Delivery Vehicle and Mechanism for Human Mesenchymal Stem Cells

Biomedical Engineering Department

A Major Qualifying Project Report:

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Abstract

Human mesenchymal stem cells (hMSCs) are an extremely valuable asset to the field of biomedical engineering as well as regenerative medicine. They are commonly used in tissue engineering because they can differentiate into a variety of cell types and are relatively easy to culture and maintain. hMSCs have been used in preclinical models for tissue engineering of bone, cartilage, muscle, marrow stroma, tendon, fat, and other connective tissues [4, 6, 7, 18, 19, 36, 40, 42, 54]. Several studies have shown that these materials show promise for rebuilding diseased or damaged tissues [24].

The difficulty involved with hMSCs arises in the delivery of the cells to the site of injured tissue. Injection is the preferred method, but it has been shown that simple injection of hMSCs in media through hypodermic needles results in extreme turbulent flow, killing the cells in the process. Biocompatible hydrogels have been previously researched as a form of carrier for hMSC transport. However, the handling and delivery of the gel, as well as the cell viability while within, remain a problem. This project aims to seek the best overall hydrogel to act as the carrier as well as a novel delivery mechanism that allows for simple, safe and convenient transport of hMSCs.
Executive Summary

Human mesenchymal stem cells (hMSCs) are an extremely valuable asset to the field of biomedical engineering as well as regenerative medicine. They are commonly used in tissue engineering because they can differentiate into a variety of cell types and are relatively easy to culture and maintain. hMSCs have been used in preclinical models for tissue engineering of bone, cartilage, muscle, marrow stroma, tendon, fat, and other connective tissues [1]. Several studies have shown that these materials show promise for rebuilding diseased or damaged tissues [2].

The difficulty involved with hMSCs arises in the delivery of the cells to the site of injured tissue. Injection is the preferred method, but it has been shown that simple injection of hMSCs in media through hypodermic needles results in extreme turbulent flow, killing the cells in the process. Biocompatible hydrogels have been previously researched as a form of carrier for hMSC transport [3]. However, the handling and delivery of the gel, as well as the cell viability while within, remain a problem. This project aims to seek the best overall hydrogel to act as the carrier as well as a novel delivery mechanism that allows for simple, safe and convenient transport of hMSCs.

The device requires the culturing of human mesenchymal stem cells and then transfer these cells into a three dimensional matrix which is in the form of a hydrogel. In order to culture these stem cells, we had to feed the cells media which included Dulbecco’s Eagle Medium, 10 % fetal bovine serum, as well as penicillin and streptomycin. This gave the cells appropriate nutrients to grow and plate. We decided to grow these cells in a T-75 flask and incubate them for a period of 5-7 days, changing the media with fresh media every 2-3 days. After the cells had fully plated on the surface of the flask, we collected the cells by applying trypsin and then
combined the cells with 0.7 g of 4 hydrogel powders (PEG, PGA, CMC, HA) along with 23mL of media. After a day of constant rotation the gel/cell matrix was formed and placed in a 24 well plate. The second part of our design required the creation of a delivery mechanism for these hMSCs/hydrogel combination. Our design team decided upon using a cartridge which has the plunger attached and holds the cells/hydrogel combination is placed in the back of the syringe, and guides it through a syringe, after which a stopper holds the cartridge in place while the hMSC/hydrogel combination continues through the syringe and dispels through a 22 gauge needle.

Our initial step was to determine which hydrogel matrices allowed for viable human mesenchymal stem cells as well as three-dimensional growth. Therefore we grew the stem cells in (PGA,PEG,CMC,HA ) in a 24 well plate and assessed there viability using the MTS assay which measures the amount of light passing through the matrix, and histology readings (H&E staining) as well.

After determining the matrix that promoted three dimensional growth with hMSCs, the next step was to test the how the design affected the viability. Therefore we compared the standard method of loading and unloading by suction of the hMSC/hydrogel composition through the needle and then ejecting it through the needle to our innovative design of loading the gel from the back and then ejecting it through the needle. By running the MTS assay, conclusions can be made as to which delivery method results in a higher viability of hMSCs.

Our results from determining which hydrogel provided a viable matrix which promoted three dimensional growth was figured out using MTS assay and histology readings. Our team eliminated PGA from the initial MTS assay when the results concluded that it was not compatible with hMSCs. CMC was eliminated through histology readings; the cells were not
mimicking the behavior as seen in the control. Our two final choices were PEG and HA, which displayed similar viability readings in the MTS assay. The histology readings suggested that HA promoted three dimension growth. Therefore HA was the selected matrix.

Next we had to prove that our design was more effective than the standard method of delivery. Therefore we performed an MTS assay comparing the standard loading and unloading with a syringe through the needle and our design which loaded the hydrogel from the back and ejected it through the needle, thus only subjecting the gel to forces in one direction (unloading). Our design team created a cartridge which can be loaded from the back of the syringe and the results confirmed that our theory and design did in fact increase viability by limiting the forces induced in the ejection phase.

Our delivery mechanism eliminates much of the viability issues presented with the standard method of loading/unloading of a hMSC/hydrogel matrix associated with shear stress. The quantitative and qualitative measurements can be easily communicated to medical professionals in need of delivering cells to a tissue where shear stress is the major constraint involved in maintaining the viability. Our team believes that this device enhance the standard method of delivery and can be modified to fit the needs of a variety of other cell lineages and can potentially aid in the healing of a variety of tissues.
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1. Introduction

The use of stem cell treatments has the potential to change the face of not only tissue engineering, but all of medicine. The ability to have these stem cells grow, proliferate, and differentiate into tissue make them extremely useful for a wide variety medical applications. A number of stem cell treatments already exist. Currently, the primary use for stem cells in medicine is for bone marrow transplantation. The future of stem cell research includes applications for cancer, spinal chord damage, haematopoiesis, baldness, missing teeth, amyotrophic lateral sclerosis (ALS), deafness, vision impairment, and tissue engineering/drug delivery [1].

Mesenchymal stem cells (MSC’s) are commonly used in tissue engineering because they can differentiate into a variety of cell types. They can be isolated from bone marrow or marrow aspirates and because they are culture-dish adherent, they can be expanded in culture while maintaining their multipotency [5]. MSC’s have been used in preclinical models for tissue engineering of bone, cartilage, muscle, marrow stroma, tendon, fat, and other connective tissues [4, 6, 7, 18, 19, 36, 40, 42, 54]. Several studies have shown that these materials show promise for rebuilding diseased or damaged tissues [24]. Culturing MSC’s is both relatively easy and inexpensive, thus making them a natural choice for study in this project.

Biodegradable polymers have been subject to extensive study in the past couple years in the fields of tissue engineering and regenerative medicine. Because of their unique mechanical properties, hydrogels in particular have the potential to have a significant impact on both of these fields [23]. Hydrogels are hydrophilic polymers that are capable of absorbing water and swelling to much greater sizes. They are inter-penetrating networks (two networks chemically crosslinked) that can undergo a reversible water swelling process that gives them unique and
useful properties. Depending on the polymers used, the way in which they are crosslinked, and side groups that can be added, the properties of these materials can be altered to fit desired needs [27].

Hydrogels were the first biomaterials designed for use in the human body. However, the initial production of hydrogels did not provide any control of their detailed structure [20]. New ideas in designing (stimuli-sensitive phase changes) hydrogels have made them relevant in tissue engineering and regenerative medicine once again [28]. Hydrogel scaffolds have been theorized to be a good mode for delivering stem cells to the site of tissue injury or disease in a 3-D manner, allowing for optimal cell proliferation. The most commonly studied hydrogels for stem cell culturing are polyethylene glycol (PEG) based. PEG is a very important biopolymer at this time because it is one of the only FDA approved polymers [22]. PEG’s copolymers are even more useful. PEG, when photo-crosslinked with fibrinogen, produces a hydrogel with an adjustable molecular design, enabling changes to be made to certain properties such as mesh size and permeability [27]. The Pluronic family [32], Polyglutamic acid (PGA) [35], PGA-PVA copolymers [37], Pullulan based hydrogels [42], hyaluronan based polymers (i.e. HA) [44], and carboxyl methyl cellulose (CMC) are other hydrogels that have potentially useful properties for stem cell delivery purposes [45].

Previously, the use of stem cell delivery to the site of injured or diseased tissue was devalued due to low cell viability rates [49]. Injection has been the most researched form of delivery. It has been shown that the shear forces inside the needle are far too great to inject cells on their own. Different media, such as saline and collagen, have been used as carriers and have shown that they can enhance cell viability [51]. However, the lack of material strength and other physical properties did keep the cells from differentiating and proliferating successfully at the
site if the injury. It is theorized that hydrogels can be a more efficient way of delivering and maintaining stem cells to the site of damaged or diseased tissues.

2. Literature Review

2.1. Stem Cells

Stem cells have the remarkable potential to develop into many different cell types in the body [1]. They serve as a repair mechanism where they can be transplanted in a human and divide limitlessly and can substitute damaged cells or replenish cells as well. When a stem cell divides it can either stay a stem cell or differentiate into a specialized cell such as a neuron, muscle cell, or cardiac cell. The three properties which separate stem cells from other cells is that they are capable of dividing and renewing themselves for long periods, they are unspecialized, and they have the ability to give rise to specialized cells [1]. The two main categories that stem cells can be divided into are embryonic stem cells and adult stem cells. Embryonic stem cells are located as the name states from the embryo, whereas adult stem cells are undifferentiated stem cells found in pools of differentiated cells in tissues or organs. The difference between the two categories of stem cells are mainly that embryonic stem cells are pluripotent, which means that they can differentiate into a variety of specialized cells, while adult stem cells are usually restricted to the organ or tissue where they were found.

2.1.1. Mesenchymal Stem Cells

Each year, millions of people suffer from tissue loss and end-stage organ failure [2]. Allogenic transplants have saved many lives. However, a shortage of organ donors limits the use of this therapy. A variety of transplants have the ability to be revolutionized by the use of
autologous stem cells as a source of donor tissue. The requirements of these cells for this application are that they should be isolated ex vivo and have multipotential precursors for diverse tissues. The adult human mesenchymal stem cell meets these requirements and is why it has been investigated as a potential substitute for organ transplantation.

Mesenchymal stem cells are non-hematopoietic cells which are derived from a variety of areas in the body. They can be derived from bone marrow, umbilical cord, the stroma of the thymus and spleen, and synovial fluid. Bone marrow is considered the most effective means of extracting mesenchymal stem cells due to its high concentration of MSCs [7].

2.1.2. Culturing Mesenchymal Stem Cells

The method of isolating and culturing MSCs was developed in the mid 1970’s by Friedenstein and has since been modified in the 1990’s [3]. This method has survived more than 30 years as the primary method of isolation and culturing. Bone marrow must be donated, and mononuclear cells (MNCs) must be prepared from the marrow through density sedimentation on Percoll and then cultivated on Petri dishes in a DMEM-LG medium with 10% fetal bovine serum. These MNCs are deprived of hematopoietic cells non-adherent to the dishes after culturing for 48 hours. The remaining cells, MSCs adhered to the dishes. The cells were spindle shaped and formed foci of more than 30 cells after culturing for 12 days. These MSCs were also homogeneous as well. [4] In order to indicate mesenchymal stem cells, alkaline phosphates positive ALP$^+$ reticular cells are associated closely with mesenchymal stem cells and ALP$^+$ staining are indicators of (MSCs) [6].

2.1.3. Differentiation in Mesenchymal Stem Cells
Mesenchymal stem cells have the ability to be induced to differentiate into bone, adipose, cartilage, muscle, and endothelium is these cells are cultured under specific permissive conditions. Mesenchymal cells can also differentiate into cells of alternate germinal derivation such as neural cells, skin, and liver, which proves how versatile mesenchymal stem cells are in regenerative medicine. Mesenchymal adult stem cells have shown the ability to differentiate into cells out of their specific organ cell lineage, but in rare cases. In terms of controlling differentiation for mesenchymal stem cells, the type of media as well as growth factors is crucial to obtain the specific cell lineage of interest.

Differentiation is caused by various factors such as growth factors. The four main growth factors that have been studied for differentiation include platelet derived growth factors (PDGF), basic fibroblast growth factors (bFGF), transforming growth factor β (TGF-β), and epidermal growth factor (EGF). For example, for chondrogenic differentiation, the MSC must centrifuge to form pellets and then growth factor-β3 was introduced. The pellets form a matrix and type II collagen was detected after 10 to 14 days. For osteogenic differentiation hMSC are derived from bone marrow and exposed to dexamethasone, β-glycerol phosphate, and ascorbate, as well as 10% FBS. Calcium accumulation is evident after 1 week. The growth factor and proteins are the determinant for differentiation for mesenchymal stem cells.

2.1.4. Applications for Mesenchymal Stem Cells

There are a variety of applications for mesenchymal stem cells due to the pluripotency of MSC’s. The major application is for orthopedic needs, such as cartilage replacement, bone fractures, and bone loss. Although there haven’t been significant tissue engineered bones or cartilage using stem cells, they have the ability to solve disorders involving osteoporosis, ligament and tendon damage. Mesenchymal stem cells can be applied to the tissue site of need
and can differentiate with the appropriate growth factors to induce differentiation. Mesenchymal stem cells can also be used to differentiate into skin cells as a skin substitute. The modern skin grafts lack some of the necessary cellular components that human skin possess, if mesenchymal stem cells can overcome this limitation it could be used for cosmetic needs, burn victims, or skin disorder substitute. Mesenchymal stem cells could be induced to promote proliferation in the liver and cure liver failure as well. It has been seen that mesenchymal stem cells can differentiate into liver cells as stated before. The potential for mesenchymal stem cells are limitless, but these cells cannot be injected without a scaffold or some type of matrix that holds these cells together. An environment to house these cells for growth and multiplication as well as transportation to the injured tissue site is necessary for an efficient stem cell delivery.

2.2. Hydrogels as Biomaterials

Hydrogels are hydrophilic polymeric materials that upon absorption of water swell to several times its size, maintain a distinct three dimensional size, and takes on unique mechanical and chemical properties. More specifically, hydrogels are crosslinked inter-penetrating networks, which can be synthesized a number of different ways. Initially, the crosslinking between polymers needed to create hydrogels was obtained through copolymerization, reaction of polymer precursors, and polymer-polymer reaction. Hydrogels were the first biopolymers designed to be used in the body [Wichterle et al, 1960]. The very first hydrogels were first introduced in the 1960’s, but were dismissed soon after because of their poor properties. The poor properties of the initial gels were due to the synthesizing methods mentioned above. They are rather simple and do not allow for specific control over the detailed structure of the polymer.

However, new and advanced methods of synthesizing hydrogels have once again made them a prevalent topic in the Biomaterial research. Among the many materials being studied
today in tissue engineering, hydrogels are receiving more attention due to their ability to retain water, good biocompatibility, low interfacial tension, and minimal mechanical and frictional irritation [29, 30]. Studies by Gong, et al, have shown that hydrogels can now be synthesized to have great mechanical strengths [60]. They can also be made with controlled porosity, which can control degradation rates as short as a couple of hours to as long as a couple of years. This is an extremely useful ability in drug delivery [8-10]. With the advancements in nanotechnology, nanotubes can reinforce the mechanical strength and structure within the hydrogel depending on the desired function [11]. Exact micropatterns can be made to the gel using by photolithography in order to enhance the gel’s specific function [12]. Nanopores and nano-scaffolding are important in tissue engineering because they provide a foundation for cell adhesion, growth, and differentiation. The development of stimuli sensitive hydrogels has the potential to be a key breakthrough in the field of drug delivery and tissue engineering. These hydrogels can change from a liquid phase to a gel phase based on pH levels and temperature changes [13-17, 28]. Some of these gels, like poly(N-isopropylacrylamide)/dextran-maleic acid (pNiPAAm) can be injected into the body as a carrier for cells, drugs, etc. easily in its liquid phase at 20 degrees Celsius. Once at the site of interest, the temperature will rise past the lower critical solution temperature (LCST) and a body temperature will congeal into a biodegradable gel, providing the physical properties needed for the particular application.

Stem cells of varying kinds have shown that they are able to remain viable, proliferate, and differentiate in certain biocompatible polymers [18]. Three dimensional scaffolds carrying bone-growth inducing proteins have been successfully used in hydrogels to help the regeneration of bone tissue [19]. Mesenchymal stem cells in particular have shown some success in differentiating into mature osteoblasts to form mineralized matrices within hydrogel scaffolds
Although, most studies have been done in vitro, there is significant data to suggest that the delivery of stem cells to the site of injured biological tissue via hydrogel injection could be an integral part of the future of regenerative medicine.

There is a wide array of hydrogels that have been studied for their usefulness in engineering. Poly(ethylene glycol), the Pluronic family, polyglutamic acid, polyvinyl alcohol, Pullulan, hyaluronan, and carboxyl methyl cellulose are the hydrogels that will be focused on for this project.

2.2.1. Poly (ethylene glycol)

Poly (ethylene glycol), or PEG, is one of the most useful and widely used polymers in terms of biomedical engineering because it is one of the few polymers that are FDA approved. It is one of the simpler polymers on its own, which makes it an easy polymer to manipulate. Below is the mer structure of PEG.

![Mer Structure of PEG](image)

**Figure 1: Mer Structure of PEG**

PEG has been shown to be nontoxic, non-immunogenic, and non-antigenic [21-23]. PEG hydrogels have been used as a scaffold for successfully repairing and regenerating tissues such as cartilage and bone [24-26]. Because of its mechanical properties and highly swollen three-dimensional shape, it is very similar to soft tissues with high water content. This allows for the
diffusion of cellular waste and necessary nutrients. PEG is bioinert and naturally non adhesive, making it a poor choice for natural cell adhesion. In order to make up for this, nanopillars can be created in the hydrogel. Kim DH et al, has shown that ultraviolet-assisted capillary lithography can be successfully used to create uniform pillars (D~150nm, h~400nm) [61]. The presence of the nanopillars led to the three-dimensional growth of cardiomyocytes.

Basic PEG hydrogels, because of their resistance to protein absorption, are unable to support adhesion of human mesenchymal stem cells on their own. However, by forming a mineral phase throughout the hydrogel, cell adhesion can be greatly improved [29]. Nuttleman et al have shown that the presence of the phosphate containing molecule ethylene glycol methacrylate phosphate (EGMP) can increase cell viability of MSCs from 15% without EGMP to 97% with EGMP. Glucosamine and dexamethasone have also been shown to affect PEG in such a way [30, 31].

PEG also has a wide array of copolymers that can be used in biomedical engineering applications. Arcaute et al, conducted a study in which stereolithography was used to construct complex bioactive PEG dimethacrylate based hydrogels. They were able to show that these hydrogels could support 87% viability of human dermal fibroblasts at two and twenty-four hours following fabrication [34]. It is reasonable to assume that provided the controlled presence of the appropriate growth factors and proteins, this gel should support mesenchymal stem cell viability and proliferation.

PEGylated fibrinogen can be controlled chemically in order meet certain desired properties. PEG diacrylate and fibrinogen can be crosslinked using photoinitiation in the presence of cells to form a dense culture of hydrogel network. Fibrinogen maintains the biofunctional backbone, while molecular changes are made to PEG, adjusting certain properties
such as mesh size and permeability [27]. Smooth muscle cells were used in the study by Dikovsky at al. These cells were shown to integrate into the PEG-DA at a constant rate and the cells showed a 91% increase after one week.

When PEG is crosslinked with another polymer, it is able to maintain its good physical properties while also gaining properties of the other polymer. A copolymer of PEG and pNiPAAm can be synthesized to create a thermo-responsive hydrogel [28]. This hydrogel can be injected at a liquid phase and congeal to a gel once inside the body. Cell detachment is able to be controlled as well, which could be helpful depending on how long the cells need to remain inside the gel at the injured tissue site.

2.2.2. Pluronic

Pluronic is actually a trademarked name for the polyoxypropylene-polyoxethylen block copolymer. It is another good biocompatible polymer with good materials and also exhibits thermal stimulated phase changes. Its thermal sensitive properties will not be of any use to us in this project because of its lower critical solution temperature.

![Figure 2: Mer Structure of Pluronic F127](image.png)

Pluronic F127 is a member of the Pluronic family that takes the form of a hydrogel. Its uses as a stem cell scaffold are almost exclusively limited to tissue engineering bone. Huang et al have constructed a Pluronic F127 based scaffold for MSCs. Their results showed that both alkaline phosphates and calcium levels increased after both seven and fourteen days of incubation, indicating osteoblastic differentiation. Using alizarin red S staining, they were able
to determine that the MSCs could survive and differentiate into osteoblasts while within the Pluronic F127 based hydrogel scaffold [33]. Pluronic F127 has also shown that it will promote alveoli tissue growth from somatic lung progenitor cells without inflammatory reaction [34].

However, another study conducted involving MSCs suspended in a Pluronic F127 hydrogel scaffold showed that the MSCs, while again promoting the formation of bone tissue in vitro, did not promote any formation of bone tissue in vivo.

2.2.3. Poly (glutamic acid)

Poly (glutamic acid), or PGA is a commonly used polymer with a relatively simple mer structure that, much like PEG, can be altered to adjust certain physical properties. PGA is a lightly hydrophilic, biodegradable polypeptide that has good physical properties (very high Young’s Modulus). When copolymerized with other biocompatible polymers, PGA can form useful hydrogels.

\[
\begin{align*}
\text{O} \\
\text{||} \\
\text{O} - \text{C} - \text{CH}_2 - \text{n}
\end{align*}
\]

\text{Poly(glycolic)acid}

Figure 3: Mer Structure of PGA

PGA has shown to be an excellent polymer for culturing specific cell types in vitro [35, 37]. While cell viability will not be a problem for a PGA based hydrogel, biocompatibility may be. Depending on the tissue of interest, PGA may trigger an immunogenic response. A study of PGA hydrogel scaffolds containing somatic lung progenitor cells showed that in vivo, an
immunocompetent host induced a foreign body response that altered the integrity of the developing tissue [35].

Because of its simple mer structure, PGA is easily copolymerized easily. A 72% sulfonated gamma-PGA (γ-PGA-S72) gel has lower swelling rates than PGA, high sulfonic acid group concentrations, and had high fibroblast growth factor activity [36]. Because of this, both cell adhesion and proliferation rates were higher than that of PGA after twenty four hours (γ-PGA-S72: 52.8%; PGA: 20.7%). PGA can also be crosslinked with Polyvinyl alcohol (PVA) to form a PGA-PVA inter penetrating network hydrogel. This gel exhibits good thermal stability and controllable swelling ratio. The tensile strength is not as high (15-30% lower) as native PVA (6.37 MPa), whereas the elongation was increased by 200 to 250%. It also has good blood compatibility and will not induce clotting when in direct contact [38].

2.2.4. Polyvinyl Alcohol

Polyvinyl alcohol (PVA) is also used on its own because of its very high Young’s modulus and ultimate tensile strength. When made into a hydrogel, theses properties can be controlled by the ratio of polymer concentration to water. By altering the number of crosslinks in its chain, highly elastic PVA gels can be obtained. PVA has also been found to support attachment and proliferation of fibroblasts [39].

![Mer Structure of PVA](image)

**Figure 4: Mer Structure of PVA**
Hydrogels have been prepared from PVA and chitosan (40 wt % chitosan content) can support fibroblast cell attachment and proliferation [40].

2.2.5. Pullulan

Pullulan is a neutral, linear, and non-immunogenic polysaccharide that has good mechanical properties and is biocompatible. Currently, the chief commercial use for pullulan is in oral hygiene films (breath fresheners) because of its edible and tasteless nature. It has also been widely used in the pharmaceutical and cosmetic industries for its functional properties that include adhesiveness, film formability, and its controlled degradability. Pullulan can also take the form of a hydrogel. Pullulan was found to be a hydrogel particularly suited for in vitro studies because of it is non-cytotoxic, degrades via enzymes, and it can be cut or molded to the desired shape easily [41, 42]. Handling and shaping of other hydrogels is difficult, but pullulan based gels can be handled, molded, and shaped with ease.

Figure 5: Mer Structure of Pullulan

Because of its high water content (90%), pullulan based hydrogels can expect limited cell adhesion on its surface. Autissier et al showed that a pullulan based gel can very well support cell viability, although with limited migration and proliferation [42]. Pullulan created with nanotubes or crosslinked with another biopolymer may provide better results.

2.2.6. Hyaluronic Acid
Hyaluronic acid is a natural carbohydrate polymer that can be found in several parts of the body, such as synovial fluid, cartilage, and skin. Because it is naturally found in the body, HA is well suited for use in many biomedical applications. HA has been used since the 1970's in ophthalmic surgeries and also to treat osteoarthritis in the knee. It is biocompatible and also can be found in the extracellular matrix of tissue, making it an increasingly popular material in tissue engineering.

![Mer Structure of HA](image)

**Figure 6: Mer Structure of HA**

HA hydrogels can support stem cell viability, proliferation, and differentiation [43]. HA was shown to be able to polymerize in situ while delivering stem cells as well as other biomolecules such as growth factors. HA, like PEG, has anti-adhesive effects and does not naturally allow protein absorption. Because of this, cell viability in vitro is not good. Therefore, the cells in the HA hydrogel must have additional extracellular matrix (ECM) proteins for adhesion and proliferation. MSCs have been shown to have an 81% cell viability rate and a round morphology in vitro after two days. While 81% viability is still not ideal, Kim et al [44] showed that bone regeneration in vivo demonstrated different results. HA hydrogels, along with the appropriate growth factors and proteins, can be used for regeneration in various tissues, such as bone, cartilage, heart, and nerve.
HA has also been crosslinked with PEGDA and modified with peptides containing the Arg-Gly-Asp sequence (RGD) to form an injectable polymer that supports cell attachment, spreading, and proliferation. Shu et al showed that in vitro, over 90% of human fibroblast cells maintained viability and 53% of the cells spread in the gel. The gel also increased cell differentiation by 33%. At day 15, the cells had proliferated over 250% of the initial count. This fibroblasts and peptides were then injected via the HA-PEGDA hydrogel into rats. As a result, the formation of the fibrous tissue was accelerated in vivo and the fibroblasts in the new fibrous tissue increased the production of procollagen within four weeks [62].

### 2.2.7 Carboxymethylcellulose

Carboxymethylcellulose (CMC) is a derivative of cellulose formed by its reaction with alkali and chloroacetic acid. At low concentrations, CMC molecules are rod-like. However, increasing the concentration causes the molecules to bend, curl up, and eventually entangle to become a thermo-reversible gel. CMC has the ability to absorb water twenty times its own weight.

![Figure 7: Mer Structure of CMC](image)

CMC has been crosslinked with superoxide dismutase and studies have conducted in vitro to determine the human fibroblast viability and proliferation. The CMC hydrogel showed a 47% viability rate after forty-eight hours and also that the cells migrated as well [45]. However,
it remains to be seen if these viability rates will be sufficient in vivo. More testing is needed on the biocompatibility of CMC and its copolymers.

2.3. Stem Cell Delivery Methods

The uses of mesenchymal stem cells (MSCs) in regenerative medicine are a promising approach for tissue engineering. MSCs have been proven to have great potential in regenerating tissues of musculoskeletal [46], cardiovascular [47], and neurological systems [48]. However, the outcomes of existing treatments have not been satisfactory. Current methods of delivery involve the use of injections and microencapsulation to implant MSC-based media into the body, however they are not efficient. By means of injection there is an immediate loss of the majority of the cells implanted due to back-flow via the injection path [49]. Also, the shear forces and turbulences generated during injection lead to low engraftment and poor functional remodeling of the cells [50]. By means of microencapsulation a large majority of biomaterials proved to have impenetrable membranes to cells. These materials restricted the use of MSCs in regenerative medicine and tissue engineering because MSCs requires intimate host-implant integration at the cellular level. As a result, the quest for a more effective approach, noninvasive by nature, is among the biggest technological challenges in regenerative medicine today.

2.3.1. Media Used in Cell Delivery

In the delivery of MSCs a variety of different media, ranging from saline, natural and synthetic hydrogels, sodium alginate, agrose [51], and polyethylene glycol [49], have been tested, however suspending the cells in saline or hydrogel, for delivery via injection, are the two most common approaches. Hydrogels of both natural and synthetic biomaterials, hyaluronan gel [52] and 2-hydroxyethyl methacrylate [53] respectively have been tested, but result in poor cell viability.
These carriers were not able to provide anchorage to the cells being delivered nor were they sufficient in protecting them against the hostile environment at the injury site, consisting of cytotoxic inflammatory cytokines [54] and matrix-eroding proteases [55]. Hyaluronan gel and 2-hydroxyethyl methacrylate when injected lead to an immediate loss of the majority of the cells implanted due to back-flow via the injection path, resulting in a low local availability of cells and an insufficient viscosity or stiffness of the hydrogel [56]. Sodium alginate, agrose and polyethylene glycol, all have impenetrable membranes to cells, restricting MSC and due to MSCs’ need for intimate host-implant integration at a cellular level and sensitivity to anchorage dependant survival-apoptosis regulation [57], the use of them results in poor cell viability [58].

2.3.2. Delivery Problems and Complications

Current methods of delivery involve the use of injections and microencapsulation to implant MSC-based media into the body, however they are not efficient. By means of injection there is an immediate loss of the majority of the cells implanted due to back-flow via the injection path resulting in a low local availability of cells and an insufficient viscosity or stiffness of the hydrogel. Also, the shear forces and turbulences generated during injection lead to low engraftment and poor functional remodeling of the cells. By means of microencapsulation a large majority of biomaterials proved to have impenetrable membranes to cells. Existing transporters were not able to sufficiently protect MSCs against the hostile environment at the injury site ensuing poor cell viability, but the use of microencapsulation proved trustworthy.

Microencapsulation is the process of entrapping cells within the boundaries of a semi-permeable membrane forming a homologous solid mass, a microsphere. This method has been used in aiding immunoisolation during allogenic or xenogenic cell transplantation. Existing microencapsulation techniques involve vigorous mechanical disturbances such as pressurized
nozzles, emulsification, or stirring during droplet generation [59], but perhaps an alternative means is achievable.

3. Project Approach

The literature review served as a backbone for our project. It gave us the necessary research to initiate ideas and clarify and define our project. In order to clarify and define our project, an initial problem statement, forming a project hypothesis, defining assumptions, and defining the specific aims of the project.

3.1. Clarification of the Initial Problem Statement

After consulting with our client and advisor as well as extensive background research, we formulated a basic problem statement for our project. “Develop an efficient delivery system for stem cells using a biopolymer.” This problem statement lacked depth and detail to produce a statement that truly described the extent of our problem. Therefore a defined problem statement was needed and thus became the first step in our design process.

Consulting with our client and advisor to formulate a clear understanding of our problem was the next step. The basic research on a variety of areas including hydrogels, delivery vehicles, and culturing stem cells had been done. Based on the problems presented in previous experiments, we have decided that our project requires the design of a new delivery method using hydrogels as a carrier. The overall designs function that the device must maintain cell viability. Cell viability is the major problem that past systems of delivery had and producing a method that increases this factor would be influential to the field of regenerative medicine. Conveying to our client that this mechanism should transport the stem cells to the area of tissue injury was important as well. One of the main obstacles to overcome was deciding which stem
cells to use for the system. After consulting with another team working on culturing stem cells and obtaining their advice, mesenchymal stem cells was a natural choice. Mesenchymal stem cells exhibit pluripotency or the ability to differentiate into a number of cell lineages as well as their accessibility and ease of differentiating. The design team formulated that an experiment transporting stem cells from Petri dish to a separate Petri dish will provide an acceptable scenario of how the hydrogel will react in the body. Therefore we formulated the following revised client statement:

“Design a mechanism for safely transporting harvested mesenchymal stem cells from a Petri dish to a site of tissue injury, maximizing cell viability while maintaining pluripotency.”

This statement portrays to our client and users exactly what the design entails and the features produced by the design.

3.2. Project Hypothesis

As stated before, the goal of our project is to design a mechanism for safely transporting harvested mesenchymal stem cells from a Petri dish to a site of tissue injury while maintaining cell viability. Current methods of delivery have problems with an efficient cell viability rate for stem cells. Upon injection, the shear forces from the needle are too great for the cells to withstand and will either crush them or induce apoptosis, causing a substantial decrease in the amount of viable cells for implantation. Another limitation of using hydrogels to transport cells via injection is that the viscosity of the hydrogel should be high enough to cause laminar flow. Turbulent flow, the antagonist to laminar flow, causes the cells to die easily due to the rotation and shear forces being applied on the area of the gel being greater.
Therefore, our team hypothesized that breaking the project down into three components.

1) Identify materials, such as the proper hydrogels, that were compatible with growing the cells.
2) Develop a delivery mechanism for ease of transfer. 3) Demonstrate feasibility for the entire system. The design team believes that a combination of these components to our project will provide a mechanism which will be more effective at maximizing the viability of the mesenchymal stem cells compared to the standard method of injection.

3.3. Project Assumptions

The hypothesis of the project requires an implementation of an innovative delivery system involving injection as well as an appropriate hydrogel that will maximize cell viability and provide a more efficient means of delivery compared to current methods. Our assumptions therefore are as follows:

- Shear forces, apoptosis, and the loss of media during implantation are the outlining problems associated with cell viability
- An appropriate hydrogel will maximize cell viability throughout the process of delivery as well as support differentiation ultimately
- Transporting the mesenchymal stem cells in hydrogel from Petri dish to Petri dish will provide an acceptable simulation of how the hydrogel will act when implanted in the body

3.4. Specific Aims

Maximizing cell viability is the major factor involving our design. Therefore our main test will involve an initial Petri dish with a predetermined amount of mesenchymal stem cells. After which the hydrogel with the MSCs will be implanted in the device and then injected onto another Petri dish and the cell viability will be recorded. If the cell viability of the
hydrogel/hMSC combination after the completion of a delivery is higher than that of cells suspended in media after the completion of a delivery, than it can be concluded that the mechanism is an efficient means of maximizing cell viability. Therefore our specific aims are as follows:

- Produce a delivery method using hMSCs suspended in hydrogel that maximizes cell viability higher than cells suspended In media.
- Design an innovative delivery mechanism which limits the forces applied on the cells.
- Implement a hydrogel which will promote cells to function accordingly in the tissue.
- Conduct and design experiments to assess the hypothesis.

4. Design

This section describes the design process to develop a hMSC delivery system utilizing hydrogels as a delivery vehicle. The design process involves three groups of individuals: clients, design team, and users. The clients, Professor Gaudette (Biomedical Engineering professor) and Al Prescott (CEO of Crescent Innovations) provided the initial problem statement of having possible mean(s) of delivering hMSC. Utilizing hydrogels as the delivery vehicle was introduced by Al Prescott who has extensive knowledge in hydrogels, and would benefit his practice greatly with the addition of this design. Both Prof. Gaudette and Al Prescott guided the design team in carrying out the design process and reaching our specific aims. The design team consisted of four biomedical engineers, Jay Shivaprakash and John Bray, who specialized in biomechanics major
at Worcester Polytechnic Institute. Wai-Mun Leung and Kene Mgbojikwe were biomedical engineers who specialized in biomaterials major at Worcester Polytechnic Institute.

The design team continued working together by following the step-by-step design process. The initial problem statement was clarified after constant feedback via meetings with the clients. Afterwards, we followed the five-stage prescriptive model in the design process in order to understand and coherently continue the process to obtain results and to determine a conclusion. Our clients provided their statements at which we formulated our problem definition. Preliminary research was conducted to provide the background literature review and conceptualize design alternatives with specifications. During the preliminary design phase, we evaluate specific stages of the testing phases. Once this is completed with nominal results, we have isolated the type of hydrogel to be used for optimal viable hMSC after transportation from cultivation process. Below is the engineering design process followed by the design team to reach specific aims.

4.1. Objectives, Functions, and Constraints

In order to achieve a true grasp of the problems and necessary components of the design project, we needed to follow the process and outline the objectives, functions, and constraints of the delivery process. The design team decided to outline the general objectives, functions, and constraints of the mechanism of delivery and the components involved that was read through preliminary literature review. After consultation with our clients, we decided to focus on the stages of the delivery process and list the objectives, functions, and constraints accordingly as can be seen in Figure 8 below.
4.1.1. Objectives, Functions, Constraints for Phase 1-A

A detailed list of the objectives and corresponding functions are listed for Phase 1-A:

Culturing and harvesting the mesenchymal stem cells can be seen in Figure 9 below.

**Detailed list of Objectives and Functions for Phase 1-A**

![Figure 9: List of Objectives and Functions for Phase 1-A](image)

- **Successfully culture and harvest hMSCs**
  - Grow in a suitable environment
  - Maintain stemness
  - Produce homogenous hMSC
  - Non-adhesive environment for hMSC

- **Maintain sterility**
  - Sterile hood and instruments
  - Sterile procedure

- **Grow appropriate amount of cells**
  - Reduce layering of hMSCs

- **Time efficient**
  - Duration of time for culturing and harvesting

- **Reusable**
  - Multiply hMSCs continually
Phase 1-A: Culturing and Harvesting Mesenchymal Stem Cells is the first stage in our design of a delivery process. The design team believed that the primary objective for this stage involved successfully culturing and harvesting hMSCs. Since this statement is broad and the term successful is very ambiguous. We decided to narrow its meaning by stating that it should grow in a suitable environment, maintain its stemness, produce homogeneous hMSCs, and create a non-adhesive environment for hMSCs. These are all necessary for proper growth of mesenchymal stem cells as reviewed in the literature. Maintaining sterility is also a necessary objective for Phase 1-A as well. If sterility is not kept in the highest regard then we could compromise the growth of mesenchymal stem cells. If any contamination is present when culturing these cells, it will jeopardize the outcome and validity of the project. Therefore it is important to keep all instruments used in the procedure sterile and use instruments under a sterile hood as much as possible. In order to efficiently test these hydrogels, it is necessary to grow an adequate amount of cells. The design team used this as an objective for this phase, and believes an appropriate amount of cells must be grown and should not be overgrown to cause layering of hMSCs. The design team wanted this procedure to be time efficient considering time is an overall constraint for the project, and the duration of time to culture stem cells and harvesting could cut into the available time. The design team wanted an efficient means to reproduce mesenchymal stem or “double” the hMSCs. Cell doubling would be cost effective and would aid the design team greatly. The various constraints associated with Phase 1-A are detailed in Figure 10 below.

**Detailed list of Constraints for Phase 1-A**
In order to successfully complete Phase 1-A, there must be a degree of sterility that must be maintained. All instruments and devices should fit under the sterile laminar flow hood and fit in the incubator as well. The time for culturing is a constraint because there is a necessary period of culturing and growing that must be done, in order to complete the project. Another constraint is the need for abundant media for growth. The media provides the hMSCs with necessary nutrients and proteins for growth; therefore it is critical to have an abundant supply of media.

4.1.2. Objectives, Functions, and Constraints for Phase 1-B

A detailed list of objectives and corresponding functions are listed in Phase 1-B: Incorporation of hydrogel with mesenchymal stem cells can be seen in Figure 11 below.
### Detailed list of Objectives and Functions for Phase 1-B

<table>
<thead>
<tr>
<th>Objective/Function</th>
<th>Description</th>
</tr>
</thead>
</table>
| **Successfully incorporate hMSCs with hydrogel** | • Promote three dimensional distribution in hydrogel  
• Maintain stemness during implantation  
• Promote proliferation in environment suitable for hMSC growth |
| **Scalable** | • Assess cell count after hydrogel has been implanted  
• Maintain cell number after implantation |
| **Sterile procedure** | • Sterile hood and instruments  
• No contamination |
| **Easy procedure** | • Time efficient  
• Minimal steps in procedure  
• Easy to incorporate hMSC into hydrogel |
| **Cost** | • Cost effective |

![Figure 11: List of Objectives and Functions for Phase 1-B](image)

These objectives relate to Phase 1-B: Incorporation of hydrogel with mesenchymal stem cells. The primary objective is successfully incorporating mesenchymal stem cells with the hydrogel. The meaning of the ambiguous term “successfully” is defined as promoting three dimensional growth in the hydrogel, maintaining stemness in the hydrogel, and promote proliferation in an environment suitable for hMSC growth. These are all necessary functions the hydrogel must maintain or assimilate in order to contain hMSC. The process should be scalable as well; the cell viability should be assessed in order to distinguish whether the hydrogel is adequate for hMSC growth. Like Phase 1-A, the procedure must be sterile for the same reasons as before. The design team believes that the procedure must be easy to perform; therefore it should be time efficient, minimal steps in procedure, and easy to incorporate hMSC into
hydrogel. The procedure should be cost effective due to budget constraints as well. The various constraints associated with Phase 1-B are detailed in Figure 12.

**Detailed list of Constraints for Phase 1-B**

- Allocate equal number of cells for testing
- Duration of time for cells to settle in hydrogel
- Fit under a sterile hood
- Fit in incubator

![Figure 12: List of Constraints for Phase 1-B](image)

The constraints for Phase 1-B show that an equal number of cells must be allocated to the tissue culture environment, whether it is a Petri dish or well plate, should be allocated properly. This is for proving the validity of the study, and to identify which hydrogel performed the best. The duration of time for cells to settle in the hydrogel is a constraint which cannot be overcome. In Phase 1-A, the reason for maintaining the highest degree of sterility was expressed and is necessary for Phase 1-B as well. Therefore it is necessary to fit under a sterile hood and fit in an incubator.

**4.1.3. Objectives, Functions, and Constraints for Phase 2**

A detailed list of objectives and corresponding functions are listed for Phase 2: Loading hydrogel into delivery mechanism in Figure 13 below.
Phase 2: Loading hydrogel into delivery mechanism requires a unique set of objectives. In order to successfully load the hydrogel into the syringe, there should be minimal media loss, maintain stemness, and maintain cell viability as well. The number of cells presented in the syringe should be maintained or increased since the hydrogel was incorporated with the hMSCs. The procedure must be scalable too; since our project deals with the cell viability of hMSC when injected using syringe, there must be a way of quantifying the cell count of hMSCs before loading occurs. Like the previous phases, the procedure should be sterile; which means using sterile instruments and there should be no adverse reaction to the syringe environment. The phase should be easy to perform, time efficient, and there should be minimal steps involved as well. The procedure should be cost effective, due to the budget constraints. A detailed list of the associated constraints can be seen in Figure 14.

**Detailed list of Objectives and Functions for Phase 2**

<table>
<thead>
<tr>
<th>Objective</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successfully load hydrogel into syringe</td>
<td>Maintain or increase cell number since incorporation of hydrogel</td>
</tr>
<tr>
<td></td>
<td>Minimize media loss</td>
</tr>
<tr>
<td></td>
<td>Maintain stemness</td>
</tr>
<tr>
<td></td>
<td>Maintain cell viability</td>
</tr>
<tr>
<td>Scalable</td>
<td>Assess cell viability (pre-injection)</td>
</tr>
<tr>
<td>Sterile procedure</td>
<td>No adverse reaction to syringe environment</td>
</tr>
<tr>
<td>Easy procedure</td>
<td>Time efficient</td>
</tr>
<tr>
<td></td>
<td>Minimal steps in procedure</td>
</tr>
<tr>
<td></td>
<td>Easy perform</td>
</tr>
<tr>
<td>Cost</td>
<td>Cost effective</td>
</tr>
</tbody>
</table>

Figure 13: List of Objectives and Functions for Phase 2

Detailed list of Constraints for Phase 2
The constraints associated with Phase 2 include the syringe must fit under the sterile hood. This is necessary for maintaining sterility and a contamination free environment for the hMSCs. Along with sterility, the syringe should accommodate the necessary environment in terms of temperature, gas mixture, and nutrients for hMSCs. For obvious reasons, the hydrogel must fit into the syringe. The duration of the procedure is a constraint which cannot be overcome. The cost for materials is a constraint, considering the associated budget constraints involved.

4.1.4. Objectives, Functions, and Constraints for Phase 3

A detailed list of objectives and corresponding functions are listed in Phase 3: Injection.
The objectives for Phase 3: Injection has been identified. The primary objective of a successful injection of hydrogel with hMSCs is defined as minimizing media loss, maintaining cell viability, and dispersing uniformly on the host. The success of the injection is dependent upon limiting shear forces of the needle wall, which is the outlining problem for the project. The process should be scalable as well, in order to find out if the shear forces had been limited, a cell viability assessment must be determined and the value should be compared to the initial value. For reasons mentioned before, the procedure must be sterile by using sterile instruments and allowing for no adverse reaction to injection. The procedure must also be time efficient and there should be minimal steps in the procedure. The process should be cost effective due to budget constraints. The associated constraints for Phase 3 can be viewed in Figure 16 below.
Detailed list of Constraints for Phase 3

<table>
<thead>
<tr>
<th>Fit under sterile hood</th>
<th>Suitable environment for hMSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of procedure</td>
<td>Cost</td>
</tr>
</tbody>
</table>

The associated constraints for the last phase of the process shows that the design team is still thinking about maintaining the highest degree of sterility because the team is working with living cells which could be jeopardize due to contamination. Therefore, the host (Petri dish, well plate) should fit under a sterile hood. The host should provide a suitable environment for hMSCs. The duration of the procedure cannot be overcome, and the cost must fit the associated budget constraint.

4.2. Analysis of Needs and Wants

In order to assess the needs and wants for our project, the design team decided to create pairwise comparison charts for each phase. A pairwise comparison chart is a technique used in Engineering Design: A Project-based Introduction. 2nd Ed (Dym, Clive, Little, Patrick. (2004), pp. 24-25) which compares objectives and associates weights to them to rank the objectives in order.
The design team decided that any objective which received a weight of 50% or higher will be considered a need, and a weight of lower than 50% will be considered a want.

4.2.1. Pairwise Comparison Chart for Phase 1-A

Table 1 shows a detailed pairwise comparison chart with associated weight analysis for Phase 1-A: Culturing and Harvesting mesenchymal stem cells.

Table 1: Detailed Pairwise Comparison Chart for Phase 1-A

<table>
<thead>
<tr>
<th></th>
<th>Successfully culture and harvest hMSCs</th>
<th>Maintain Sterility</th>
<th>Grow appropriate amount of cells</th>
<th>Time efficient</th>
<th>Reusable (splitting)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successfully culture and harvest hMSCs</td>
<td>X</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Maintain Sterility</td>
<td>0</td>
<td>X</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Grow appropriate amount of cells</td>
<td>0</td>
<td>0</td>
<td>X</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Time efficient</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>X</td>
<td>0</td>
</tr>
<tr>
<td>Reusable (splitting)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>X</td>
</tr>
</tbody>
</table>

Successfully culture and harvest hMSC = 5/15 = 33.3% = NEED
Maintain Sterility = 4/15 = 26.7% = NEED
Grow appropriate amount of cells = 3/15 = 20% = NEED
Time efficient = 1/15 = 6.67% = WANT
Reusable = 2/15 = 13.3% = WANT
As can be seen the needs associated with Phase 1-A: Culturing and Harvesting Mesenchymal Stem Cells are successfully culturing and harvesting hMSCs, maintaining sterility, and growing appropriate amount of cells. Obviously it is necessary to successfully culture and harvest hMSC in order to proceed to the next phase. Sterility has been mentioned several times in this section, and through the analysis proves to be an outlining feature in Phase 1-A. The design team needs to grow appropriate amounts of cells in order to have a great enough supply to test and distinguish which hydrogel performs the best.

### 4.2.2. Pairwise Comparison Chart for Phase 1-B

Table 2 shows a detailed pairwise comparison chart with associated weight analysis for Phase 1-B: Incorporation of hydrogel with mesenchymal stem cells.

**Table 2: Detailed Pairwise Comparison Chart for Phase 1-B**

<table>
<thead>
<tr>
<th></th>
<th>Successfully incorporate hMSC with hydrogel</th>
<th>Scalable</th>
<th>Sterile procedure</th>
<th>Easy Procedure</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successfully incorporate hMSC with hydrogel</td>
<td>X</td>
<td>½</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Scalable</td>
<td>½</td>
<td>X</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sterile procedure</td>
<td>0</td>
<td>0</td>
<td>X</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Easy procedure</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>X</td>
<td>½</td>
</tr>
<tr>
<td>Cost</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>½</td>
<td>X</td>
</tr>
</tbody>
</table>
Successfully incorporate hMSC with hydrogel = 4.5/15 = 30% = NEED
Scalable = 4.5/15 = 30% = NEED
Sterile procedure = 3/15 = 20% = NEED
Easy procedure = 1.5/4 = 10% = WANT
Cost = 1.5/4 = 10% = WANT

For Phase 1-B: Incorporation of hydrogel with mesenchymal stem cells the pairwise comparison chart shows that successfully incorporating hMSCs with hydrogel, scalable, and sterile procedure are the associated needs for the phase. Successful incorporation of hMSC with hydrogel is necessary in order to proceed to the next phase and is very important to assess if the hydrogel will be able to promote growth for hMSCs. Scalability is necessary in order to assess qualitatively or quantitatively how effective these hydrogels perform and all hMSC to be viable with hydrogel. A sterile procedure for reasons mentioned previously in the section, which is why it is analyzed as a need.

### 4.2.3. Pairwise Comparison Chart for Phase 2

Table 3 shows a detailed pairwise comparison chart with associated weight analysis for Phase 2: Load hydrogel into delivery mechanism.

**Table 3: Detailed Pairwise Comparison Chart for Phase 2**

<table>
<thead>
<tr>
<th></th>
<th>Successfully load hydrogel into syringe</th>
<th>Scalable</th>
<th>Sterile procedure</th>
<th>Easy Procedure</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successfully load hydrogel into syringe</td>
<td>X</td>
<td>½</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Scalable</td>
<td>½</td>
<td>X</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sterile procedure</td>
<td>0</td>
<td>0</td>
<td>X</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Phase 2: Loading hydrogel into delivery mechanism shows that the associated needs are successfully loading hydrogel into syringe, scalable, and sterile procedure. In order to proceed to the next phase, successful loading of the hydrogel into the syringe must be met. The process needs to be scalable, in order to obtain results and prove the validity of the study. A sterile procedure is necessary because of the reasons mentioned before in the section.

4.2.4. Pairwise Comparison Chart for Phase 3

Table 4 shows a detailed pairwise comparison chart with associated weight analysis for Phase 3: Injection.

<table>
<thead>
<tr>
<th></th>
<th>Successful injection of hydrogel with hMSCs</th>
<th>Scalable</th>
<th>Sterile procedure</th>
<th>Easy Procedure</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successful injection of hydrogel with hMSCs</td>
<td>X</td>
<td>½</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Scalable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.3. Design Specifications

Successful injection of hydrogel with hMSCs = 4.5/15 = 30% = NEED
Scalable = 4.5/15 = 30% = NEED
Sterile procedure = 3/15 = 20% = NEED
Easy procedure = 1.5/15 = 10% = WANT
Cost = 1.5/15 = 10% = WANT

For Phase 3: Injection the needs are as stated; successful injection of hydrogel with hMSCs, scalable, and sterile procedure. A successful injection of hydrogel with hMSCs has been defined before, and is important to conclude the process. Scalability is equally as important because the project goal is to assess the cell viability and find out which hydrogel reduces the cell viability after injection the best, therefore a need for quantifying or visually analyzing the cell viability is necessary. A sterile procedure is necessary as well for the reasons mentioned before in the section, and is why it has been assigned as a need.

Thus far in this chapter, we have sufficiently identified the objectives and functions of our project. With this having been done, we are now able to establish the specifications of our project which will describe certain goals that the design must meet in order to be successful.
Again, our specifications will be congruent with the rest of our design process and be broken down into three phases:

**Phase 1A and 1B: culturing human mesenchymal stem cells and incorporation with hydrogels.**

**Phase 2: Transportation of hydrogel containing mesenchymal stem cells into delivery mechanism**

**Phase 3: Injection of hydrogel containing mesenchymal stem cells through a needle.**

The specifications are described in detail in the next section. The sections describe the particular specifications of each phase, the method in which they will be tested, and the criteria that must be met in order to be considered a success.

### 4.3.1. Design Specifications for Phase 1A

In order to grow a sufficient amount of hMSCs, the team decided to use T-75 flask, to create a large bank of mesenchymal stem cells. In order to ensure cell growth and stemness we used Dulbecco’s Modified Eagle’s Media (DMEM) along with 10% fetal bovine serum and penicillin as well to feed the cells and allow to plate on the T-75 flask. To ensure viability, our team decided to change the media and supplement the flask with fresh media every 3 days. A sterile procedure was used by performing all experiments under a hood, with proper sterile procedures taken into account such as spraying instruments with 70% IPA and using pre-packaged pipettes.

**Design Specifications for Phase 1-A**

- Cell Viability based histology and MTS Assay
- Sterility based on histology
4.3.2. Design Specifications for Phase 1B

In order to optimize the design of our device, we decided to incorporate hydrogel with mesenchymal stem cells in a 24 well plate. We believed the 24 well plate would allow us to maximize the number of hydrogels while using the limited number of cells we had. In order to assess cell viability we tested all combination of cells and hydrogel using an MTS assay which measures absorbance of light through the gel. Next, we must acquire a linear relationship of the cell viability of increasing cell number. From this relationship, we then can correlate the MTS results to the estimated cell number. Thus, the absorbance level can be converted to cell number which can be compared to the initial count. In order to ensure stemness and migration, we decided to place the cells on top of the hydrogel, in order for the cells to fully disperse through the gel and inter mix within the gel thoroughly.

Design Specification for Phase 1-B

- 2000 cells per 750 µL incorporated into hydrogel
- Cell viability must be at 60 % from initial count after 10 days based on histology and MTS Assay
- Three-dimensional distribution based on observation via inverted microscope

4.3.3. Design Specifications for Phase 2

In order to ensure that our design was optimal to our objective, constrains, and functions, several design modifications needed to be made. We decided that the best way maximize cell viability while minimizing volume loss is to limit the amount of times the hydrogel was put under pressure or forces were induced on the gel. Another specification is that this design is
designed for various needle gauges. We also require that our design be used using sterile methods as well as used strictly under a sterile hood.

**Design Specifications for Phase 2**

- Cell viability based on histology and MTS Assay
- Compatible with existing needles
- Less than 20% residual hydrogel volume

**4.3.4. Design Specifications for Phase 3**

A safe effective delivery was the ultimate goal of this device. In order to ensure that we had to make sure there was enough pressure to force the gel through the needle. We decided that the needle can be at various diameters using the Reynolds number equation. Also we need to make sure the viability is not compromised, by doing transferring from Petri dish to Petri dish and using MTS assay and histology.

**Design Specifications for Phase 3**

- Cell viability must be 45% compared to original count based on histology and MTS Assay
- Flow profile based on Reynold’s number, below 3000 for laminar flow.

**4.4. Revised Client Statement**

Our initial client statement was undefined and incomplete

“Design a mechanism for safely transporting harvested mesenchymal stem cells from a Petri dish to a site of tissue injury, maximizing cell viability while maintaining pluripotency.”
Having completed a detailed analysis of the objectives, functions, constraints, and specifications, we now have a much clearer of the design space in which our delivery system can be created.

We are now able to form a more concise client statement.

“Design and develop a hydrogel based system for safely transporting harvested human mesenchymal stem cells from a culture flask to the site of tissue injury, maximizing cell viability while maintaining pluripotency. The human mesenchymal stem cells should be incorporated into a hydrogel at a density of 2000 cells per 750 µl and maintain 60% cell viability after 10 days. The cells must be transported to the delivery mechanism while maintaining this cell viability and pluripotency. Upon delivery from the mechanism to the injured tissue site, 45% of cells must remain viable, as well as be located within the hydrogel to promote migration and integration with the surrounding tissue. The hydrogel itself must maintain its structural integrity through the delivery process, protecting the cells in the mechanism as well as at the site of injury until degradation.”

4.5. Conceptual Designs

At first our conceptual designs focused on the needle head. Our team thought that manipulating the forces induced on the needle head could have a positive effect on the viability of the cells. Here are some of the designs that were constructed and a brief statement about each design.

Figure 17: Conceptual Design 1
Conceptual design 1 was a surface treated polymer lining for the needle head. This design was implemented to reduce the shear forces on the cells as well as create a flexible lining which could form to the gel.

**Figure 18: Conceptual Design 2**

Conceptual design 2 used a multi-porous needle which was created to limit the shear force induced on the cells by allowing for the gel to perfuse out of the holes and allow for more surface area for ejection.

After consulting and proceeding through the design process, the design team decided to gear our conceptual designs towards phase 2 of the process which was the loading phase. We
formulated out conceptual designs based on the objectives that were formulated moths ago such as reducing media loss, reducing shear force, and cell viability. The following are some conceptual designs geared towards the loading phase as well as a brief description of each.

**Figure 19: Conceptual Design 3**

Conceptual design 3 was created to use a cartridge that can be loaded from the rear. This delivery method exposes the cells to forces in one direction, ejection.
4.6. Design Matrix for Conceptual Designs

After formulating the conceptual designs, the next step was to evaluate the conceptual designs based on objectives and functions, and choose the appropriate design. The objectives and functions the design team thought was necessary for the design to possess include: maintain hMSC viability, easy to load, simple to use, cost effective, compatible with existing instruments, decrease media loss. As can be seen from the table, the cartridge design was the nest design and is what we used as our final design.

Table 5: Design Matrix for Conceptual Design

<table>
<thead>
<tr>
<th></th>
<th>CD1</th>
<th>CD2</th>
<th>CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintains hMSC viability</td>
<td>3</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Easy to load</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Simple to use</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Cost effective</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Compatible with existing instruments</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Decreases media loss</td>
<td>3</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>23</td>
<td>38</td>
</tr>
</tbody>
</table>

The cartridge design (Figure 19: conceptual design 3) maintains hMSCs viability better then the other conceptual designs because of forces are induced only in the ejection phase. The cartridge design is easy to load because the cartridge can be easily loaded in the back and is user friendly, it requires less force then the other methods of loading and unloading of the gel through the needle. The design is simple to use as explained before, and extremely user friendly. It is cost effective because it does not require manufacturing like the other conceptual designs. The
design is compatible with existing instruments because it can easily fit in the incubator and the gel can actually grow within the cartridge in the incubator. The design also decreases media loss better than the other conceptual designs because the cartridge protects the gel from media loss and guides until ejection.

4.7. Final Design

The final design was created based on the conceptual design. The figures explain how the final design works.

**Figure 20: Cartridge design in test tube rack**

Cartridge design can easily fit in a test tube rack which can fit within an incubator therefore allowing the gels and cells to grow within the design for easy implementation into the syringe. Note how the plunger is attached to the cartridge.
Figure 21: Align the syringe and cartridge

Cartridge aligns with the lumen of the syringe in the back for easy loading that requires little force.

Figure 22: Insert cartridge into syringe

This is user friendly and again requires little force to implement. It is easy to load and takes away the shear forces induced normally by the standard method of delivery.
The cartridge guides the cells in the gel through out until it reaches the needle. This allows for minimal media loss and secures that the cells will only be exposed to forces when ejected.

5. Methods

In this chapter we discuss the process to achieve the objective of our project: the transportation of hMSCs while retaining a sufficient level of cell viability. We divided our project into two parts, designing a delivery vehicle and mechanism. In part one, designing a delivery vehicle, we explain the steps for: culturing hMSCs, the fabrication of hydrogel, and the incorporation of hMSCs into hydrogel. In part two, designing a delivery mechanism, we explain the steps for: the transportation of the hMSC seeded hydrogel via a syringe, and the computational fluid mechanics required.
5.1. Part one: Delivery vehicle

In this section we discuss the procedure and protocols for the culturing of hMSCs, the fabrication of the hydrogel, and the integration of the hMSCs with the hydrogel. All procedures were performed in a sterile environment within a Class II A/B3 Biological Safety Cabinet, hood. hMSCs seeded in hydrogels were compared to hMSCs in media.

5.1.1. Culturing hMSCs

We obtained hMSC P-14 in a cryovial, frozen in a liquid nitrogen cryotank from Glenn Gaudette (a Professor at WPI). Using proper thawing protocols (Appendix A), obtained from Gaudette laboratory, the cells were thawed and placed into tissue-culture treated BD Flacon T-75 flask. Briefly, the cells were seeded with a 0.2 µm vented blue plug seal cap and DMEM (Dulbecco’s Modified Eagle’s Medium) containing 10% Fetal Bovine Serum and 1% Penicillin-Streptomycin Solution was used as the media. During the thawing procedure, at a follow-up time, a cell count was taken following the proper cell counting protocol (Appendix B) obtained from Gaudette laboratory.

Cells were placed and stored in an incubator at 37°C (5% CO2) for a period of 7 days, changing the media at days 1, 4, and 7. On day 7, T-Flask containing cells (hMSC_f) were viewed under an inverted microscope and it was found that the flask was over-populated by plated hMSCs. DMEM inside the hMSC_f was removed with a Pasteur pipette and 5 ml of Trypsin (from Mediatech Inc, Herndon VA, 02771) was added once to hMSC_f. After 5 minutes, the hMSC detached from the surface of the T-Flask. Afterwards 5 ml of DMEM was added to hMSC_f to
inhibit Trypsin activity. This solution was removed from hMSC$_f$ with the pipette and placed in a 15 ml conical tube. Following steps 5-8 of the thawing protocol, the solution was placed in two new T-75 flasks and into an incubator.

Cell media inside hMSC$_f$ were changed on the same days as initial hMSC$_f$, day 1, 4 and 7, after plating and splitting performed as seen fit.

**5.1.2. Fabrication of hydrogel**

We obtained four hydrogels, 0.7 g PGA, 5.8 g PEG, 7.0 g CMC, and 0.7 g HA, in powdered form contained within 15 ml conical tubes from Al Prescott (CEO of Crescent Innovations) and stored them at room temperature. Four sterile 50 ml beakers containing stir bars were placed on a scale under a hood. The scale was calibrated to disregard the weight and 0.7 g of each hydrogel was measured. Next, 23 ml of fresh DMEM was added into their respective beakers making an approximate 3% concentration. The beakers were covered with a sterile gauze and aluminum foil to prevent evaporation and then placed on a stir plate running at 200-300 rpm for 20 hours. Afterwards, a gel was formed and stored in an incubator at 37°C. The procedure was performed under the supervision and instructions of Al Prescott.

**5.1.3. Integration of hMSCs into hydrogel & testing for viability**

Media inside hMSC$_f$ was removed with a Pasteur pipette and 5 ml of Trypsin was added to the hMSC$_f$. After 5 minutes, the hMSC detached and another 5 ml of DMEM was added to hMSC$_f$. The solution was removed from hMSC$_f$ using a pipette and placed in a 15ml conical tube. Steps 5 and 6 from the thawing protocol were followed, but a new T-Flask was not used. After re-suspension, for uniform cell distribution, 1ml of hMSC-DMEM solution was placed in each well of a tissue culture treated Multiwell 24 well plates. Each row, with 4 wells per row,
was designated to specific hydrogels and 1ml of hydrogel solution was place into their respective wells. The last 2 rows were left with only hMSC-DMEM solution to serve as the controls. For Visual description can be seen in Appendix C.

**5.1.3.1. MTS Assay**

MTS Assay solution was added to the each well of the Multiwell 24 well plate containing hMSC seeded hydrogel. 0.22ml of MTS Assay solution was added to wells that contained hMSC seeded hydrogel. 0.11ml of MTS Assay solution was added to wells that contained only hMSCs in media solution. Well plate was placed back into incubator for 2 hours to attain suitable absorbance readings. 300µml was taken from each well on the Multiwell 24 well plate and place into wells on a Multiwell 96 well plate filling 3 wells, with 100µml in each. 96 well plate was placed into Spectramax 250 to assess absorbance levels. For further detail see Appendix B

**5.1.3.2. Histology Staining**

For each well of the 24-well plate, we first removed most of the solution using pipette. Then we fixed the cell with approximately1ml of paraformaldehyde per well for ~10minutes. After 10 minutes, we rinse the well with tap water and removed the mixture with vacuum pipette. Next, we added approximately1ml hematoxylin into each well and let it sit there for ~4minutes. After 4minutes, we repeatedly rinse the well with tap water and used a vacuum pipette to remove the contents from the edge of the well until blue staining can be seen uniformly in the center of the well.
5.2. Part two: Delivery mechanism

In this section we discuss the procedure and protocols for the transportation of hMSC seeded hydrogel via a syringe. All procedures were performed in a sterile environment within a Class II A/B3 Biological Safety Cabinet, hood. HMSCs seeded in hydrogel were compared to hMSCs that did not pass through a syringe.

5.2.1. Syringe Methods

HMSC seeded hydrogel were placed into 5cc syringe (from Becton Dickinson & Co., Franklin Lakes, New Jersey, 07417-1884) using two different techniques, loaded and standard, and then expelled out of a 22G1 Precision Glide Needle (from Becton Dickinson & Co., Franklin Lakes, New Jersey, 07417-1884). Solution was then assessed with histology staining and MTS assay to provide results on which method maintained cell viability.

5.2.1.1. Standard method

In the standard method, hMSCs seeded hydrogel in a 24 well-plate were withdrawn and expelled through the needle.

5.2.1.2. Loaded method

In the loaded method hMSCs seeded hydrogel in a 24 well-plate were withdrawn with a 10 ml pipette. Solution was expelled into the syringe, loading it from the back end with the plunger removed. Once loaded with the hMSCs seeded hydrogel, the plunger is placed back and the solution expelled out through the needle.
5.2.2. Computational fluid mechanics

In order to better understand exactly what forces and motions cause cell lysis, we analyzed the fluid mechanics of our delivery vehicles upon passage through hypodermic needles of various gauges. We used tested our varying delivery methods using 18, 22, and 27 gauge needles. We expelled normal media through each needle using normal, non-excessive force. We measured the time it took to expel 1.5ml of media through a 22 gauge needle. The average time of non-excessive expulsion was 4.12 seconds. For the sake of simplicity, we rounded this time down to 4 seconds. Based on the length and diameter of the 22 gauge needle, we calculated the volumetric flow rate. We used this rate as a base for the rest of our calculations.

We first calculated the Reynolds number for our gels. The following equation was used in order to determine the Reynolds number:

\[ \text{Re} = \frac{\rho VL}{\mu} \]

In this calculation, \( \rho \) denotes the fluid density of the vehicle, \( V \) is the mean fluid velocity, \( L \) is the length and \( \mu \) is the viscosity of the vehicle.

We used the following We then calculated the pressure gradient throughout the length of the needle using the following equation, where \( \frac{dV}{dt} \) is volumetric flow rate through the needle, \( \mu \) again is viscosity of the fluid vehicle, \( R \) is the radius of the needle and \( L \) is the length again.

\[ \Delta P = \left( \frac{\partial V}{\partial t} \right) \left( \frac{8 \mu}{\pi R^4} \right) (L) \]

Based on this value, we calculated the shear forces acting between the fluid particles using this equation. Again, \( R \) is the radius, \( \Delta P \) denotes the change in pressure, and \( \Delta x \) denotes position of the fluid being measured with respect to the distance from the wall of the needle.

\[ \tau = \left( \frac{R}{2} \right) \left( \frac{\Delta P}{\Delta x} \right) \]

6. Results

6.1. Part 1: Fabrication of Hydrogels

During the fabrication of each hydrogel, CMC, PEG, and HA had the same red coloration from the DMEM (10% fetal bovine serum) solution that used as the media for hydration of the
hydrogels. PGA did not have the red coloration, but when the hydrogel came in contact with DMEM, it began to change from red to light pink and remained at a yellow coloration. DMEM solution used in providing nutrients to hMSCs also has a pH indicator which causes the DMEM solution to change color when an environment is too acidic (yellow) for an hMSCs culture. After constant mixing (of the solution and hydrogel in powder form) for 19 hours over a magnetic stir plate, the solution became viscous. The viscosity was later quantitatively evaluated, but HA was the most viscous of all four hydrogels. PEG was the least viscous. PGA was eliminated from further testing because of its high acidic properties indicated with the change in coloration from red to yellow during the fabrication of the hydrogel.

6.2. Hydrogel incorporation with hMSCs

The follow sections describe the results of MTS assay and histological testing that was conducted in this study.

6.2.1. Part 1: Incorporation into T-flask and cell count

The hydrogels that were incorporated into four T-flasks of hMSC culture and incubated for a period of 13 days acquired cell counts which are seen in Table 6 below:
### Table 6: Cell Count of hMSCs after incorporation within hydrogels via Trypan Blue Staining

<table>
<thead>
<tr>
<th></th>
<th>PEG</th>
<th>CMC</th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td># live cells</td>
<td>357</td>
<td>15</td>
<td>129</td>
</tr>
<tr>
<td># dead cells</td>
<td>6</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>#Totals cells</td>
<td>363</td>
<td>38</td>
<td>132</td>
</tr>
<tr>
<td>Cell Viability %</td>
<td>98.3</td>
<td>39.5</td>
<td>94.5</td>
</tr>
</tbody>
</table>

(PGA hydrogel and data were discard for suspicion of contamination)

6.2.1.1. **Incorporation into 24-well plates and MTS assay**

Hydrogels were placed into 24-well cell culture plates. Three main tests groups were setup. Test group one consist of 1 ml hydrogel and 1 ml of cell culture which were placed into the well in that sequence. Eight wells were made with two wells associated with each hydrogel. Test group two consists of 1 ml of cell culture and 1 ml of hydrogel which were placed into the well in that sequence. Eight wells were also made with two wells associated with each hydrogel. Test group three consists of eight wells each with 1ml of cell culture as the negative control.

The 24-well plate was incubated for six days and then MTS Assay was performed. Each well in the 24-well plate were distributed into 3 wells of the 96-well plate. Each well consists .2 ml of solution from the 24-well plate. The absorbance levels of each hydrogel in test group one and two, and control can be seen in Figure 24 and 25. The calculations and raw data can be seen in Appendix D.
Adding hydrogel before the incorporation of hMSCs (Hydrogel then Cell) resulted in higher absorbance levels, which meant that there were more cells. Therefore we conducted further testing with this sequence of incorporation. Because of a small sample size (n=2), more test samples were created that consisted of hydrogel then cell method of incorporation of hMSCs and hydrogel. Figure 26 shows the absorbance levels from a MTS assay with a larger sample size (n=5) of hydrogel then cell. The calculations and raw data can be seen in Appendix E.
6.2.1.2. Incorporation into 24-well plates and Histology

Histology results show that hMSCs incorporated with HA has the most distinct visibility of hMSCs. It was difficult to determine the three-dimensional distribution between hMSCs incorporated in HA, CMC, and PEG. The two-dimensional image from digital photograph did, however, portrayed layers which can be seen in Figure 27 and Figure 28 which are indicated with the green boxes. Figure 27 is picture of our control group where hMSCs are in cell media without incorporation into hydrogel where layers of plated hMSC are seen indicated in green. There were plated hMSCs behind the purple hematoxylin stained hMSCs. Figure 28 is picture of HA hydrogel with hMSCs incorporation. Plated hMSCs were not visible in CMC hydrogel, but dead cell matter was visible which are indicated in red in Figure 29, thus eliminating it as a possible delivery vehicle. The hMSCs within PEG hydrogel were visible but not as distinct as the hMSCs of HA hydrogel. There were not much purple staining from the hematoxylin, but three-dimensional distribution could be seen which are indicated in green in Figure 30.
Figure 27: Histological result of control group without hydrogel and green boxes indicate layering of hMSCs

Figure 28: Histological result of hMSCs with HA hydrogel and green boxes indicate layering of hMSCs
Figure 29: Histological result of hMSCs with CMC hydrogel and red boxes indicate dead hMSCs

Figure 30: Histological result of hMSCs with PEG hydrogel and green boxes indicate layering of hMSCs
6.2.2. Part 2: Transportation of hMSC-hydrogel solution and MTS Assay

Based upon the cell number of the MTS Assay and histological results, we have concluded that HA and PEG hydrogel should be further tested and CMC was eliminated as a possible delivery vehicle because it resulted in dead cell matter as seen in Figure 29. We experimented with the methods of transferring the hMSC-hydrogel serum, mentioned in chapter 5.2.1. The MTS assay results of the both methods with HA and PEG hydrogel can be seen in Figure 31 with the six different test groups and the raw data can be seen in Appendix F. Overall, the loaded methods of both PEG and HA did give as much cell number as compared with the standard methods. However, loaded control (hMSCs in cell media) had the highest cell number with over 45,000 cells. Therefore, with conflicting results we decided to conduct computational fluid mechanics and histological testing of PEG and HA via loaded and standard methods. Also, the statistical p-value of 0.011284 was computed using ANOVA which compared Loaded HA to standard HA. The computation can be seen in Appendix F.

![Cell Viability of Transfer method (n=4)](image)

Figure 31: Cell Viability of Transfer Methods of HA and PEG
6.2.2.1. Computational Fluid Mechanics

We conducted computational fluid mechanics to evaluate PEG’s and HA’s Reynolds number to determine which one had the most turbulent flow which induced stress force on the hMSCs when passing through the needle. HA and PEG, therefore, were tested for their viscosity via viscometer. The viscosity is needed to determine the Reynolds number, unitless value, to assess whether our hydrogel creates a turbulent or laminar flow. The viscosity of PEG is \( \mu_{\text{PEG}} = 0.001 \text{ Pa}\cdot\text{s} \) and HA is \( \mu_{\text{HA}} = 25.283 \text{ Pa}\cdot\text{s} \) (calculations are in Appendices G and H). The design team decided to calculate the force through the plunger as a proof of concept proving when incorporating the delivery vehicle with the mechanism it proved to be better than then standard method of delivery (calculations are in Appendix G). We used the diameter of a 22-gauge needle to determine the Reynolds number of PEG and HA. The \( \text{Re}_{\text{PEG}} = 11056 \) and \( \text{Re}_{\text{HA}} = 2.357 \). Overall, HA resulted in a laminar flow and PEG resulted in a turbulent flow.

6.2.2.2. Transportation of hMSC-hydrogel solution and Histology

The histological results can be seen in Figure 32-37 of the six test groups. Correlated to the cell viability of the transfer methods, standard method with HA had visibility of the hMSCs which is indicated in green in Figure 32. However, loaded method with HA also had visible hMSCs which are indicated in green in Figure 33. The standard and loaded methods of PEG had dead cell matter which was indicated red in Figure 34 and 35. The standard and loaded methods of control also resulted in dead cell matter which is indicated in red in Figure 36 and 37.
Figure 32: Histological result of standard method with HA and green boxes indicate viable hMSCs. Many hMSCs, that appeared viable, were found throughout the HA.

Figure 33: Histological result of loaded method of HA and green boxes indicate viable hMSCs, which were numerous in the HA.
Figure 34: Histological result of standard method with PEG and red boxes indicate dead hMSCs.

Figure 35: Histological result of loaded method of PEG and red boxes indicate dead hMSCs.
Figure 36: Histological result of standard method with hMSCs in media and red boxes indicate dead hMSCs.

Figure 37: Histological result of loaded method with hMSCs in media and red boxes indicate dead hMSCs.
From the cell viability and histological results from using the two different transfer methods, we can see that the hMSCs were adversely affected when passing though the needle during the standard method of delivery which can be seen in Figure 36 and 37. Also, when the hMSCs were incorporated into HA hydrogel, the cell viability was greater than without any hydrogel incorporation. Qualitatively, more hMSC visibility was seen when incorporated with HA hydrogel than with PEG. Based on these results, we have decided that HA hydrogel is the most optimal delivery vehicle to transport the hMSCs.

6.2.2.3. Loaded and standard method of delivery of hMSCs within HA hydrogel and MTS Assay

Another MTS Assay was conducted to determine whether standard or loaded methods of delivery maintained the most cell viability. The calculations and raw data can be seen in Appendix J. The results can be seen in Figure 38 where it showed that HA under loaded method of delivery had the highest cell number.

![Cell Viability of Transfer Method (n=9)](image)

**Figure 38: Cell Viability of Transfer Methods of HA.**
6.2.2.4. **Loaded and standard method of delivery of hMSCs within HA hydrogel and Histology**

The histological results can be seen in Figure 39-43. For this test the control group, in Figure 39 consists of hMSCs that were not incorporated with hydrogel and was not delivery by standard or loaded methods. The hMSCs of the control group was transported to 24-well plates using a pipette. The control group served to compare the histological results from test groups: Standard hMSCs within HA, Standard hMSCs in media, Loaded hMSCs within HA, and Loaded hMSCs in Media. Both Figure 41 and 43, hMSCs in media, without incorporation with hydrogel, did not have similar cell morphology as our control group. However, Figure 40 and 42 had similar cell morphology with control when incorporated with HA. The loaded method of hMSCs incorporated with HA had the most similarity with the control group.

![Figure 39: Histological result of Control group not delivered and not incorporated with hydrogel](image-url)
Figure 40: Histological result of standard method of hMSCs within HA

Figure 41: Histological result of standard method of hMSCs in media
Figure 42: Histological result of loaded method of hMSCs within HA

Figure 43: Histological result of loaded method of hMSCs in media
7. Analysis and Discussion

7.1. Preliminary Delivery Vehicle Analysis

The MTS assay, histology readings, and three-dimensional distribution were the tests which aided in our decision to use hyaluronic acid (HA) as our hydrogel and delivery vehicle of choice.

In our preliminary MTS assay using hMSC suspended on top of PEG, HA, CMC, and PGA hydrogels. After incorporating the hMSCs with PGA it was clearly evident that the acidic nature of PGA limited the viability of hMSCs as well as the film of lysed matter on top of the gel. These observations along with consultation from our clients allowed us to limit the use of PGA. The design team then decided to proceed with the other three hydrogels and conduct an MTS assay. In the MTS assay the team decided to conduct the MTS assay by incorporating hMSC on a 24 well plate, with half being suspended on top of the gel and half suspended on the bottom of the gel. This would eventually prove which hydrogel intermixed or provided three dimensional distributions of hMSCs as well as which method we should use in further testing. The results showed that incorporating the cells suspended on top of the hydrogel resulted in the highest viability and cell number, therefore the team decided to proceed with this method of incorporation.

The results show that CMC did not provide a suitable viable environment for hMSCs with its initial 39.5 % viability outlined in Table 6 (Results section). Our hypothesis was further proven by the histology readings using H & E stain showing hMSCs in the CMC matrix in Figure 29 (Results section). Comparing hMSCs in the CMC matrix to PEG, HA, and the control, the cells do not look as uniform or disperse as they do in other gels. The cells ball up and look as
if they are dead from the histology readings. This allowed us to rule out CMC as a possible delivery vehicle.

This left the design team with two choices for possible delivery vehicles which was PEG and HA. Since both had similar readings for MTS assay and histology readings compared to the control, we decided to proceed with these two gels to secondary testing involving three dimensional distribution, fluid mechanics testing, and processing the gels with hMSCs through two different delivery mechanisms.

7.2. Secondary Delivery Vehicle Analysis
In order to distinguish which delivery vehicle supported cell viability with providing three dimensional distributions, further testing had to be done. To understand, the nature of these gels it is important to describe the viscosity. The HA hydrogel is very thick and heavy and moves like molasses, whereas PEG is like water and moves very rapidly. Therefore from observation it was clearly evident that HA provided three dimensional distribution of hMSCs compared to PEG. Yet we had to prove this results which will be further explained.

Our design team wanted to first figure out which gel caused turbulent flow compared to laminar flow. Turbulent flow causes rotation of fluid particles within the pipe which causes constant shearing along the pipe wall and this is what limits the viability of cells when ejected through a needle which acts as our pipe. Laminar flow allows for a fluid linear ejection of the fluid particles and limits shearing occurring and benefits viability of cells. A diagram of turbulent vs. laminar flow is provided in Appendix (I). Therefore we decided to use a Viscometer (find details from Al) and test the viscosities of PEG and HA. Our team decided to calculate the Reynold’s number of each hydrogel. The Reynold’s number determines whether a fluid is laminar or turbulent, if a fluid has a Reynold’s number above 300 than it is considered turbulent.
As mentioned in chapter 6.2.2.1, HA had a Reynold’s number considerably lower than 3000, therefore, categorizing it as laminar. PEG had a Reynold’s number significantly higher than 3000 categorizing it as turbulent.

Or next step was to proceed to the actual test of comparing PEG and HA within two different delivery mechanisms. We decided to use the standard method of delivery which involves using a syringe and loading the gel through the needle and ejecting it out through the needle. The other mechanism was pipetting the hydrogel/hMSC combination and ejecting it in the back of the syringe and ejecting it through the needle. The results shown in Figure 31 and Figure 32 (Results section) prove that HA provides a better delivery vehicle for hMSCs. The histology readings show PEG which has lysed matter compared to HA which still has uniform and disperse cells after delivery seen in Figure 28.

This concluded our decision to use HA as our delivery vehicle. The design team had to next think of a unique delivery mechanism to complete the project goals.

7.3. Preliminary Delivery Mechanism Analysis

After deciding upon a suitable delivery vehicle, our next step was to create a delivery mechanism. The design team decided upon using conceptual design three which was using a cartridge that is loaded from the rear of the syringe for a variety of reasons. It is cost effective in that little manufacturing is needed, which benefits the users tremendously. It is compatible with existing medical instruments. It fits in a test tube rack, incubator, and under a sterile hood. It can vary in size for different sized syringes as well. It is easy to load because the hMSCs in the hydrogel matrix are grown in the cartridge and then easily loaded in the rear. The cartridge decreases media loss and maintains the viability of the cells by only exposing it to forces when ejected. We decided to conduct preliminary MTS test by comparing our design to the standard
method of delivery. The standard method of delivery is defined as loading the hydrogel through the needle and ejecting it though the needle as well. As can be seen in Figure 38 (Results section) the results show that our design maintains viability at a greater rate than the standard method. This concludes that our design only exposes the cells to forces in the ejection state. This also proves that forces induced on cells limits their viability.

### 7.4. Secondary Delivery Mechanism Analysis

After concluding that our design maintains cell viability by exposing the cells to forces when being ejected, we wanted to combine the delivery vehicle in the delivery mechanism and relate them to the standard method of delivery. Three separate tests were conducted using MTS and histology: standard method of delivery comparing hMSCs in media vs. hMSCs in HA hydrogel, loaded method of delivery comparing hMSCs in media vs. hMSCs in HA hydrogel, and loaded method of delivering hMSCs in HA hydrogel vs. standard method of delivering hMSCs in HA hydrogel (Figures 39-43, Results section). As can be seen through the histology pictures the loaded delivery mechanism using HA hydrogel as a delivery vehicle shows the most cells which are viable compared to the other methods. The MTS assay also validates this theory as well. Therefore it is safe to say that the combination of using HA hydrogel as a delivery vehicle and loading it from the rear therefore exposing it to forces ejected from the rear is clearly a better method than the standard method of delivery. The unique aspect about the device is that the cells in the delivery vehicle can be grown in the cartridge in an incubator and then loaded from the rear and injected to the injured tissue.

### 7.5. Limitations of Testing Methods
Some of the testing methods we used had limitations. Due to the high viscosity of hyaluronic acid and the slow rate of diffusion, only limited testing methods could be used for assessing cell viability. Another limitation of using hydrogel is a proper way of assessing three dimensional distribution due to the gel like nature of HA. These topics will be discussed in detail.

HA hydrogel is a highly viscous matrix that compares to molasses in its movement. It does not allow diffusion quickly because of this high viscosity. This limited the testing protocols we could use. For example, we tried to use LIVE/DEAD assay but the dye could only be applied for a period of 45 minutes before killing the cells. When the design team tried to use this method, they could not visualize any cells under a fluorescent microscope and the theory was because the dye could not diffuse through the gel. Therefore we were limited to the only method of quantifying cell viability which was the MTS assay. Though MTS assay quantifies how many cells are located in the gel and does not distinguish whether these cells are dead or alive, this along with histology was a sufficient enough method to distinguish cell viability.

Another limitation is qualifying three dimensional distribution. Because of the three dimensional nature it was hard to show pictures of this on a two dimensional scale. The usual method is to take slices of the gel and show different levels of the material to prove three dimensional distribution of cells. Our first thought was free drying the hydrogel and then taking slices of the gel, but this would show dead cells which would disprove our viability. Because of the gel like nature of the hydrogel, it was difficult to display the three dimensional nature of the cells in the allotted time. Therefore expressing the three dimensional nature verbally was the only method of relaying the three dimensional nature.
8. Conclusions

Based on the testing we had done and our analysis that has been discussed in previous chapters, we were able to interoperate our results and determine conclusions that can summarize our attempts to design a suitable vehicle and mechanism for delivering hMSCs.

Both PEG and HA proved to be suitable materials in which to contain hMSCs. After being cultured, they were able to maintain cell viability as well as allow for proliferation within the gels. However, only HA was able to do so in a three dimensional manner, which, according to our literature review, allows for greater success in producing new tissue at the site of injury. We determined this to be true based on our histology results, shown in figure --, and by observing cells at different depths using a microscope.

Our computational fluid mechanics analysis suggests that cell previous cell viability difficulties through delivery are caused by the internal shear forces between the fluid particles themselves cause by extreme turbulent flow. As a result, it is important that the hydrogel delivery vehicle be viscous. The viscosity of the vehicle allows for safer transport of our hMSCs by promoting a more laminar flow during injection through a hypodermic needle. Our MTS assay and histology results supported our assessment, showing that cell viability after being passed through a needle is significantly higher in HA, a viscous gel, than in media, a fluid with similar viscosity to water.

The same results show that cells are compromised due to the forces caused by forces created between the fluid vehicle and the wall of the needle. Each time the cells were passed through the needle, regardless of the vehicle, cell viability was lost. Based on this assumption, we naturally determined that it was important to limit the amount of passes through a needle. Our design of a cartridge loaded mechanism into the syringe to allow for easy culturing of cells
within the gel as well as easy and immediate delivery to the site of injured tissue. Upon testing our mechanism in comparison with standard delivery methods using hMSC seeded hydrogels, we confirmed that our design in fact allowed for greater cell viability than standard procedures.

Overall, we developed a suitable delivery vehicle that was able to allow for cell viability while promoting three-dimensional distribution and providing protection upon delivery through a hypodermic needle. The second part of our project resulted in the design of a novel delivery mechanism in which stem cells can be cultured within a viscous hydrogel vehicle inside of a cartridge-like cell culturing well. When needed, the cartridge, complete with cell seeded gel, can be taken from the incubator and inserted into a pre-fit syringe and be ready for immediate injection to the injury site. Our complete design allows for an efficient, effective, and easy method for quickly delivering stem cells from the laboratory to operation room and into the site of injury of the patient.

9. Recommendations

After creating our final designs, the design team discussed possible methods of improving the design and the methods of testing. As stated in the discussion and analysis section we had limitations in some of the methods of testing. The design team came up with possible solutions for these limitations and a possible addition to the design.

Quantifying cell viability was a limitation throughout the design. Due to the thick viscosity of the hydrogel, diffusion of dye became a problem and limited us using LIVE/DEAD assay and other assays to quantify cell viability. Our design team recommendation would be to use flow cytometry to quantify viability due to its ability to singly count live and dead cells. Due
to time and budget constraints, we were not able to use this method, but believe it would enhance the ability to truly quantify cell viability.

Showing a picture of three dimensional distribution throughout the hydrogel was another limitation the design team experienced in the project. The design team’s recommendation was to freeze the hydrogel and use a dye to depict the cells in the gel, and cut vertical sections of the gel and show that three dimensional distribution occurred.

The design team also wanted to check if the cells when delivered targeted the specific site of injury and made sure the cells help aid in the process of healing and not migrate to other parts of the body. Therefore the team recommends delivery the hydrogel and hMSCs with a chondrogenic differentiation media into animals with articular defects and doing in vivo studies to see if healing occurs after 6 months. This would truly promote our design as a potential solution to problems associated with delivering stem cells.

A design recommendation the team came up with is creating a space saving cartridge with a screw in plunger. This would allow for the cartridges to have more room in the incubator, and could serve as a well plate which can be taken out and serve as a cartridge and loaded into the syringe.
10. References


# Appendix A: Culturing hMSCs Protocol

<table>
<thead>
<tr>
<th>Protocol Name</th>
<th>Thawing hMSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Objectives and Specifications</strong></td>
<td>Thaw human mesenchymal stem cells (hMSCs) so that they can be used for experiments</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Materials and Equipment</strong></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>1. DMEM (Dulbecco’s Modified Eagle’s Medium), 10% FBS, 1% P/S with all supplements (serum = FBS, L-glutamine, antibiotics = P/S), 37°C. Stored at -20°C.</td>
<td></td>
</tr>
<tr>
<td>2. Pipets: 25mL, 10mL, 5mL Serological Pipets.</td>
<td></td>
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<tr>
<td>3. Miscellaneous items: Sterile culture flasks, 15mL conical tubes, 70% Isopropyl Alcohol (IPA), Pipet Aid, Lab marker for labeling.</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Procedure</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Spray inside surface of hood with 70% IPA. Spray all exterior surfaces of containers to be brought into hood with 70% IPA. Set up all necessary items in hood.</td>
<td></td>
</tr>
<tr>
<td>2. Remove stored cryovial(s) containing cells from Liquid Nitrogen cryotank/Dry ice. <em>Optional:</em> Wipe cryovial(s) with 70% IPA and in sterile field, briefly twist cap a quarter turn to relieve pressure, then retighten.</td>
<td></td>
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<tr>
<td>3. Thaw cells rapidly by immediately immersing vial(s) into 37°C water bath. Do not submerge them completely and watch them closely. Gently agitate for approx. 2 min (no longer than 3 min). <em>Note:</em> Most cell death occurs between −50°C and 0°C when thawing.</td>
<td></td>
</tr>
<tr>
<td>4. When fully thawed (all ice crystals melted), remove vial(s) immediately, wipe dry then spray outside of vial(s) thoroughly with 70% IPA before bringing cells into the hood. Transfer thawed cell suspension into 15mL tube containing 5mL pre-warmed media to dilute.</td>
<td></td>
</tr>
<tr>
<td>5. Centrifuge cells at 1000 rpm for 5 minutes to remove any residual DMSO. While cells are being spun down, set up new T75 flasks and add 13mL of DMEM to each. Allow temperature to equilibrate to 37°C.</td>
<td></td>
</tr>
<tr>
<td>6. Decant supernatant; Resuspend cell pellet in minimum volume of fresh pre-warmed media. Perform CELL COUNTING. Seed cells by transferring the appropriate amount of cell suspension into new culture flask(s) with fresh medium. <em>Note:</em> Amount of suspension transferred will depend on the density at which cells were frozen and desired cell density for new seed.</td>
<td></td>
</tr>
<tr>
<td>7. Place cells in incubator, and replace with equal volume of fresh medium after 24 hrs to remove any (floating) dead cells. Observe cells daily for growth (confluence reached by ~1 week) and freedom from contamination. Media to be changed every 3-4 days.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8. Clean up hood and spray down surface with 70% IPA. Close it and turn on UV light.</td>
</tr>
<tr>
<td>---</td>
<td>-------------------------------------------------------------------</td>
</tr>
<tr>
<td>References</td>
<td>Protocol obtained from Gaudette laboratory</td>
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<tr>
<td>Protocol Name</td>
<td><strong>Subculturing hMSCs</strong></td>
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</tr>
<tr>
<td><strong>Objectives and Specifications</strong></td>
<td>Culture hMSCs so that they can be used for experiments</td>
</tr>
</tbody>
</table>
| **Materials and Equipment** | 1. DMEM (Dulbecco’s Modified Eagle’s Medium), 10% FBS, 1% P/S with all supplements (serum = FBS, L-glutamine, antibiotics = P/S), 37°C. Stored at 4°C.  
2. 0.25% Trypsin, 37°C. Stored at -20°C. (Not to be left in water bath for extended period of time.)  
3. Pipets: 25mL, 10mL, 5mL Serological Pipets, 5mL aspirating Pasteur Pipets.  
4. Miscellaneous items: Sterile culture flasks for seeding, 70% Isopropyl Alcohol (IPA), Pipet Aid, Lab marker for labeling. |
| **Procedure** | 1. Spray inside surface of hood with 70% IPA. Spray all exterior surfaces of containers to be brought into hood with 70% IPA. Set up all necessary items in hood.  
2. Remove all media from T75 flasks with hMSCs (P# donor #) previously cultured at high density. Remember: Handle flasks vertically as to not allow media to enter neck of dish, specially designed for CO2 exchange.  
3. Add 5mL Trypsin gently to bottom edge/corner of flask (as to not shock/dislodge cells). Rock flask gently to ensure full coating of bottom surface. Check cells under microscope to make sure they are detaching from flask and have “rounded-up” morphology and are “flying around”. (Trypsin is a protease that acts to degrade protein.)  
4. When all cells appear round, add 5 mL fresh media (DMEM, 10%FBS), and thoroughly wash flask to gather up all cells from the bottom of the flask by gently triturating up and down while tilting the flask. Note: No need to aspirate trypsin since DMEM will inactivate its proteolytic action.  
5. Centrifuge cells at 1000rpm for 5 minutes.  
6. Decant supernatant and resuspend cell pellet in minimum volume of fresh pre-warmed media. Determine number cells according to CELL COUNTING protocol.  
7. Add appropriate volume (depending on % confluency) of cell suspension to fresh flasks. Gently rock/swirl flask to spread out cells.  
8. Place cells in incubator and observe daily for growth (toward confluency) and freedom from contamination. Media to be changed every 3-4 days.  
9. Clean up hood and spray down surface with 70% IPA. Close it and turn on UV light. |
| References | Protocol obtained from Gaudette laboratory |
# Appendix B: Cell Counting and MTS Assay Protocols

<table>
<thead>
<tr>
<th>Protocol Name</th>
<th>Cell Counting</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Objectives and Specifications</strong></td>
<td>Determine concentration of cells in suspension</td>
</tr>
</tbody>
</table>
| **Materials and Equipment** | 1. Hemacytometer  
2. Trypan Blue  
3. PBS  
4. Cell counter; 10μL pipettes |
| **Procedure** | 1. Prepare a 1:10 dilution (thus dilution factor = 10) of cell suspension to be counted as follows: Place 50 μL Trypan Blue + 40μL non-sterile PBS + 10μL cell suspension in a small Eppendorf tube. Triturate gently as to increase accuracy of count. *Note: Trypan Blue is toxic and a potential carcinogen so extra care should be taken with its use.*  
2. Prepare the hemacytometer by placing a clean coverslip onto its center grid section (mirror-like polished surface with wells). Both should be cleaned with ethanol prior to use.  
3. Carefully load a small amount (~10μL) of cell suspension into the wells underneath and on each end of the coverslip. *Note: A hemacytometer is a specialized glass slide with a 3x3 grid pattern etched upon it whose volume is known. When covered by a coverslip, cells spread out due to capillary action.*  
4. Using a microscope, cells are counted within each of squares of the hemacytometer grid to obtain a measure of cell concentration as follows: |

Count all viable cells in each of the 4 corner fields adjacent to the center square (i.e. squares that lie along a diagonal, here 1, 3, 7 & 9) for each side of hemacytometer for a total of 8 fields. Adopt a rule for counting cells that fall on grid lines to eliminate duplicate counts (i.e. count cells on left or top lines of a square, but not those on bottom or right lines). *Note: Dead cells appear blue as stained by Trypan Blue and should be excluded from the count, while viable cells appear bright and do not take up the dye unless exposed to it for an extended period of time after which they may absorb it and...*
appear non-viable.

5. Use the following equations with numbers attained from count to calculate cell concentrations. Final count or actual cell density in cells/mL → Eqn: \( C_1V_1 = C_2V_2 \)

\[
\text{average count per field} \\
\frac{\# \text{ viable cells}}{\text{mL}} = \left[ \frac{\# \text{ viable cells}}{\text{total } \# \text{ fields}} \right] \times \text{dilution factor (10)} \times 10^4
\]

\[
\text{Total } \# \text{ viable cells} = \# \text{ viable cells/mL} \times \text{original vol from which sample removed} = C_1 \times V_1
\]

\[
\text{Final resuspension volume (} \# \text{ mL of cells to add)} = \frac{\text{total } \# \text{ cells/target cell density}}{C_2}
\]

\[
\% \text{ viability} = \frac{\# \text{ viable cells}}{\text{total } \# \text{ cells}} \times 100
\]

*Note: Must perform dead count for total # cells.*

<table>
<thead>
<tr>
<th>Protocol Name</th>
<th>MTS Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Objectives and Specifications</strong></td>
<td>Determine concentration of cells in suspension</td>
</tr>
<tr>
<td><strong>Materials and Equipment</strong></td>
<td>1. CellTiter 96® AQueous One Solution</td>
</tr>
<tr>
<td></td>
<td>2. 96 well plates</td>
</tr>
<tr>
<td></td>
<td>3. Pipettes; pipette tips; lab marker;</td>
</tr>
<tr>
<td><strong>Procedure</strong></td>
<td>1. Thaw the CellTiter 96® AQueous One Solution Reagent. It should take approximately 90 minutes at room temperature on the bench top, or 10 minutes in a water bath at 37°C, to completely thaw the 20ml size.</td>
</tr>
<tr>
<td></td>
<td>2. Pipet 20(\mu)l of CellTiter 96® AQueous One Solution Reagent into each well of the 96-well assay plate containing the samples in 100(\mu)l of culture medium. If culture volume is larger than 100(\mu)l, add solution to obtain total ratio of 1:5.</td>
</tr>
<tr>
<td></td>
<td>3. Incubate the plate for 2 hours at 37°C in a humidified, 5% CO2 atmosphere.</td>
</tr>
<tr>
<td></td>
<td>4. Record the absorbance at 490nm using a 96-well plate reader.</td>
</tr>
<tr>
<td><strong>References</strong></td>
<td>Protocol modified from manufacturer's instructions.</td>
</tr>
</tbody>
</table>
Appendix C: Integration of hMSCs into hydrogel

Figure 44: Two variations of incorporation of hMSCs into hydrogel within 24-well plate.
Appendix D: MTS Assay Data of Incorporation of hMSCS into 24-well plates with hydrogels (n=2).

Table 7: Raw data of MTS Assay of the two variations of incorporation (n=2)

<table>
<thead>
<tr>
<th>Hydrogel then cell n=2</th>
<th>PEG</th>
<th>PGA</th>
<th>CMC</th>
<th>HA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.217</td>
<td>0.115</td>
<td>0.561</td>
<td>0.65</td>
<td>0.555</td>
</tr>
<tr>
<td></td>
<td>0.247</td>
<td>0.126</td>
<td>0.324</td>
<td>0.475</td>
<td>0.414</td>
</tr>
<tr>
<td></td>
<td>0.239</td>
<td>0.126</td>
<td>0.137</td>
<td>0.514</td>
<td>0.688</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>0.198</td>
<td>0.119</td>
<td>0.392</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td>0.209</td>
<td>0.196</td>
<td>0.308</td>
<td>0.596</td>
</tr>
<tr>
<td></td>
<td>0.207</td>
<td>0.124</td>
<td>0.184</td>
<td>0.375</td>
<td>0.479</td>
</tr>
</tbody>
</table>

| Avg absorbance | 0.221667 | 0.149667 | 0.192 | 0.43 | 0.559381 |
| STDEV | 0.018173 | 0.042041 | 0.080402 | 0.104367 | 0.099687 |

<table>
<thead>
<tr>
<th>Cell then Hydrogel n=2</th>
<th>PEG</th>
<th>PGA</th>
<th>CMC</th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.203</td>
<td>0.174</td>
<td>0.128</td>
<td>0.215</td>
</tr>
<tr>
<td></td>
<td>0.218</td>
<td>0.133</td>
<td>0.144</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.221</td>
<td>0.137</td>
<td>0.187</td>
<td>0.168</td>
</tr>
<tr>
<td></td>
<td>0.208</td>
<td>0.117</td>
<td>0.098</td>
<td>0.121</td>
</tr>
<tr>
<td></td>
<td>0.228</td>
<td>0.21</td>
<td>0.157</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.229</td>
<td>0.128</td>
<td>0.29</td>
<td>0.267</td>
</tr>
</tbody>
</table>
Appendix E: MTS Assay Data of Incorporation of hMSCS into 24-well plates with hydrogels then cell suspension (n=5).

<table>
<thead>
<tr>
<th>Plate</th>
<th>Plate#1</th>
<th>1.1</th>
<th>PlateForm</th>
<th>Endpoint</th>
<th>Absorbance</th>
<th>Raw</th>
<th>FALSE</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PEG</td>
<td>PEG</td>
<td>HA</td>
<td>HA</td>
<td>CMC</td>
<td>CMC</td>
<td>Control</td>
</tr>
<tr>
<td>n=5</td>
<td></td>
<td>0.414</td>
<td>0.39</td>
<td>0.414</td>
<td>0.34</td>
<td>0.272</td>
<td>0.357</td>
<td>0.355</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.363</td>
<td>0.381</td>
<td>0.337</td>
<td>0.361</td>
<td>0.255</td>
<td>0.357</td>
<td>0.294</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.383</td>
<td>0.412</td>
<td>0.413</td>
<td>0.252</td>
<td>0.333</td>
<td>0.286</td>
<td>0.271</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.41</td>
<td>0.46</td>
<td>0.461</td>
<td>0.211</td>
<td>0.378</td>
<td>0.26</td>
<td>0.288</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.376</td>
<td>0.39</td>
<td>0.395</td>
<td>0.391</td>
<td>0.367</td>
<td>0.231</td>
<td>0.266</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.366</td>
<td>0.438</td>
<td>0.426</td>
<td>0.414</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.368</td>
<td>0.39</td>
<td>0.414</td>
<td>0.398</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.383</td>
<td>0.367</td>
<td>0.317</td>
<td>0.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.363</td>
<td>0.345</td>
<td>0.389</td>
<td>0.285</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.342</td>
<td>0.343</td>
<td>0.316</td>
<td>0.259</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.327</td>
<td>0.233</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8: Raw data of MTS Assay of hydrogel then cell incorporation (n=5)
Appendix F: MTS Assay Data of Transportation of hMSC-hydrogel solution

Table 9: Raw data of cell viability of transfer method (n=2)

<table>
<thead>
<tr>
<th></th>
<th>Avg Absorbance</th>
<th>Avg Cell number</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 ml cell suspension</td>
<td>0.207667</td>
<td>17500</td>
</tr>
<tr>
<td>1.0 ml cell suspension</td>
<td>0.301</td>
<td>35000</td>
</tr>
<tr>
<td>1.5 ml cell suspension</td>
<td>0.345167</td>
<td>52500</td>
</tr>
<tr>
<td>2.0 ml cell suspension</td>
<td>0.552233</td>
<td>70000</td>
</tr>
<tr>
<td>2.5 ml cell suspension</td>
<td>0.365967</td>
<td>87500</td>
</tr>
<tr>
<td>3.0 ml cell suspension</td>
<td>0.477667</td>
<td>105000</td>
</tr>
</tbody>
</table>

Table 9: Raw data of cell viability of transfer method (n=2)

<table>
<thead>
<tr>
<th></th>
<th>Absorbance</th>
<th>Raw</th>
<th>FALSE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature(C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.8</td>
<td>0.195</td>
<td>0.246</td>
<td>0.281</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>0.213</td>
<td>0.217</td>
</tr>
<tr>
<td></td>
<td>0.264</td>
<td>0.266</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>0.28</td>
<td>0.237</td>
<td>0.224</td>
</tr>
</tbody>
</table>

|               |           |     |          |
| HA            | PEG       |     | Control  |

Figure 45: Equation to determine cell number for cell viability of transfer method of HA and PEG

y = 154863x - 9834

Cell number Vs. Absorbance

Vary Absorbance

Linear (Vary Absorbance)
<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>2</td>
<td>0.355</td>
<td>0.1775</td>
<td>0.000613</td>
</tr>
<tr>
<td>Column 2</td>
<td>2</td>
<td>0.574</td>
<td>0.287</td>
<td>0.000128</td>
</tr>
<tr>
<td>Column 3</td>
<td>2</td>
<td>0.596</td>
<td>0.298</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

**ANOVA**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.017754</td>
<td>2</td>
<td>0.008877</td>
<td>28.31632</td>
<td>0.011284</td>
<td>9.552094</td>
</tr>
<tr>
<td>Within Groups</td>
<td>0.000941</td>
<td>3</td>
<td>0.000314</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.018695</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 10: Anova results of HA Loaded
Appendix G: Force Calculation of Plunger

\[ P_1 V_1 = P_2 V_2 \quad P_2 = \frac{P_1 V_1}{V_2} = \text{Force Calculations: Plunger} \]

\[ P_{18} = \left( 5.682 \times 10^5 \text{Pa} \right) \left( \pi \left( 6.35 \times 10^{-4} \text{m} \right)^2 \right) \left( 0.081 \right) \left( 1.5 \times 10^{-4} \text{m}^3 \right) = 1.828 \times 10^4 \text{Pa} \]

\[ F_{\text{Plunger}} = \left( 1.828 \times 10^4 \text{Pa} \right) \pi \left( 0.06 \text{m} \right) = 20.45 \text{N} \]

\[ P_{22} = \left( 7.77 \times 10^7 \text{Pa} \right) \pi \left( 5.362 \times 10^{-4} \text{m} \right)^2 \left( 0.081 \right)^2 \left( 1.5 \times 10^{-4} \text{m}^3 \right) = 4.87 \times 10^8 \text{Pa} \]

\[ F_{P_{22}} = 500 \text{N} \]

\[ P_{27} = \left( 5.288 \times 10^5 \text{Pa} \right) \pi \left( 2.05 \times 10^{-4} \text{m} \right)^2 \left( 0.081 \right)^2 \left( 1.5 \times 10^{-4} \text{m}^3 \right) = 5.84 \times 10^5 \text{Pa} \]

\[ F = 66 \text{N} \]
Appendix H: Computational Fluid Mechanics calculations

Memorandum

Corporate Headquarters
16 Lake Shore Drive North
Westford, MA 01886
(978) 764-8604
alprescott@crescentinnovations.com

Research and Development Facility
10 New Bond Street
Worcester, MA 01606

To: MQP Group
From: Al Prescott
Date: January 31, 2008

Re: HA Gel Viscoisty

Gentlemen,

As you will recall, the other day we measured the viscosity of the PEG and HA hydrogels that you have used in the recent cell culture studies. This memo will document the data we generated and show you how we determine viscosity from it.

First, we take the calibration data, which consists of the three cSts of known viscosity. The data is below in table 1.

Table 1

<table>
<thead>
<tr>
<th>RPM</th>
<th>30,000</th>
<th>12500</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Scale</td>
<td>% Scale</td>
<td>% Scale</td>
</tr>
<tr>
<td>0.5</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>7.5</td>
<td>3.5</td>
<td>0.5</td>
</tr>
<tr>
<td>2.5</td>
<td>18</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>36.5</td>
<td>16</td>
<td>1.5</td>
</tr>
<tr>
<td>10</td>
<td>72</td>
<td>32</td>
<td>2.8</td>
</tr>
<tr>
<td>20</td>
<td>64</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>12.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1400</td>
<td>25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Once this data is entered into a spreadsheet, we can graph each RPM, with the % Scale number on the X axis, and the viscosity values as the Y axis. When you do this, each data set can have a line linearly regressed, and the equation displayed as shown in Figure #1.
Now, we take the data we generated from the HA gel, which is in Table 2.

Table 2

<table>
<thead>
<tr>
<th>RPM</th>
<th>% Scale</th>
<th>Visc, cps</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>3.5</td>
<td>25,396</td>
</tr>
<tr>
<td>1</td>
<td>6.5</td>
<td>21,372</td>
</tr>
<tr>
<td>2.5</td>
<td>10</td>
<td>16,070</td>
</tr>
<tr>
<td>5</td>
<td>14.8</td>
<td>13,336</td>
</tr>
<tr>
<td>10</td>
<td>20.5</td>
<td>7,986</td>
</tr>
<tr>
<td>20</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>36.2</td>
<td></td>
</tr>
</tbody>
</table>

In this table, the RPM was known, the % scale was read off the viscometer, and the viscosity at each RPM is determined by taking the equations from figure #1, and programming them into the visccy, cps column, where \( x \) = % Scale, and \( y \) is the resulting viscosity.

As mentioned before, the key viscosity # is at the 0.5 RPM, but I have attached a graph of the Viscosity versus RPM, which is analogous to Viscosity versus Rate of Shear. Not the graph in figure #2 follows that of a classic shear thinning non-Newtonian fluid.
I strongly suggest you practice generating each of these graph is excel (or other program) so that you can reproduce the results and are comfortable with them.
\[ \frac{dV}{dt} = \frac{1.5 \times 10^{-6} \text{ m}^3}{4.0 \text{ s}} = \frac{3.75 \times 10^{-7} \text{ m}^2}{5} = \frac{JV}{\nu t} \]

\[ V_{\text{vel}} = \frac{\left( \frac{dV}{dt} \right) \frac{1}{A}}{\left( \frac{1}{L} \right)} = \frac{3.75 \times 10^{-7} \text{ m}^2}{5} \left( \frac{25.392}{\text{ N} \cdot \text{ m}^2} \right) \left( \frac{0.0381}{\text{ m}} \right) = \Delta P_{\text{vel}} \]

\[ \Delta P_{\text{vel}} = 5.682 \times 10^6 \text{ N/m}^2 \]

\[ \tau_{\text{vel}} = \frac{6.35 \times 10^{-4} \text{ m}}{2} \cdot \frac{5.682 \times 10^6 \text{ N/m}^2}{0.0381 \text{ m}} = 4.735 \times 10^4 \text{ N/m}^2 = \tau_{\text{vel}} \]

\[ V_{\text{vel}} = \frac{\left( \frac{dV}{dt} \right) \frac{1}{A}}{\left( \frac{1}{L} \right)} = 3.75 \times 10^{-7} \text{ m}^2 \frac{25.392}{5} \left( \frac{0.0381}{\text{ m}} \right) = 0.0744 \text{ m/s} \]

\[ 1.075 \text{ m/s} = V_{\text{vel}} \]

\[ \Delta P_{\text{vel}} = \left( \frac{\partial V}{\partial t} \right) \frac{8\mu}{\pi R^4} \left( \frac{1}{L} \right) = 3.75 \times 10^{-7} \text{ m}^2 \frac{25.392}{5} \left( \frac{0.0381}{\text{ m}} \right) \]

\[ \tau_{\text{vel}} = \frac{3.302 \times 10^{-4} \text{ m}}{2} \cdot \frac{7.7 \times 10^7 \text{ Pa}}{0.0381 \text{ m}} = 3.367 \times 10^5 \text{ N/m}^2 \]

\[ V_{\text{vel}} = \frac{3.75 \times 10^{-7} \text{ m}^2}{5} = 2.84 \text{ m/s} = V_{\text{vel}} \]

\[ \Delta P_{\text{vel}} = \left( \frac{\partial V}{\partial t} \right) \frac{8\mu}{\pi R^4} \left( \frac{1}{L} \right) = 3.75 \times 10^{-7} \text{ m}^2 \frac{25.392}{5} \left( \frac{0.0381}{\text{ m}} \right) \]

\[ \tau_{\text{vel}} = \frac{2.05 \times 10^{-4} \text{ m}}{2} \cdot \frac{5.23 \times 10^7 \text{ Pa}}{0.0381 \text{ m}} = 1.407 \times 10^6 \text{ N/m}^2 \]

\[ \tau_{\text{vel}} = 1.407 \times 10^6 \text{ N/m}^2 \]

\[ \tau_{\text{vel}} = 1.28 \times 10^7 \text{ N/m}^2 \]

\[ V = 1.5 \times 10^{-6} \text{ m/s} \]

\[ dV = 4.0 \text{ s} \]

\[ L = 1.5 \text{ m} \]

\[ \mu = 25.392 \text{ N} \cdot \text{ m}^{-2} \]

\[ A_{\text{vel}} = 1.28 \times 10^7 \text{ N/m}^2 \]

\[ A_{\text{vel}} = 3.423 \times 10^{-4} \text{ m} \]

\[ A_{\text{vel}} = 3.423 \times 10^{-4} \text{ m} \]

\[ R_{\text{vel}} = 6.37 \times 10^6 \]

\[ R_{\text{vel}} = 3.34 \times 10^6 \]

\[ R_{\text{vel}} = 2.05 \times 10^{-4} \]

\[ 106 \]
\[ \Delta P = \frac{(3.75 \times 10^{-7} \text{ m}^2)}{1} \times \frac{8(25.392 \text{ kg/s})}{\pi (0.038 \text{ m})} \times \frac{(0.038 \text{ m})}{3} = 5.18 \times 10^{-7} \text{ Pa} \]

\[ \tau_{23,1'} = \frac{(3.362 \times 10^{-4} \text{ m}) \times (5.18 \times 10^{-7} \text{ Pa})}{2} = 3.367 \times 10^5 \text{ N/m}^2 \]

\[ \Delta P_{27,0''} = \frac{(3.75 \times 10^{-7} \text{ m}^2)}{1} \times \frac{8(25.392 \text{ kg/s})}{\pi (2.05 \times 10^{-4} \text{ m})} \times \frac{(0.038 \text{ m})}{3} = 1.744 \times 10^8 \text{ Pa} \]

\[ \tau_{27,0''} = \frac{(2.05 \times 10^{-4} \text{ m}) \times (1.744 \times 10^8 \text{ Pa})}{1} = 1.407 \times 10^6 \text{ N/m}^2 \]

Pressure in Syringe (flow rate through 23,1' needle, high force on plunger

\[ \Delta P = \frac{(3.75 \times 10^{-7} \text{ m}^2)}{1} \times \frac{8(25.392 \text{ kg/s})}{\pi (0.019 \text{ m})} \times \frac{(0.013 \text{ m})}{1} = 15.44 \text{ N/m}^2 \]

Syringe
\[ V = 1.5 \times 10^{-6} \text{ m}^3 \]
\[ r = 0.006 \text{ m} \]
\[ L = 0.132 \text{ cm} \]

Flow rate through 23,1' needle, high force on plunger
\[ V = 1.5 \times 10^{-6} \text{ m}^3 \quad \frac{\Delta V}{\Delta t} = \frac{(1.5 \times 10^{-6} \text{ m}^3)}{(2.304 \text{ s})} = 6.39 \times 10^{-7} \text{ m}^3 \]

\[ \Delta P_{23,1'} = \frac{(6.39 \times 10^{-7} \text{ m}^3)}{1} \times \frac{8(25.392 \text{ kg/s})}{\pi (0.006 \text{ m})} \times \frac{(0.013 \text{ m})}{1} = 42.4 \text{ N/m}^2 \]

Flow rate through 27,0'' needle, high force on plunger
\[ \frac{\Delta V}{\Delta t} = \frac{(1.5 \times 10^{-6} \text{ m}^3)}{(12.756 \text{ s})} = 1.176 \times 10^{-7} \text{ m}^3 \]

\[ \Delta P_{27,0''} = \frac{(1.176 \times 10^{-7} \text{ m}^3)}{1} \times \frac{8(25.392 \text{ kg/s})}{\pi (0.006 \text{ m})} \times \frac{(0.013 \text{ m})}{1} = 77.8 \text{ N/m}^2 \]

Don't trust this yet...
\[
\frac{Re}{\mu} = \frac{\rho V_s L}{\mu} \\
\mu = \text{fluid viscosity (N s/m}^2) \quad \rho = \text{fluid density (kg m}^{-3}) \\
V_s = \text{mean fluid velocity (m/s)} \\
P = \text{fluid density (kg m}^{-3}) \\
L = \text{length of needle (m)} \\
\]

**HA Reynolds Calculations**

\[
Re_{PA} = \frac{(1017 \text{ Kg/m}^2)(1.286 \text{ m/s})(0.0381 \text{ m})}{(25.3 \text{ N/s m}^{-3})} = 4.4517 \\
Re_{22} = \frac{(1017 \text{ Kg/m}^2)(1.095 \text{ m/s})(0.0381 \text{ m})}{(25.3 \text{ N/s m}^{-3})} = 1.671 \\
Re_{27} = \frac{(1017 \text{ Kg/m}^2)(2.844 \text{ m/s})(0.0381 \text{ m})}{(25.3 \text{ N/s m}^{-3})} = 4.334 \\
\]

**H₂O/PEG Reynolds Calculations**

\[
Re_{16} = \frac{(993.3 \text{ Kg/m}^2)(1.296 \text{ m/s})(0.0381 \text{ m})}{(1 \times 10^{-3} \text{ N/s m}^{-3})} = 11202 \\
Re_{22} = \frac{(993.3 \text{ Kg/m}^2)(1.015 \text{ m/s})(0.0381 \text{ m})}{(1 \times 10^{-3} \text{ N/s m}^{-3})} = 41439 \\
Re_{27} = \frac{(993.3 \text{ Kg/m}^2)(2.898 \text{ m/s})(0.0381 \text{ m})}{(1 \times 10^{-3} \text{ N/s m}^{-3})} = 107479 \\
\]

**HA Laminar flow through hypodermic needle**

\[\rightarrow V \text{ (m/s)}\]
Appendix I: Visual Description of Laminar and Turbulent Flow

Figure 46: Laminar v.s. Turbulent Flow
Appendix J: MTS Assay Data of loaded and standard methods of delivery of hMSCs within HA hydrogel

| Table 11: Raw data of loaded and standard methods of delivery of hMSCs within HA hydrogel |

<table>
<thead>
<tr>
<th>Method</th>
<th>Raw data of loaded and standard methods of delivery of hMSCs within HA hydrogel</th>
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</table>

<table>
<thead>
<tr>
<th>HA Needle in Needle out</th>
<th>Avg. Cell Number</th>
<th>7.82x10⁶</th>
<th>7.82x10⁶</th>
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</thead>
<tbody>
<tr>
<td>HA Loaded Needle out</td>
<td>1.25x10⁶</td>
<td>1.25x10⁶</td>
<td>1.25x10⁶</td>
</tr>
<tr>
<td>Control Needle out</td>
<td>1.0x10⁶</td>
<td>1.0x10⁶</td>
<td>1.0x10⁶</td>
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<tr>
<td>HA Loaded/Needle out</td>
<td>2.5x10⁶</td>
<td>2.5x10⁶</td>
<td>2.5x10⁶</td>
</tr>
<tr>
<td>Control Loaded/Needle</td>
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</tbody>
</table>

Figure 47: Equation to determine cell number for cell viability of transfer method of HA
### Anova: Single Factor

#### SUMMARY

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<th>Average</th>
<th>Variance</th>
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#### ANOVA

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<th>df</th>
<th>MS</th>
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<th>P-value</th>
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</table>

*Table 12: ANOVA results of HA Loaded*
Appendix K: Conceptual Designs of Needle

Figure 48: Syringe Needle with hydrogel interior coating to reduce shear forces acting on hMSCs during withdrawal and expulsion.

Figure 49: Syringe needle with multiple holes to reduce shear force and hMSCs loss during expulsion.
Appendix L: Cartridge Conceptual designs

Figure 50: Cartridge type syringe with interchangeable needle head.

Figure 51: Syringe with Cartridge inserted from the back. O-ring provides air-tight seal to prevent leaking during expulsion.
Appendix M: Final Cartridge Design

Step 1: Take one of the cartridges from the rack.

Step 2: Align the syringe and cartridge with plunger.

Step 3: Insert Cartridge into syringe. Contents.

Step 4: Press plunger to eject