nih 3t3 cells due to safety issues
-background of fibroblasts, migration to wound sites,
-add space between text on migration slide to make it easier to read
-how fibroblasts move along gradients into the wound site, PDGF as a chemoattractant, forms focal adhesion and contracts at the rear edge to "walk" towards the site

-why do we care about migration? Tissue repair, old age causes dysfunction
-add space on this slide as well
-patent review- font change to make it consistent
-prior art- discuss graphene movement study,
-knowledge gap- original HL60→ nih cells

-make sure bolding is consistent as well

-trade studies: mention all of them but only show cell type and target in main presentation
-cell trade study NIH was the cheapest and easiest in terms of paperwork
-categories explained for target trade study:
-trade study needs to be finished for scores, we want to use PDGFRbeta as a final solution

-design implementation: protocol description
-need to cut down text, use animation to display lines individually, helps with audience engagement

-images connected with arrows to show protocol process
-using microscope to image cells and verify transfection
    -smaller arrows are not as effective for directing information
    -could cut the count tool image
    -make font and format exact across all slides

-advantages of new assay and design properties
-fluorescence readout→ standard curve → cell count→ t-test
    -proportion test instead of t-test if using %s (z-test)
    -need to figure out sample count to determine which test to run(z>30 samples)

-Gantt chart: summer and spring condensed, timeline of transition laid out
-move CDR back and move NIH research up
-summary slide: add bullet for lab work impacts
-make sentence titles as short as possible

Give Narges at least a day to review for last-minute changes- send her version we submit on Monday
Work on CDR and protocols

Present targets as is- if really pressed we can say we had limited time

Capstone Design Meeting Minutes Document

Project Title: Former HL60 Cell Race

Student Team Members: Jon Kim, Jacob Miller, Chris Schmidt, Victoria Rivera, Keshav Kotteswaran, Harris Goodwin

Sponsor: NA

Faculty Advisor: Narges Yazdani

Date of meeting: February 21, 2024

Location of meeting: BCC

Attendees: All of Team J + Narges

Designated Secretary: Keshav Kotteswaran

Minutes

-Protocols started

-Budget finalized with agreed upon chemotaxis assay

-Need to add methods trade study to supplementary slides

Review current state of presentation- each discuss the slides we contributed to

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-project shifted to nih 3t3 cells due to safety issues

-background of fibroblasts, migration to wound sites,

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Capstone Design Meeting Minutes Document

Project Title: Former HL60 Cell Race

Student Team Members: Jon Kim, Jacob Miller, Chris Schmidt, Victoria Rivera, Keshav Kotteswaran, Harris Goodwin

Sponsor: NA

Faculty Advisor: Narges Yazdani

Date of meeting: March 13, 2024

Location of meeting: Online Via Teams

Attendees: All of Team J + Narges

Designated Secretary: Keshav Kotteswaran

Minutes

Summary of state of the project:

- Approved Biosafety paperwork, working on submitting Trello orders to get in lab
- Would like to go to lab and get familiarized before materials arrive
- Working on getting our protocols approved by Sage etc.
    - Need to submit them soon and get into lab to get familiarized
- Waiting for figuring out how shared supplies will go
    - Potentially have one team order all the shared supplies if possible
    - Split and share costs after arrival
- Need to wait for approvals and inform Narges before next steps
- Comments on revised version of CDR to go over
- No comments/grade from presentation yet
- Need to set meeting with other team today to decide on orders
    - Waiting until Friday could push back ordering to next week/ compound wait with shipping time
- Emphasis on minimal timing and getting into the lab soon to get comfortable

Capstone Design Meeting Minutes Document

Project Title: Former HL60 Cell Race

Student Team Members: Jon Kim, Jacob Miller, Chris Schmidt, Victoria Rivera, Keshav Kotteswaran, Harris Goodwin

Sponsor: NA

Faculty Advisor: Narges Yazdani

Date of meeting: March 20, 2024

Location of meeting: BCC

Attendees: All of Team J + Narges

Designated Secretary: Harris Goodwin

Minutes

- Meet ahead of time for experiments
    - Schedule TA times week before and make sure people are available
- ETA of materials
    - PDGF-AA will take 21 business days to arrive, scheduling it for April 15 arrival
        - Backup plans include using FBS or just the migration inducer provided in the chemotaxis assay
- We can do bacterial culture and extract plasmids and culture cells
- Ensure that everything is ready to be loaded into the assay the day PDGF arrives
    - Plasmids are isolated and cells are transfected, cultured and ready to be loaded into the assay
- Maybe connect with the reps of the vendor to inquire about expedited delivery
- Don't worry about the grade, give it the best effort and that is all that matters
- Be proud of the project and the work we have done and be able to talk about it
    - Lots of stories we can tell regarding it, try to get a result to discuss
- Lipofection in Narges's experience is easy, just follow the protocol
- It took her to about a week
- Transfection optimizations?

    - Narges always chooses the middle point of given ranges
    - Middle of the range tends to work for most systems and is a good starting point
- Narges suggested transfection parameters
    - DNA of 750ng
    - 3.5uL of Lipofectamine LTX
    - 1uL of PLUS reagent
- Use amount to be able to have extra, always have a backup plan, don't use all the reagent for one run
- Start plasmid isolation early, as soon as we can
- Always plan for repetition of experiments and have a plan B

Comments about the experiment timeline

- Bacterial culture/plasmid isolation
    - Nanodrop for measuring concentration of RNA
    - Existing protocol for nanodrop plates
    - Ask Sage or Kathryn about how to determine DNA concentration
    - If there is not a method to determine DNA concentration, some labs (Sara Rouhanifard) could be of assistance
    - Check to make sure the software has the protocol on it
- Lipofection
    - Get to 50-80% confluency
    - Cells express the protein the plasmid is transfecting
    - 6-well plate makes most since if it's feasible
        - Make sure to have enough media, transfection reagents, pDNA etc.
- Chemotaxis assay
    - How to make standard curve
    - Make sure to know if they include the standards
    - Since the plate for making a standard curve is a different plate than the chemotaxis assay plate, we do not need to generate the standard curve instantantly
- Don't overload days with experiments, could get overwhelming
- If the estimation for an experiment is 2 hours, budget 4 hours for being in lab
    - This means when planning experiments (if some are done same day)
    - Booking TAs for supervision
- Could do standard curve on a day where we are only passaging cells
- Have maximum one experiment planned for a day

Project Title: Former HL60 Cell Race

Student Team Members: Jon Kim, Jacob Miller, Chris Schmidt, Victoria Rivera, Keshav Kotteswaran, Harris Goodwin

Sponsor: NA

Faculty Advisor: Narges Yazdani

Date of meeting: March 27, 2024

Location of meeting: BCC

Attendees: All of Team J + Narges

Designated Secretary: Jon Kim

Minutes

- Working on the comments on the protocols
- Cell culture protocol
    - Need to fix order of cell counting and spinning down cells
    - Need to work out cell counting
        - Count cells in four corners, average them (sum and divide by four) multiply by dilution factor (2x), multiply by 10^4
- Try to expand cells on saturday and harvest plasmid
- Narges suggests one group work on cells and the other group work on bacteria to avoid contamination

Capstone Design Meeting Minutes Document

Project Title: Former HL60 Cell Race

Student Team Members: Jon Kim, Jacob Miller, Chris Schmidt, Victoria Rivera, Keshav Kotteswaran, Harris Goodwin

Sponsor: NA

Faculty Advisor: Narges Yazdani

Date of meeting: April 5, 2024

Location of meeting: BCC

Attendees: All of Team J + Narges

Designated Secretary:

Minutes

- Current schedule slated to finish on next Wednesday
- Try to talk to Saige and see flexibility on working more on Saturday
- Try to negotiate poster deadline as well if things don't go as expected

Appendix C – Bill of Materials/Budget

Item Name	Base Cost	Tax	Shipping Cost	Total Cost	Vendor	Preferred Ve	American Express?	Product Link
QIAprep Spin Miniprep Kit	$125.00	17.72	$0**	$142.72	QIAgen	No	Yes	https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/pl
LB Broth, 2x 500mL	$73.30	$0(with lipo)	$0**	$73.30	Thermo Fisher	No	Yes	https://www.thermofisher.com/order/catalog/product/10855001
PDGFRalpha Plasmid	$85	TBD*	TBD*	$85.00	AddGene	No	Yes	https://www.addgene.org/66787/
PDGFRbeta plasmid	$85	TBD*	TBD*	$85.00	AddGene	No	Yes	https://www.addgene.org/66790/
Lipofectamine LTX Kit with PLUS, 0.3mL	$259.65	$43.97	$105	408.12	Thermo Fisher	No	Yes	https://www.thermofisher.com/order/catalog/product/15338030
Opti-MEM Reduced Serum Media	$64.65	$3.88	$0**	68.53	Thermo Fisher	No	Yes	https://www.thermofisher.com/order/catalog/product/31985070
Cell Culture Media(DMEM) 2x500mL	$113.30	$0(with lipo)	$0**	$113.30	Thermo Fisher	No	Yes	https://www.thermofisher.com/order/catalog/product/10564011
Chemotaxis Assay	$640	TBD*	TBD*	$640	Abcam	No	Yes	https://www.abcam.com/products/chip-kits/cell-migrationchemotaxis-assay-kit-96-well-8-m-ab235673.html
Hygromycin(select after transfection)	$132	$11.88	$58	201.88	Fisher Scientific	No	Yes	https://www.fishersci.com/shop/products/hygromycin-b-50-mg-ml-distilled-water-sterile-filtered/AAJ67371XF#?ke
Cotton Swabs	$3	$0.17	$7	9.77	Amazon	Yes	Yes	https://www.amazon.com/Amazon-Basics-Cotton-Swabs-500ct/dp/B09541P9WH?psc=1&ref_=fplfs&smid=ATVPDK
Recombinant Mouse PDGF-AA(chemoattra	$392	$0(with lipo)	$0**	$392	Thermo Fisher	No	Yes	https://www.thermofisher.com/proteins/product/Mouse-PDGF-AA-Recombinant-Protein/14-8989-80
Penicillin-Strepotmycin-100mL	$26	TBD*	TBD*	$26	Thermo Fisher	no	yes	https://www.thermofisher.com/order/catalog/product/15140122
			Total	$2,245.62				
			Total with split costs	$1,670.29				
			Total without assay kit	$1,030.29				

Appendix D: Biosafety Registration Paperwork

Biosafety Registration Main Text:

Project / Capstone Team Name: HL60 Cell Team #1(Group J)

Brief Summary of Bioengineering Capstone Project

Write a high-level overview of and summary of the objectives of your project.

Our project is a Bioengineering undergraduate Capstone project which will involve the use of NIH 3T3 murine fibroblasts as a model for wound healing measured using a chemotaxis migration assay. Each team will develop a novel strategy to modify their cells in order to improve physical, biological, and/or chemical features related to chemotaxis and cell movement. All lab work for this project will be completed by April 24th 2024. Materials used for this project will be disposed of using appropriate waste streams on or before this date.

Description of Experimental and Procedural Details

Describe your experiments and procedures, focusing on steps taken to limit risk of exposure of personnel to hazardous materials, including biohazards. A step-by-step protocol is NOT required here.

The Description of Experimental and Procedural Details must include transport, PPE (personal protective equipment), waste handling, centrifugation, exposure response, and spill response language. Template langstuage appears below in blue font. Select the sections relevant to your proposed work (e.g. if your work does not involve human cell lines or other materials, do not include the Human Materials Handling language in your Description of Experimental and Procedural Details). Edit the template language as needed to be specific to your project.

NIH3T3 Cell Line Maintenance

Complete culture media for these cells will be formulated with Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS).

The NIH3T3 cells will be sourced from the Bioengineering Teaching Lab as they are already grown and stored there for another course. We will take one vial/flask and scale up the cells for our project. NIH3T3 cells will be cultured in typical cell culture flasks in complete culture media. When passaging, spent cell culture media will be removed, cells washed with PBS, and then a Trypsin-EDTA solution added to dissociate the cells from the flask. Cells will be counted, centrifuged and resuspended in fresh complete culture media before being introduced into a new culture flask. Passaging would take place every 3-4 days. Any waste produced while culturing the NIH3T3 cell lines will be treated with bleach at a 1:10 ratio for a contact time of 30 minutes before being disposed of.

After transfection with any antibiotic resistance gene, the culture medium will be supplemented with the appropriate amount of the relevant antibiotic. The antibiotics being considered for use are Hygromycin at 200ug/mL and Neomycin at 400ug/mL The purpose of the antibiotic supplemented media is to select for cells transfected with the gene of interest and antibiotic resistance by killing the cells without antibiotic resistance. A maximum of eight T75 flasks will be maintained simultaneously with a maximum volume of 20 mL of media per flask. All work with NIH3T3 cells will be done in a Biosafety Cabinet which will be disinfected according to the relevant RG1 procedures discussed below. Waste produced will also be treated according to RG1 procedures with

solid waste being disposed of in the appropriate biowaste collection bin and liquid waste will be treated with bleach to a concentration of 10% for a minimum contact time of 30 minutes before disposal.

Bacterial Plasmid Culture Protocol:

To isolate our plasmid of interest from the agar stab we will use a commercially available kit similar to the PureLink™ HiPure Plasmid Miniprep Kit from Thermo Fisher.

The following protocol will outline the selection of our plasmids from their initial bacterial agar stab form. The first step is to obtain a Luria Broth (LB) agar plate and use a sterile pipette tip to transfer bacteria from the stab culture to the surface of the plate by creating a streak. Two more pipette tips will be used to create subsequent streaks from the initial streak. The plate will then be incubated at 37°C for at least 12 hours to recover individual colonies of the bacteria. The next phase will involve inoculating the bacteria colonies from the plate into a liquid LB culture. Liquid LB will be made up from LB agar powder at an appropriate volume (e.g. 500mL). Antibiotics will be added at an appropriate concentration for the plasmid being purified (EGFR, PDGFRalpha, and PDGFRbeta require Hygromycin at 200ug/mL and TIAM1 requires Neomycin at 400ug/mL). Using a sterile pipette tip, a single colony will be selected from the agar plate and placed in the tube with the liquid culture and antibiotic. This culture will be covered in foil and incubated at 37°C for at least 12 hours to allow the bacteria to grow. Once growth is observed the next day, the bacteria will be stored as a glycerol stock if work is not continuing the next day. If work continues, we will begin with the protocol described in the mini-prep kit in order to isolate the plasmid of interest. All work with bacteria will be performed inside Biosafety Cabinets to avoid contamination and exposure. Proper waste disposal and cleanup procedures for RG1 material will be followed during and after any procedures.

Lipofection

Lipofection is a nonviral means of introducing genetic material into the NIH 3T3 cells. This process involves mixing a reagent containing lipid particles with the desired genetic material (RNA or plasmid). Potential genes for lipofection use include plasmids for PDGFRalpha, PDGFRbeta, Tiam1, and EGFR which correspond to our potential targets in the cell for influencing movement. One or multiple of these targets will be chosen based on budgetary and time restraints. PDGFRalpha, PDGFRbeta, Tiam1, and EGFR will all be knocked in if chosen. The links to the plasmids for these genes to be used in lipofection can be found in our rDNA biosafety form. The lipid includes particles that carry the nucleic acids into the nucleus where they can be expressed by the cell. This could then be followed with antibiotic selection for a pure successfully transfected population if antibiotic resistance is included on the plasmid being transfected. Antibiotics that could be used to screen the transduced cells include Hygromycin and Neomycin since the resistance genes for these antibiotics are included in our plasmids of interest (i.e. all plasmids used confer resistance to either Hygromycin or Neomycin) Further details on the antibiotic selection can be found in the Bacterial Plasmid Culture Protocol section and the Cell Line Maintenance Section. This process would involve recombinant nucleic acids and a murine cell line so the safety protocols defined for human material and RG1 materials would be followed. All work with our cell lines and bacteria will be done in sterile BSC to prevent contamination. A brief protocol for the lipofection procedure is given below.

Lipofection Procedure:

This procedure is derived from the Lipofectamine LTX Protocol by Thermo Fisher. To start we will seed our NIH3T3 cells to be between 70% and 90% confluent at the time of lipofection in a 96-well plate. The Lipofectamine LTX reagent will then be diluted in Opti-MEM medium (0.15uL in 5uL x 2). A master mix of DNA will be prepared by diluting 0.2ug of our plasmid of interest in 10uL of Opti-MEM along with 0.4uL of PLUS reagent. The diluted DNA master mix will then be added to the diluted Lipofectamine LTX in a 1:1 ratio (5uL:5uL). This mixture will be incubated for 15 minutes at room temperature. 10uL of the DNA-lipid complex will be added to each well of cells and then incubated for 3 days at 37 °C. For any siRNA, we will follow the same procedure described above. However, we will **not** add PLUS reagent in the siRNA dilution step.

All reagents for this procedure are included in the Lipofectamine LTX Reagent Kit except for Opti-MEM medium.

Chemotaxis Assay

To determine the effectiveness of our modifications to the NIH3T3 cells, we will conduct a chemotaxis assay. In this assay, we will compare the ability of unmodified control cells and modified cells to migrate in the presence of a chemoattractant. We will use FBS and Platelet-Derived Growth Factor (PDGF) as chemoattractants in our tests. The Cell Migration/Chemotaxis Assay Kit will be purchased from Abcam. A procedure based on the one provided by Abcam will be generated to more closely fit our specific needs. The assay's readout is through fluorescence which will be quantified using a plate reader present in the Bioengineering Teaching Lab (ISEC255).

Rough Timeline For Lab Work in ISEC255

3/10-3/16
Receive Cells/Begin culturing
3/17-3/23
Transfect Cells with plasmids
3/24-4/6
Run chemotaxis and protein expression assays
4/6-End of Project
Empty lab space and dispose of relevant materials appropriately

PPE:
Lab coat, eye protection, and nitrile gloves will be worn at all times when handling this material. Gloves will be changed upon contamination. Hands will be washed after removing gloves and before leaving the lab. Lab coats will be sent for laundering at the end of each work day. Researchers will inspect lab coats for contamination and spot treat with disinfectant if applicable before sending out for laundering.
Double gloving will be used at all times by team members when working with human cells and/or pathogens. When using double-gloves, the outer gloves will be removed prior to exiting the biosafety cabinet. This will minimize the potential to spread contamination outside of the biosafety cabinet.

BIOHAZARD TRANSPORT:
Any time that hazardous biological materials are transported out of the ISEC 255 teaching lab, they will be first sealed in a leakproof, shatterproof transport container that also contains disposable absorbent material such as paper towels inside. The container will be labeled on the outside with a biosafety symbol sticker. After sealing, the container will be decontaminated for a minimum 10-minute contact time with 70% ethanol or 1:10 or 1:100 bleach (prepared fresh daily). The container will then be transported out of the lab by a researcher who is not wearing gloves or lab coat. This process will be repeated to return to ISEC 255. The container will be decontaminated inside and out after use.

70% ethanol will be used to wipe the surface contaminated with cell culture in the biosafety cabinet, but 1:10 bleach will be used when discarding cell culture that is not used.

70% ethanol will be used to thoroughly decontaminate areas following decontamination with 10% bleach when working with CRISPR/Cas9 gene editing protocols.

Remaining DMSO reagent or samples containing DMSO will be discarded in a separate hazardous waste container.

HUMAN MATERIALS HANDLING:
Human materials used in this project will be handled using Universal Precautions (i.e. they will be handled as if they are infectious). Handling will conform to BSL2/RG2 requirements.

SURFACE AND EQUIPMENT DECONTAMINATION:
choose the correct procedure for your materials

Our group will follow the standards for RG2 material decontamination as shown below for all of our materials.

RG2 materials including all human materials:
All work surfaces, biosafety cabinets, and other equipment will be decontaminated using 1:10 or 1:100 bleach solution for a 10-minute contact time followed by 70% ethanol. Bleach solutions will be prepared fresh daily as 1 part household bleach in 9 parts water.

BIOLOGICAL WASTE MANAGEMENT:

---Solid Waste---

--Non-sharps:

-Plastic bin collection:
Non-sharp solid biohazardous waste will be collected in a red, plastic biowaste collection bin that is lined with a red biohazard bag and has a lid. The lid must be closed at all times except when waste is being added to the pedal bin. When full, the bags will be tied with a single, gooseneck-style, water-tight knot and placed in a cardboard biohazard burn box that is double lined with red biohazard bags and has a lid. The box will then be sealed with tape on all edges. The box will be labeled with the appropriate generator label, lab location, PI name and phone number. Only boxes that are fully packaged and taped on all edges will be transported to the ISEC biowaste room.

-Burn box collection:
Non-sharp solid biohazardous waste will be collected in a cardboard biohazard burn box that is double lined with red biohazard bags and has a lid. The lid will fully cover the box at all times except when waste is being added. When full, the bags will be tied with a single, gooseneck-style, water-tight knot. The box will then be sealed with tape on all edges. The box will be labeled with the appropriate generator label, lab location, PI name and phone number. Only boxes that are fully packaged and taped on all edges will be transported to the building biowaste room.

--Pseudosharps:

-Pseudosharps include intact serological pipets and other non-sharp biological waste that could puncture a red biohazard bag. All pseudosharps will either be collected in a Bio-Bin or Pipette Keeper at all times except when waste is being added. This includes serological pipets and microscope slides. Bio-Bins and Pipette Keepers will be kept closed except when waste is being added. Once full, the Bio-Bin or Pipette Keeper will be sealed according to the instructions printed on them once. It will then be placed into a cardboard biohazard burn box that is double lined with red biohazard bags. When full, the bags will be tied with a single, gooseneck-style, water-tight knot and placed in a cardboard, biohazard burn box that is double lined with red biohazard bags and has a lid. The box will then be sealed with tape on all edges. The box will be labeled with the appropriate generator label, lab location, PI name and phone number. Only boxes that are fully packaged and taped on all edges will be transported to the building biowaste room.

--Sharps:
Avoid the use of sharps when working with biohazards (e.g. needles and syringes or glass Pasteur pipettes). Biological sharps waste includes needles, broken glass, blades, and other true sharps waste that is contaminated with biological material. All biohazardous sharps will be collected in red, hard-walled sharps containers labeled with a biohazard symbol and with a lid. The containers will be labeled with PI's name and laboratory location. The containers will be kept closed at all times except when waste is being added.
Once the container reaches 2/3 full, the lid will be locked and the container checked for labeling. It will then be placed into a cardboard biohazard burn box that is double lined with red biohazard bags. When full, the bags will be tied with a single, gooseneck-style, water-tight knot and placed in a cardboard, biohazard burn box that is double lined with red biohazard bags and has a lid. The box will then be sealed with tape on all edges. The box will be labeled with the appropriate generator label, lab location, PI name and phone number. Only boxes that are fully packaged and taped on all edges will be transported to the building biowaste room.

---Liquid waste---

Liquid biological waste contains biological contaminants that can be deactivated with bleach. Liquid biological waste DOES NOT contain any chemicals or substances that are incompatible with bleach and/or that cannot be disposed of down a laboratory sink drain after deactivation with bleach.

Vacuum collection:

*Since vacuum aspiration is not supported in the Bioengineering teaching lab, we will be relying on the following non-vacuum collection method to discard liquid biological waste.

All liquid biological waste collected via vacuum must be collected into a trap container containing enough household bleach for a final concentration of 1 part household bleach to 9 parts of liquid waste. The bleach must be added fresh daily before vacuum liquid collection begins. The trap container must be directly connected to a second trap container that serves as emergency overflow. An in-line HEPA or 0.2um membrane filter must be connected between the emergency overflow trap and the port to the in-house vacuum system or vacuum pump. After collection, liquid waste must remain in contact with the bleach for no less than 30 minutes. Then, the liquid waste may be brought to the laboratory sink for disposal down the drain with large volumes of tap water. Glassware and waste must never be left in the sink, particularly if the sink is used for handwashing or if it has an eyewash station. Waste will only be disposed of in the sink if it has been determined to be non-hazardous for both biohazards and chemical hazards.

Non-vacuum collection:

*Note: We will be using the following protocol for discarding liquid biological waste since vacuum aspiration is not supported in the Bioengineering teaching lab.

All liquid biological waste collected without vacuum (e.g. by pipetting into a waste container) must be collected into a waste container with a screw cap containing enough household bleach for a final concentration of 1 part household bleach to 9 parts of liquid waste. The bleach must be added fresh daily before liquid collection begins. After collection, liquid waste must remain in contact with the bleach for no less than 30 minutes. Then, the liquid waste may be brought to the laboratory sink for disposal down the drain with large volumes of tap water. Glassware and waste must never be left in the sink, particularly if the sink is used for handwashing or if it has an

eyewash station. Waste will only be disposed of in the sink if it has been determined to be non-hazardous for both biohazards and chemical hazards.

BIOHAZARD EXPOSURE RESPONSE:

---Puncture, cut, or other exposure via broken skin---
1. Immediately after exposure, the affected area will be washed with soap and water for no less than 15 minutes.
2. Then, the exposure will be immediately reported to the NUPD emergency line at 617-373-3333 or using the emergency reporting tool on the SafeZone app.
3. First responders will be provided with printed copies of the appropriate biosafety registration, the SDS of any chemicals involved in the exposure, and any other relevant information.
4. The PI, DSO, and EHS (617-373-2769, ehs@northeastern.edu, biosafety@northeastern.edu) will be notified of the incident within 24 hours of the exposure.

---Exposure to the eyes---
1. Immediately after exposure, the eyes will be flushed for no less than 15 minutes using the laboratory eyewash.
2. The exposure will be reported to the NUPD emergency line at 617-373-3333 or using the emergency reporting tool on the SafeZone app.
3. First responders will be provided with printed copies of the appropriate biosafety registration, the SDS of any chemicals involved in the exposure, and any other relevant information.
4. The PI, DSO, and EHS w(617-373-2769, ehs@northeastern.edu, biosafety@northeastern.edu) will be notified of the incident within 24 hours of the exposure.

---Exposure to the body---
1. Immediately after exposure, the affected area(s) will be flushed for no less than 15 minutes using the laboratory safety shower.
2. The exposure will be reported to the NUPD emergency line at 617-373-3333 or using the emergency reporting tool on the SafeZone app.
3. First responders will be provided with printed copies of the appropriate biosafety registration, the SDS of any chemicals involved in the exposure, and any other relevant information.
4. The PI, DSO, and EHS (617-373-2769, ehs@northeastern.edu, biosafety@northeastern.edu) will be notified of the incident within 24 hours of the exposure.

BIOHAZARD SPILL RESPONSE:

The lab will be immediately evacuated for 30 minutes to allow aerosols to settle in the event of a spill.

---If the researcher(s) feel comfortable responding to the spill:
1. Absorbent material such as paper towels will be placed on spill.
2. Then, household bleach will be poured around the perimeter of the spill.
3. Household bleach will then be poured in concentric circles from the spill perimeter inward until the entire spill area is in contact with bleach.
4. The spill area will remain in contact with the bleach for 20 minutes.
5. Then, additional absorbent material will be used to soak up all of the bleached spill.
6. Spill cleanup material will be collected in a cardboard biohazard burn box that is double lined with red biohazard bags.

7. The spill area will be given a final surface decontamination with 10% bleach (1 part bleach to 9 parts water) for a 10-minute contact time.
8. At the end of spill cleanup, the red biohazard bags will be tied with a single, gooseneck-style, water-tight knot.
9. The box will then be sealed with tape on all edges.
10. The box will be labeled with the appropriate generator label, lab location, PI name and phone number.
11. Only boxes that are fully packaged and taped on all edges will be transported to the building biowaste room.

---For large spills and if the researchers do not feel comfortable responding to the spill:
1. The researcher(s) will evacuate the area and notify those working nearby to evacuate.
2. Then, the spill will be immediately reported to the NUPD emergency line at 617-373-3333 or using the emergency reporting tool on the SafeZone app.
3. First responders will be provided with printed copies of the appropriate biosafety registration, the SDS of any chemicals involved in the spill, and any other relevant information.
4. The PI, DSO, and EHS (617-373-2769, ehs@northeastern.edu, biosafety@northeastern.edu) will be notified of the incident within 24 hours of the spill.

CENTRIFUGE USE WITH BIOHAZARDOUS MATERIALS:

The following steps are required when centrifuging BSL2/RG2 and higher materials and are good practice when centrifuging BSL1/RG1 materials.
1. When used with RG2 materials, only centrifuges with a solid cover and an interlock to prevent opening while in operation will be used.
2. Tubes, safety rotors, and safety buckets will be loaded inside the biosafety cabinet.
3. Centrifuge tubes will not be overfilled.
4. Prior to loading into the centrifuge, tubes will be wiped down with disinfectant.
5. If using a swinging bucket rotor, the buckets will be sealed with containment lids designed for use with biohazardous materials. If used a fixed rotor, the rotor will be sealed using a rotor lid designed for use with biohazardous materials.
6. After centrifuging, the centrifuge will sit unopened for no less than 10 minutes to allow aerosols to settle.
7. Buckets or rotors will be transferred to the biosafety cabinet (BSC) before the lids are unsealed.
8. Buckets and rotors will be decontaminated after each use.

Biosafety Registration rDNA

Project / Capstone Team Name: HL60 Cell Team #1(Group J)

Plasmids Used

Name	Gene(s)/ Insert(s)	Viral origin? (answer non viral or list the virus of origin)	Commercially available? (yes/no)	Gene(s)/ Insert(s)	Nature of sequence(s)? (cDNA, siRNA, miRNA, genomic DNA, or other)	Viral Particle Production? (yes/no)	Additional details, including url/link
pCMV3-C-GFPSpark-EGFR	EGFR	Nonviral	Yes	EGFR	cDNA	No	https://www.sinobiological.com/cdna-clone/mouse-egfr-mg51091 (Cat: MG51091-ACG)
PCDNA3.4-TIAM1	TIAM1	Nonviral	Yes	TIAM1	cDNA	No	https://www.addgene.org/190510/
pcDNA5FRT-EF-Pdgfralpha-EGFPN	PDGFRalpha	Nonviral	Yes	PDGFRalpha	cDNA	No	https://www.addgene.org/66787/
pcDNA5FRT-EF-Pdgfrbeta-EGFPN	PDGFRbeta	Nonviral	Yes	PDGFRbeta	cDNA	No	https://www.addgene.org/66790/

The reference for each of the Sections mentioned in the questions below is the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* available here: https://osp.od.nih.gov/biotechnology/nih-guidelines/

Form Questions

1) Do any of your experiments involve the deliberate transfer of a drug resistance trait to micro-organisms that are not known to acquire the trait naturally (yes or no)?:
No
2) Do any of your experiments involve recombinant or synthetic nucleic acid sequences that are deliberately created for biosynthesis of molecules toxic in vertebrates at an LD50 of less than 100 ng/kg body weight (yes or no)?:
No
3) Do you conduct experiments in which recombinant or synthetic nucleic acids are transferred into human subjects? (E.g. Gene therapy studies, vaccination studies) (yes or no):
No
4) Does your research involve the introduction of recombinant or synthetic nucleic acid molecules into Risk Group 2, 3, 4 or Restricted Agents? (yes or no):
Yes
5) Does your research involve the cloning of recombinant or synthetic nucleic acids from risk group 2, 3, 4 or restricted agents cloned into a nonpathogenic prokaryotic or lower eukaryotic host vector system? (yes or no):
Yes
6) Do your experiments involve the use of infectious or defective DNA or RNA Viruses in tissue culture systems? (This includes the use of a packaging cell line(s) to generate viral particles for transduction) (yes or no):
No
7) Do your experiments involve whole animals? (yes or no):
No
8) Do your experiments involve plants containing recombinant or synthetic nucleic acid molecules? (yes or no):
No
9) Do your experiments involve growing cultures of organisms containing recombinant, synthetic recombinant, or synthetic nucleic acid molecules in excess of 10 liters in a single growth vessel? (yes or no):
No
10) Do you perform experiments with influenza viruses generated by recombinant or synthetic methods? (yes or no):
No
11) Are you conducting any recombinant or synthetic nucleic acid experiments that are not classified under Sections III-A, III-B, III-C, III-D, III-F? (yes or no):
No

Exempt Experiments

1) Are any experiments conducted under your research exempt under NIH Guidelines Section III-F-1? (yes or no):
No
2) Are any experiments conducted under your research exempt under NIH Guidelines Section III-F-2? (yes or no):
No
3) Are any experiments conducted under your research exempt under NIH Guidelines Section III-F-3? (yes or no):
No
4) Are any experiments conducted under your research exempt under NIH Guidelines Section III-F-4? (yes or no):
No
5) Are any experiments conducted under your research exempt under NIH Guidelines Section III-F-5? (yes or no):
No
6) Are any experiments conducted under your research exempt under NIH Guidelines Section III-F-6? (yes or no):
No
7) Are any experiments conducted under your research exempt under NIH Guidelines Section III-F-7? (yes or no):
No
8) Are any experiments conducted under your research exempt under NIH Guidelines Section III-F-8? (yes or no):
No

Biosafety Registration Cells and Tissues

Project / Capstone Team Name:_HL60 Cell Team #1(Group J)

Cell lines used: NIH 3T3 murine fibroblasts

Cell Line Name	Cell Type/Origin	Species of Organism	Source Type (human, nonhuman primate, nonprimate animal, or arthropod)	Immortalized (yes or no; non-immortalized cells are primary cells)	Viral Packaging (yes or no)	Source of cells (colleague, commercial supplier, hospital/clinical lab, human subject, orin-house)	Details of source of cells, including url/link
NIH 3T3 murine fibroblast	Mouse fibroblast cell line	Mus musculus, Mouse	Animal	Yes	No	Colleague	https://www.atcc.org/products/crl-1658

Appendix E: List of Relevant Standards

ISO/TS 23511:2023 Biotechnology

https://www.iso.org/standard/75854.html

This document defines terms related to cell line authentication in the field of biotechnology. It describes the general principles, detection strategies and analytical methods for cell line authentication. It specifies requirements and key considerations for method selection, quality control parameters, data analysis and reporting.

This document is applicable to routine inspection of cell lines in culture and in storage in the fields of basic research, translational studies and product manufacturing. It is also applicable to cell line origin validation in academic and industrial laboratories, cell banks and manufacturing sites. It is primarily applicable to mammalian cells, including human cells.

ASTM E3072-22a Standard Terminology for Industrial Biotechnology and Synthetic Biology

https://compass.astm.org/document/?contentCode=ASTM%7CE3072-22A%7Cen-US

This standard provides an overview of the common terminology for individuals to become aware of when becoming involved in work that is related to biotechnology and synthetic biology. As stated in the documentation for this standard, it is the user's responsibility to establish appropriate safety measures, become aware of appropriate health and environmental policies when working with certain substances provided in this list, and understand the regulatory limitations prior to using such substances in the lab. This standard provides an overview of terminology related to genetics and gene editing, such as genome editing, genome-edited organism, and genome, which are relevant to understand for our project as we focus on genetically altering a murine fibroblast cell line, NIH 3T3 cells, to improve their migration pathways.

UNE UNE-EN 12740:2000 Biotechnology - Laboratories for research, development and analysis - Guidance for handling, inactivating and testing of waste

https://compass.astm.org/document/?contentCode=UNE%7CUNE-EN%2012740%3A2000%7Cen-US

This standard discusses an overview of methods that can be used for proper disposal of hazardous waste products from laboratory work. It focuses on methods for handling and inactivation of waste products to reduce the likelihood of risks that can occur for animals, humans, and the environment from exposure to harmful contaminants or organisms that are present in the discarded laboratory substances and equipment. Our project takes this into consideration, which is also detailed in our biosafety registration paperwork, for how we plan to properly discard our materials and decontaminate workspaces used in the lab. We will also take into consideration other factors, such as the amount of time used for laboratory equipment to help preserve energy.

UNE UNE-EN 12683:1999 Biotechnology - Modified organisms for application in the environment - Guidance for the characterization of the genetically modified organism by analysis of the molecular stability of the genomic modification

https://compass.astm.org/document/?contentCode=UNE%7CUNE-EN%2012683%3A1999%7Cen-US

This standard provides an overview for properly measuring the molecular stability behind genetic modifications induced in an organism of choice. Some factors to consider are

reproducibility of the experiment, understanding how this functions in biological systems, and how it interferes with life cycle and external factors. Assessing the molecular stability of a genetic modification can be done as early as the genomic and transcriptional levels. Looking at the overview of the terminology provided in this document, an organism is identified as something that can replicate or transfer genetic material, and a genetically modified organism is defined as an organism that is genetically altered in such a way that it does not occur through natural biological processes. By these definitions, we are genetically altering a murine fibroblast cell line, which does undergo replication, and we are genetically altering the cell to induce expression of molecules to levels that are not naturally expressed. We will compare the modified cells to a control, wild-type cell line, and we will analyze the molecular stability of the transfected cell line through selection procedures. We will assess how well the transfected cell line survives when culturing them and assess the stability of the modified cell line when analyzing their response in a chemotaxis assay. We hope to understand how the genetic modifications impact chemotactic pathways of fibroblasts on a molecular level, which can give potential insight for how this may help humans during wound healing.

UNE UNE-EN 12682:1999 Biotechnology - Modified organisms for application in the environment - Guidance for the characterization of the genetically modified organism by analysis of the functional expression of the genomic modification

https://compass.astm.org/document/?contentCode=UNE%7CUNE-EN%2012682%3A1999%7Cen-US

This standard provides an overview of methods to measure changes in functionality of a genetically modified organism. One of the methods it discusses is analyzing how external factors may affect the genetically modified organism, and we have considered various external factors for the purposes of our project, such as considering how heating our cells may influence the speed of our genetically modified cells when measuring chemotaxis. With the original maze race concept, we also considered factors such as the addition of a hypertonic solution to improve the modified cells' efficiency in navigating through narrow channels. We're also considering cell culture conditions that can influence transfection efficiency, and we are adjusting the content of the cell media as an external factor by adding an antibiotic to select for transfected cells. In addition, this standard discusses the importance of measuring a data signal to assess changes in functionality of the modified organism, including changes in position and movement. We are planning to quantitatively measure the chemotaxis efficiency of the modified cells with a chemotaxis assay and assess their changes in movement based on percentage of cells that migrate toward the bottom chamber, which contains the chemoattractant.

UNE UNE-EN 12741:2000 Biotechnology - Laboratories for research, development and analysis - Guidance for biotechnology laboratory operations

https://compass.astm.org/document/?contentCode=UNE%7CUNE-EN%2012741%3A2000%7Cen-US

This standard provides documentation of the proper regulatory measures that need to be taken to conduct laboratory work that is safe for the employees working in the lab. It also provides information about regulatory measures for proper disposal of waste to help protect animals and the environment. Proper containment protocols should be followed when working with hazardous materials, and we will be following specific containment and decontamination protocols, as discussed in our biosafety registration paperwork, when working with cell culture, isolating plasmids, performing a lipofection protocol, and working with reagents when running a chemotaxis assay. Also, we will ensure proper disposal of lab materials when finished to avoid harmful exposure to contaminated materials for others working in the lab and to avoid harmful exposure of chemical and biological substances to the environment.

STP26067S Containment and Regulations for Safe Biotechnology

https://compass.astm.org/document/?contentCode=ASTM%7CSTP26067S%7Cen-US

This standard discusses the proper containment and regulatory procedures that need to be followed when working with materials that pose various risk levels. Biological materials are classified from moderate to severe risks. We will be following specific containment and the respective regulatory procedures when working with biological materials and will ensure proper decontamination and disposal of our reagents and other materials when finished to avoid harmful exposure of contaminants to other members that work in the lab.

ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories

https://www.iso.org/standard/66912.html

This standard focuses on general testing and calibration guidelines that can apply to our research. This standard is to ensure the credibility, quality, and proficiency of work done in the lab. This can translate to both our cell modifications as well as cell reading steps.

Appendix E: Raw Data

Standard Curve Fluorescence Reading:

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.362	0.368	0.344	0.315	0.317	0.294	0.109	0.133	0.037	0.665	0.212	0.219	530
	0.472	0.466	0.45	0.458	0.455	0.471	0.108	0.131	0.037	0.929	0.596	0.605	590
B	0.292	0.293	0.281	0.261	0.265	0.265	0.296	0.298	0.038	0.048	0.051	0.048	530
	0.467	0.485	0.479	0.496	0.489	0.494	0.299	0.294	0.037	0.046	0.051	0.047	590
C	0.261	0.261	0.26	0.239	0.246	0.244	0.092	0.214	0.037	0.048	0.057	0.048	530
	0.484	0.49	0.499	0.504	0.511	0.514	0.086	0.213	0.037	0.046	0.056	0.047	590
D	0.222	0.223	0.218	0.217	0.211	0.211	0.375	0.357	0.037	0.048	0.048	0.048	530
	0.519	0.509	0.514	0.518	0.505	0.514	0.353	0.345	0.037	0.047	0.047	0.048	590
E	0.209	0.209	0.209	0.207	0.207	0.205	0.036	0.037	0.037	0.048	0.048	0.049	530
	0.531	0.524	0.526	0.526	0.535	0.554	0.036	0.036	0.036	0.047	0.047	0.048	590
F	0.19	0.182	0.165	0.201	0.172	0.161	0.224	0.355	0.388	0.048	0.048	0.048	530
	0.494	0.47	0.419	0.54	0.447	0.422	0.208	0.334	0.383	0.047	0.048	0.047	590
G	0.206	0.218	0.216	0.22	0.217	0.196	0.037	0.455	0.055	0.049	0.049	0.048	530
	0.557	0.592	0.586	0.602	0.584	0.512	0.037	0.437	0.056	0.048	0.048	0.047	590
H	0.048	0.047	0.049	0.048	0.047	0.048	0.047	0.048	0.037	0.048	0.048	0.048	530
	0.047	0.046	0.047	0.048	0.046	0.048	0.047	0.048	0.036	0.048	0.048	0.047	590

Chemotaxis Assay Fluorescence Reading

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.177	0.102	0.102	0.106	0.156	0.14	0.152	0.136	0.105	0.105	0.103	0.103	530
	0.308	0.227	0.222	0.232	0.351	0.303	0.325	0.302	0.239	0.236	0.231	0.231	590
B	0.1	0.114	0.107	0.117	0.155	0.14	0.144	0.128	0.1	0.12	0.14	0.108	530
	0.221	0.256	0.238	0.271	0.338	0.313	0.323	0.297	0.228	0.253	0.325	0.239	590
C	0.119	0.119	0.105	0.098	0.148	0.12	0.125	0.13	0.11	0.102	0.11	0.13	530
	0.27	0.263	0.232	0.221	0.36	0.286	0.299	0.294	0.237	0.216	0.241	0.259	590
D	0.253	0.056	0.05	0.05	0.24	0.051	0.055	0.048	0.049	0.049	0.049	0.05	530
	0.137	0.055	0.049	0.05	0.109	0.05	0.055	0.048	0.047	0.048	0.049	0.049	590
E	0.051	0.05	0.049	0.049	0.048	0.05	0.051	0.05	0.058	0.058	0.049	0.054	530
	0.05	0.049	0.048	0.047	0.049	0.048	0.049	0.049	0.057	0.057	0.049	0.053	590

F	0.071	0.058	0.056	0.051	0.048	0.05	0.049	0.053	0.049	0.05	0.049	0.052	530
	0.069	0.056	0.055	0.049	0.048	0.05	0.048	0.052	0.047	0.05	0.048	0.051	590
G	0.05	0.056	0.053	0.049	0.051	0.05	0.049	0.057	0.053	0.05	0.053	0.05	530
	0.049	0.055	0.052	0.049	0.05	0.049	0.047	0.055	0.053	0.05	0.053	0.049	590
H	0.062	0.051	0.093	0.052	0.049	0.051	0.052	0.054	0.05	0.05	0.049	0.049	530
	0.061	0.05	0.087	0.051	0.048	0.05	0.051	0.052	0.048	0.049	0.048	0.048	590