Project Number: GXP-0702

Design of a High-Throughput Molecular Diffusion Device to Study Molecular Transport through Collagen Membranes

A Major Qualifying Project Report:

Submitted to the Faculty

Of the

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

By

Rachael Buteau

Thomas Jenket

Christina Mezzone

April 26, 2007

Approved:

Prof. George Pins, Major Advisor

Katie Bush, Co-Advisor

TABLE OF CONTENTS

AUTHORSHIP PAGE

All authors for various sections are listed below. Editing and proofreading of the entire document was completed by the members listed below for each section.

Section	Author(s)	Editor(s)
TITLE PAGE	Christina Mezzone	ALL
TABLE OF CONTENTS	Christina Mezzone	ALL
AUTHORSHIP PAGE	Christina Mezzone	ALL
ACKNOWLEDGEMENTS	N/A	
ABSTRACT	N/A	
TABLE OF FIGURES	Christina Mezzone	ALL
TABLE OF TABLES	Christina Mezzone	ALL
TABLE OF TABLES	Christina Mezzone	ALL
1.0 INTRODUCTION	Christina Mezzone	ALL
2.0 BACKGROUND		ALL
2.1 Basal Lamina Scaffold	Christina Mezzone	
2.1.1 Structure of the basal lamina		
2.1.2 Composition of the basal lamina		
2.1.3 Functions of the basal lamina		
2.2 Basal lamina within the body	Christina Mezzone	
2.2.1 Muscle Cells		
2.2.2 Kidneys		
2.2.3 Epithelial Tissue		
2.3 Importance of the Basal Lamina to Tissue Engineering	Christina Mezzone	
2.4 Characterization of Membranes by Diffusion Studies		
2.4.1 Molecules	Christina Mezzone	
2.4.2 Validation Methods	Thomas Jenket	
2.4.2.1 Spectrophotometry		
2.4.2.2 Enzyme-linked Immunosorbent Assay (ELISA)		
2.4.2.3 High-performance Lipid Chromatography (HPLC)		
2.4.2.4 Gas Chromatography (GC)		
2.5 Devices	Rachael Buteau	
2.6 Current devices available to purchase	Thomas Jenket	
2.7 Device Materials	Rachael Buteau	

3.0 PROJECT APPROACH	Rachael Buteau and Christina Mezzone	ALL
3.1 Project Hypothesis		
3.2 Project Assumptions		
3.3 Project Aims and Specifications		
4.0 DESIGN		ALL
4.1 Clarification of Design Goals	Rachael Buteau	
4.1.1 Establishing Project Goals	Rachael Buteau	
4.1.2 Constraints, User Requirements, and Functions	Rachael Buteau	
4.1.3 Weighting Objectives	Rachael Buteau	
4.1.4 Revised Client Statement	Rachael Buteau	
4.2 Brainstorming	Rachael Buteau	
4.2.1 Brainstorming Session	Rachael Buteau	
4.2.2 Morphological Chart	Rachael Buteau and Christina Mezzone	
4.2.3 Pros and Cons for Means of Each Feature/Function	Rachael Buteau	
4.3 Preliminary Design	Rachael Buteau	
4.3.1Metrics	Christina Mezzone	
4.3.2 Decision Matrices	Christina Mezzone	
4.4 Proposed Final Design	Rachael Buteau	
4.4.1 Model	Rachael Buteau	
4.5 Modifications	Rachael Buteau	
4.5.1 Cartridge on one side	Rachael Buteau	
4.5.2 Components	Rachael Buteau	
4.5.3 Manufacturing Modifications	Rachael Buteau	
4.6 Prototype	Rachael Buteau	
4.6.1 Manufacturing Modifications	Rachael Buteau	
4.6.2 Design Modifications	Rachael Buteau	
4.7 Final Design	Rachael Buteau	
4.7.1 Components of System	Rachael Buteau	
4.7.2 Fabrication Specifications	Rachael Buteau	
5.0 METHODOLOGY		ALL
5.1 Molecules for Studies	Rachael Buteau	
5.2 Assays for Testing		
5.2.1 BCA	Thomas Jenket	

5.2.2 Glucose	Christina Mezzone	
5.3 Preparation of Collagen Membranes	Christina Mezzone	
5.3.1 Pore Size		
5.4 Experimental Design		
5.4.1 Assembly of Experimental System	Christina Mezzone	
5.4.1.1 PermeGear®	Christina Mezzone	
5.4.1.2 High-throughput System	Christina Mezzone	
5.4.2 Experimental Protocols	Christina Mezzone	
5.4.3 Readings	Christina Mezzone	
5.4.4 Calculations for Diffusion Coefficients	Thomas Jenket	
5.5 Preliminary Validation	Christina Mezzone	
5.5.1 Leakage		
5.5.2 Temperature Control		
5.5.3 Mixing		
5.5.4 PermeGear® Studies		
5.6 High-throughput System Studies	Christina Mezzone	
6.0 RESULTS	Christina Mezzone	CM, TJ
6.1 Specifying Membrane Porosity		
6.2 Validation Results		
6.2.1 Leakage		
6.2.2 Temperature Control		
6.2.3 Mixing		
6.3 PermeGear® Studies		
6.4 High-throughput Experimental Results		
6.5 Graphical Representation of Diffusion Studies		
6.5.1 Glucose		
6.5.2 BSA		
6.5.3 Gamma Globulin		
6.6 Calculated Diffusion Coefficients		
7.0 ANALYSIS AND DISCUSSION	Christina Mezzone	TJ
7.1 Preliminary Trials		
7.2 Diffusion Trial Comparisons		
7.3 PermeGear® vs. High-throughput System		
7.4 Comparison to Previous Research		
7.4.1 Glucose		
7.4.2 BSA		

Appendix AB - Clamp Base	Rachael Buteau	
Appendix AC - Clamp Assembly	Rachael Buteau	
Appendix AD - Rat Tail Tendon Collagen Membrane Protocol	Transcribed by Christina Mezzone	
Appendix AE - PermeGear® Assembly Protocol	Christina Mezzone	
Appendix AF - High-Throughput Assembly System Protocol	Christina Mezzone	
Appendix AG- Protocol for Making Solutions of 5 mg/ml	Thomas Jenket	
Appendix AH - Diffusion Assay for Glucose	Christina Mezzone	
Appendix AI - Diffusion Assay for Bovine Serum Albumin	Christina Mezzone	
Appendix AJ - Diffusion Assay for Gamma Globulin	Christina Mezzone	
Appendix AK - BCA Protocol for BSA	Thomas Jenket	
Appendix AL - BCA Protocol for Gamma Globulin	Thomas Jenket	
Appendix AM- Glucose (HK) Assay	Christina Mezzone	
Appendix AN - Plate Reader and Softmax Pro Software Protocol	Christina Mezzone	
Appendix AO - Pore Size Analysis		
Appendix AP - Standard Results for Glucose, BSA and Gamma Globulin	Christina Mezzone	
Appendix AQ - Results for PermeGear® Trials: Glucose	Christina Mezzone	
Appendix AR - Results for PermeGear® Trials: BSA	Christina Mezzone	
Appendix AS - Results for PermeGear® Trials: Gamma Globulin	Christina Mezzone	
Appendix AT - Results for High-throughput System: Glucose using Dialysis Membranes	Christina Mezzone	
Appendix AU - Results for High-throughput System: Glucose Using SACE Membranes	Christina Mezzone	
Appendix AV - Results for High-throughput System: BSA Using SAC Membranes	Christina Mezzone	
Appendix AW - Results for High-throughput System: Gamma Globulin Using SAC Membranes	Christina Mezzone	
Appendix AX – Rearranged Fick's Law Variable Substitutions	Christina Mezzone	

Appendix AY – Calculations for Rearranged Fick's Law: Glucose	Christina Mezzone	
Appendix AZ – Calculations for Rearranged Fick's Law: BSA	Christina Mezzone	
Appendix BA – Calculations for Rearranged Fick's Law: Gamma Globulin	Christina Mezzone	
Appendix BB –Analysis of PermeGear® vs. High-throughput System: Glucose	Christina Mezzone	
Appendix BC –Analysis of PermeGear® vs. High-throughput System: BSA	Christina Mezzone	
Appendix BD –Analysis of PermeGear® vs. High-throughput System: Gamma Globulin	Christina Mezzone	

ACKNOWLEDGEMENTS

Our team would like to thank Professor Pins along with Katie Bush for their support and advice throughout the entire time of this project. We would also like to thank Mike O'Donnell for his help in the development of our high-throughput system. We would also like to acknowledge Kevin Cornwell and Stuart Howes for their support within the lab.

ABSTRACT

Diffusion studies allow scientists to gain a better understanding of the function and structure of membranes. However, these diffusion studies can be time consuming and costly when using current devices on the market. By designing and validating an affordable high-throughput molecular diffusion system time and money needed to characterize membranes can be reduced. Our device was validated using glucose (180 D), bovine serum albumin (BSA, 50,000 D), and gamma globulin (150,000 D); three molecules with a large range in molecular size. After comparison of calculated diffusion coefficients using our device and a marketed device, diffusion coefficients were shown to be the same for both devices. Using our device, membrane diffusion trials could be carried out simultaneously using 10 membranes compared to the current device which only allows one. The calculated coefficients were 5.58 $x10^{-6}$ cm^2/s, 7.08 $x10^{-8}$ cm^2/s, and 4.83 $x10^{-8}$ cm^2/s for glucose, BSA, and gamma globulin, respectively. These findings are comparable to published diffusion coefficient values, suggesting that our device will serve as a facile tool to rapidly characterize membranes being developed for the design of engineered tissues.

TABLE OF FIGURES

TABLE OF TABLES

1.0 INTRODUCTION

The basal lamina is a thin layer of extracellular matrix proteins that separates sheets of cells from the surrounding connective tissue within many organs [1-3]. This matrix component provides the foundation for cells to grow and differentiate upon and maintains the structural support system for the surrounding tissue. Its structure varies depending on its location within the body, such as in epithelial tissue, muscle and skin [1]. In addition to the basal lamina's physical support for the surrounding tissue and new tissue growth, it also acts as a selectively permeable barrier to various molecules within the body [1, 3, 4].

Any damage that afflicts various tissues and organs may affect the performance of the basal lamina. The basal lamina can even loose its ability to maintain the normal tissue structure and prevent the re-growth of new tissue within the area. Tissue engineers are currently researching means to treat this problem. Their ultimate goal is to develop a synthetic material that can be placed within the body to replace the natural basal lamina when it is harmed and can no longer carry out its functions. However, tissue engineers have made minimal progress in the development of a material/structure that is able to provide the necessary support to the surrounding tissue, create a selective barrier for molecules and allow new cellular/tissue growth to occur.

Tissue engineers are in the progress of trying to understand the basic functions of the basal lamina before developing a structure that can be placed within the body to repair the loss of the basement membrane and carry out its specific functions [5]. In order to begin the development stage of a synthetic basal lamina, these researchers have focused on studying the diffusive properties of these membranes. The *in vitro* studies on various types of porous membranes that have a similar structure and porosity to the basal

lamina have been conducted by tissue engineers and may provide the desired results. These tests may uncover the information needed to develop a material that can act as a barrier for certain molecules while allowing others to enter and exit the surrounding tissue and organs for a particular area of the body.

Currently, many types of diffusion studies are being performed on various types of membranes in order to achieve this goal. These studies use different molecules, membrane materials, and porosities to better understand the diffusion processes that occur within the body. These *in vitro* experiments also help tissue engineers to specifically create a material that has similar diffusion properties to the basal lamina. Many diffusion chambers have been created and are marketed to carry out these studies. One particular device is the PermeGear Side-by-Side Diffusion Chamber created by PermeGear, Inc [6]. This device places the membrane of choice between a donor and receiver chamber that can be filled with a molecular solution of known concentration and a buffer solution in order to create a concentration gradient that drives diffusion through the membrane. However, it is difficult to simultaneously carry out multiple experiments due to the fact that this device only has one set of chambers. Harvard Apparatus has a system that allows three studies to be performed simultaneously but the device is very expensive. Also, its user-friendliness and reliability have also been found to be problematic; therefore, there is a demand for a different type of device that allows for more testing to be carried out concurrently.

The goal of our project is to develop a high-throughput device to study the diffusion rate of various molecules through membranes. The project specifically aims to quantitatively define diffusion through collagen membranes to establish a more definitive

understanding of its functions and to develop a basal lamina for skin substitutes. The steps of engineering design will be utilized in order to develop and validate a diffusion system that meets our client's needs and desires. To do this, we constructed a high-throughput molecular diffusion device to characterize molecular diffusion through membranes. In order to validate the system, various experiments were conducted with the device using molecules of varying molecular weight to study their diffusion rates through both dialysis and collagen membranes. The dialysis membrane studies acted as a standard for the rate of diffusion for each of the molecules used within the studies. Diffusion studies were performed using the PermeGear® device to act as a standard for our device. The data was analyzed and the diffusivity of each molecule through the collagen membranes is reported. This data helps in characterizing the collagen membranes and is carried out in a more timely fashion with use of the multi-chamber system that can carry out multiple diffusion experiments simultaneously.

2.0 BRIEF CLINICAL AND ENGINEERING MOTIVATION FOR PROJECT

2.1 Basal lamina scaffold

The basal lamina is a thin layer of extracellular matrix proteins in tissue that separates sheets of cells from the surrounding connective tissue within many organs [1-3]. It has been found that this layer usually develops from parenchymal cells [1]. One layer of the surface provides the ability for cells to grow and differentiate, while the other provides support and adherence to the surrounding connective tissue [4]. The basal lamina has a distinct structure and a variety of functions that are dependent on its surrounding tissue.

2.1.1 Structure of basal lamina

There are three different forms the basal lamina takes on in its locations within the body. The structure may surround cells, reside underneath epithelia, or develop between cell sheets as seen in Figure 1 [1].

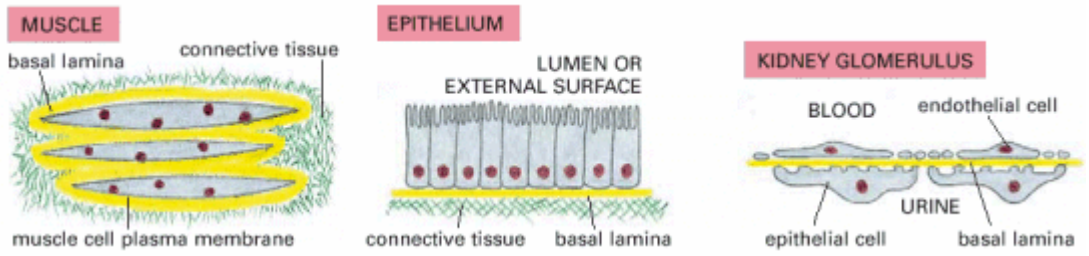


Figure 1: Organization of the Basal Lamina [1]

The basal lamina has a central dense region referred to as the lamina densa and a less dense region known as the lamina lucida [3]. The lamina lucida abuts the plasma membrane. When the basal lamina is located between cell sheets, the side adjacent to the

plasma membrane is the lucida interna while the opposite side is the subbasal lamina. The thickness of the basal lamina varies, depending on the tissue it resides within. For example, the thickness of the basement membrane within the kidneys ranges from 240 to 340 nm while that of the skin is 50 to 90 nm [3]. This size difference correlates to the different structures and organization of the tissues, as well as the functions the basal lamina performs in each location.

The porosity of the basal lamina varies depending on the area that the scaffold is located within the body. In previous studies, researchers have tried to develop membranes with a specific pore size similar to the area where the membrane would be located [7]. However, none of the current types of matrices are able to be applied and used as a basal lamina due to the need for the basal lamina to be able to work with the surrounding tissue and respond to various signals that may be sent to the area.

The basal lamina within some tissue has a more complex three dimensional structure. These structures can contain invaginations, referred to as rete ridges and papillary projections, which increase the surface area of the tissue. The role of these invaginations is to increase the biomechanical stability of the scaffold. Invaginations can effect cell proliferation and differentiation within the tissue as well [2]. Overall, the basal lamina contains various components that assist the basement membrane to maintain its form.

2.1.2 Composition of basal lamina

Although the composition of the basal lamina varies from tissue to tissue, there are a few main components that reside within all forms. During initial development, laminin molecules make up the basal lamina within the body [1]. Laminin plays a key

part in the structural role of the basement membrane, specifically for cell attachment. Laminin is a large flexible glycoprotein with three polypeptide chains held together by disulfide bonds. After the development phase, it has been found that type IV collagen makes up 40-65 % of all basal lamina [3]. Type V collagen has also been found to reside within the basal lamina of smooth muscle cells. The non-collagenous components in mature basement membranes including the glycoproteins are laminin, entactin, fibronectin, and proteoglycans [3]. Entactin is only located within certain membranes such as the nerve, muscles and kidneys but has an unknown function within basal lamina at this time. Fibronectin plays a role in cell attachment and structure of the basal lamina. The proteoglycans of the basal lamina dictate the selective filtration of the membrane. All of these components of the basal lamina work together to help carry out particular functions within the body.

2.1.3 Functions of basal lamina

There are three main functions of the basal lamina. One of these is its ability to act as a scaffold for tissue growth [1, 4]. It maintains the site for tissue re-growth after cell loss by allowing new cells grow along this scaffold. Within this process, the basal lamina also guides cell polarity, cell metabolism, and the organization of surrounding proteins [1]. If the basal lamina does not remain intact after injury, any new cellular re-growth will result in scar tissue and loss of function [4]. Another function of the basal lamina is to act as a barrier to control the selective permeability of the membrane. Two places where the permeability of the membrane is used to control diffusion of dissolved molecules are the kidneys and in tubular basement membranes. Not only does this control the filtration aspect of the tissue but also the movement of cells within or around

the area [1]. The third function of the basal lamina is that it provides structural support to the surrounding area by linking epithelium to the underlying matrix or to another cell layer depending on its location within the body.

2.2. Basal lamina within the body

Basal lamina resides within many tissues and organs of the body including muscles, lungs, kidneys, pancreas, the nervous system, liver, and the skin. Within each area, the basal lamina has a specific role in maintaining the structure of the area while providing scaffolding for the surrounding tissue. Depending on the location within the body, there are many differences in interactions between basal lamina and the surrounding tissue. A few examples of this can be shown within the muscle cells, kidneys, and epithelium.

2.2.1 Muscle cells

Within muscle, the basal lamina remains the scaffold upon which new cells can grow after resulting in an injury. It maintains the spatial relationship between both capillaries and fibers within the muscle [4]. The new growth of cells often results in the thickening of the basal lamina which leads to the removal of the older basal lamina. During cellular re-growth, muscle cells proliferate through basal lamina tubes which are part of the muscle structure. The basal lamina is able to separate the cells from the underlying connective tissue [1].

2.2.2 Kidneys

The basal lamina within the kidneys has a significant role in maintaining the order of cell re-growth and participates in their filtering process. It lies within the glomerulus

of the kidneys between direct contact with both blood and urine. It is a key decision maker in choosing which molecules will pass into the urine from the blood [1]. Molecules that are greater than 7 nm in diameter (anything larger than serum albumin) rarely cross through this barrier [3]. Permeability of this barrier is dictated by molecular size and charge. The heparan sulfate proteoglycans of the basal lamina play a key role in the filtration function. Without these chains, the lamina's filtering properties would be destroyed [1].

2.2.3 Epithelial tissue

There are two types of epithelial tissue: glandular tissue and covering/lining tissue. Glandular tissue is the secreting portion of a gland that is found in such places as the thyroid and sweat glands. Covering and lining tissue can be found within internal organs and lining major tracts within the body such as the digestive tract and the respiratory tracts. The basement membrane of epithelial tissue is a layer of extracellular material that takes a role in the development of the tissue. Not only does it act as an anchoring system but it also gives the cells an area to grow. It allows for both the epithelium and the connective tissue to remain connected. It also provides a surface for cell proliferation and migration to occur on during growth and wound healing. The basal lamina also prevents the invasion of large molecules into the connective tissue by acting as a selective barrier. For example, the basal lamina prevents fibroblasts in the underlying connective tissue from contacting epithelial cells within the skin [1]. However, the basal lamina does not prevent the movement of macrophages or lymphocytes.

2.2.3.1 Basal Lamina of skin

One area of focus within research of basal lamina has been the basal lamina acting as the connector between the dermal and epidermal layer within skin. The basal lamina not only provides a connection between the layers but also acts as a barrier to different cells intermingling, such as keratinocytes and fibroblasts. The structure permits movement of immune cells between the layers. Also, the basal lamina acts as a guide for cell proliferation and differentiation [4]. The proliferation and differentiation of the cells within this area is influenced by the topography of the basal lamina [8], [9].

There is an importance to understanding the mechanism of interaction between the dermal fibroblasts, the basal lamina and keratinocytes. When looking at native skin, there are specific keratins that are expressed during keratinocyte proliferation and differentiation. These proteins have been used as markers to study keratinocyte proliferation quantitatively. It has been found that growth factors and cytokines play a key role in stimulating the growth of keratinocytes. Fibroblasts produce these growth factors and cytokines which directly correlate to the amount of epithelial re-growth due to paracrine signaling [10]. This phenomenon shows the effect that the dermal fibroblasts have on epidermal layer growth. Since the basal lamina provides both structural and key functions to not only the skin but various parts of the body, it has become a major area of research for tissue engineers.

2.3 Importance of Basal Lamina to Tissue Engineering

Tissue engineers research various materials develop substitutes for injured tissues and organs that need repair. Researchers desire to find a material/design that will allow the tissues and organs to heal and return to a state where they can carry out their natural

functions [5]. Development of artificial tissues containing basal lamina analogs are very important for tissue engineering due to the critical functions they perform. The ability of the basal lamina to control the maintenance, regulation, and regeneration of various tissues and organs has been the driving force behind tissue engineers to produce a similar, synthetic structure that can carry out these functions. However, limited success has been made when trying to develop an engineered material that meets the standards of the basal lamina. It has been difficult for tissue engineers to develop a scaffold that can function as a selective barrier. Some success has been found in substitutes for skin and cartilage often using cultured cells and biomaterials [11, 12].

Due to the complexity of the basement membrane, tissue engineers need to understand the details of what this scaffold provides for the body before trying to create a material that will be able to support and perform the functions of the basal lamina. One key area of focus is the regulation properties of the membranous structure. Understanding the mechanism of molecular diffusion for various membranes in an *in vitro* environment will allow tissue engineers to characterize this property of membranes. These results can lead to the creation of a material that can act as a barrier to some molecules while allowing select molecules to enter and exit the surrounding tissues and organs. By researching membranes with porosity similar to various basal lamina and understanding their regulation parameters, tissue engineers will be one step closer to creating a scaffold that will allow the restoration of various tissues and organs. Tissue engineers are currently carrying out various diffusion studies on membranes in order to apply their findings on a larger scale to characterize and develop a synthetic, implantable basal lamina.

2.4 Characterization of Membranes by Diffusion Studies

2.4.1 Molecules

Many studies have been completed using various membranes in order to understand their diffusivity properties. In order to gain a better understanding of the membrane's parameters, various molecules have been used to simulate the size variety of molecules that diffuse through the basal lamina allow tissue engineers to gain a better understanding of the membrane's parameters. The results of these studies also can be applied to the basal lamina throughout the body. Each location of the basal lamina in the body allows molecules of various sizes and polarity to diffuse through it, depending on the basal lamina's structure and porosity.

In 1988, Gilbert examined the diffusion properties of collagen matrices in order to further the research of developing a drug delivery system that could use macromolecules [13]. Collagen was the material that was chosen for the membrane, due to its biocompatibility. Within this study, collagen membranes were designed in a variety of ways including native and non-native quaternary structure, porous fibrils, and dense aggregate membranes. Various types of crosslinking were used to create these membranes as well. The constructed membranes were placed within a two-cell diffusion chamber and a variety of molecules were used to characterize the parameters of the membrane. Samples were taken out of the receiver chamber within the diffusion cell and ultraviolet spectroscopy was used to determine the concentrations of the samples. From this data, permeation coefficients were determined from each type of molecule used within the study. The molecules were found to have the diffusion coefficients found in Table 1. This study found that membranes that were crosslinked with polyglycerol

polyglycidal ether (PPE) had a higher diffusivity coefficient than those that were crosslinked with glutaraldehyde. The study suggests that the diffusion coefficients for the collagen membranes crosslinked with PPE were higher because the chains were more flexible than those in the glutaraldehyde crosslinked membranes.

Table 1: Diffusion coefficients for various molecules across porous random fibril collagen membranes crosslinked with glutaraldehyde and PPE

Molecule	D for Gluteraldehyde Crosslinking (cm^2/s)	D for PPE Crosslinking (cm^2/s)
BSA	1.48×10^{7}	3.35×10^{7}
Lysozyme	8.19×10^{7}	8.71×10^{7}
Carbonic anhydrase	4.22×10^{7}	6.49×10^{7}
Ovalbumin	2.67×10^{7}	4.92×10^{7}

Desai reported many findings about fabricated membranes. Desai developed a micromachining method able to develop porous, silicone membranes with controlled porosity. This study determined how various porosity and permeability of membranes could help further the creation of an immunoisolation membrane [14]. Within this study, membranes were developed with a range of porosities: 18 nm, 66 nm and 78 nm. These membranes each were then characterized by their diffusion parameters. The permeability of insulin, glucose, and immunogamma globulin (IgG) was studied using a mini-diffusion chamber. The membrane was placed in between the two chambers, which allowed passive diffusion to occur. One side of the chamber contained a particular molecule in solution and the other contained just the buffer solution. Samples were taken from the buffer solution during the passive diffusion process in order to determine the concentration flux occurring over time. This method was carried out using both glucose and insulin; however, IgG was first fluorescently labeled before carrying out the

experiment. A biocapsule was used to study the diffusion rate of IgG through the membrane and the samples taken were read with a spectrofluroimeter. Both insulin and glucose were found to diffuse through the membrane at a significant rate when comparing each molecule's concentration of the various samples over time. IgG was not completely blocked but was greatly hindered in its diffusion through the membrane; the diffusion rate was less than other studies cited that tried to block IgG. Although changing the pore size of the membrane to a smaller size prevents the diffusion of IgG, it also has an effect on the diffusion of both glucose and insulin. Desai stated that the ability to maintain the membrane immunoisolation effect long term may be better than to have a totally immunoisolation membrane.

Desai completed another study to better understand the diffusion parameters of membranes. The goal was to develop a biocapsule that could be used to encapsulate insulin-secreting cells [15]. The membranes used within this study had a defined pore size of 24 nm. Using these membranes, the diffusion rate of glucose through the membrane was studied using a mini-molecular diffusion device again. Using a starting concentration of 6.66g/dl, diffusion across the membrane was allowed to occur as samples were removed from the receptor chamber of the diffusion device. These samples were analyzed using a spectrophotometer. Glucose studies with albumin in solution were also evaluated during these diffusion studies. Insulin diffusion was examined using a similar protocol as well. From these studies, Desai and Leoni found that the rate of glucose diffusion through the membrane was not decreased nor was it impeded in the presence of albumin. This was a key finding because it showed that the membrane may sustain and be able to carry out its functions for a longer period of time. The diffusion of

glucose was driven by Fick's law. Insulin also was able to easily flow through the membrane; however, it initially had a slow diffusion rate that eventually increased.

More *in vitro* studies were completed by Leoni in order better define the diffusion parameters of the membrane before *in vivo* testing was conducted. The study used a mini diffusion chamber to test the diffusion rates for microfabricated membranes with various pore sizes: 7 nm, 13 nm, 20 nm, 49 nm [16]. Three different sized molecules were chosen to better characterize the membranes. Glucose (180 D), human albumin (67 kD) and immunoglobulin G (150 kD) were individually used to define the diffusion parameters. A colorimetric assay was used to determine the concentration of glucose within the receiver chamber. A Bradford assay was used to find the albumin concentration, and an ELISA was used to determine the concentration of IgG in the samples taken during the experiment. This data was analyzed with Fick's first law to determine the diffusion rates of each molecule. The diffusion rate was correlated to the pore size of the membrane being used as well. For both glucose and albumin, the diffusion rates increased over time and also linearly increased as pore size increased. At a smaller pore size, the data seem to conflict with the calculations found with Fick's law. This proved that below a certain pore size this law could not be applied to characterize diffusion. IgG was excluded from diffusing through the membranes for most of the pore sizes. It was concluded that having a pore size 2-5 times larger than the molecule in use will still allow the diffusion of that particular molecule to carry out Fickian diffusion. At too small or too large a pore size, the diffusion of the molecules can be inhibited or skewed.

There have been a few diffusion studies focusing on the use of glucose as well. Myung et al, studied the diffusion rate of glucose through dialysis membrane (MWCO:14 kD), finding a diffusion coefficient of 3.4×10^{-7} cm^2/s [17]. These dialysis membrane findings were used as a benchmark for more glucose diffusion studies through various types of corneas within Myung's research. Another study completed by Liu et al. looked at the diffusion of glucose and bovine serum albumin (BSA) through collagen membranes [18]. The membranes used within these studies were EDC/NHS crosslinked membranes. This, along with many other types of crosslinking, can affect the porosity along with the thickness of the membrane, which are just a few parameters the diffusion coefficients are dependent on.

Lastly, but most importantly, diffusion has been looked at within native skin. Khalil et al. studied the diffusion of glucose through various layers of native skin [19]. Within cadaver dermis, the diffusion coefficient of glucose was found to be 2.64×10^{-6} cm^2/s. The diffusivity values were also found for various epidermal layers that underwent various treatments before being studied, such as tape stripping. These studies were able to determine the diffusion coefficients for each layer of skin while also understanding how various structural components of the skin can effect diffusion.

All of this research aims to characterize membrane transport using various molecules. In order to determine these transport parameters, validation methods must be followed. These validation techniques are used to determine the concentrations of the samples being removed from the chambers of device, which can then be used to calculate the diffusion coefficient of the particular molecules used within the study.

2.4.2 Validation Methods

To characterize the properties of the membranes, protein assays are important to determine the concentration, or changes in concentrations, as molecules pass through the membrane. There are several methods available to choose from that have been well documented and work in different ways. Absorption, colorimetric assays, high performance liquid chromatography (HPLC), and gas chromatography are four different methods discussed in this section.

2.4.2.1 Absorption

Absorption assays are performed with a spectrophotometer, using different wavelengths to measure how much of the light is absorbed. The Lambert-Beer law mathematically expresses how light is absorbed by matter. This law relates the amount of material in the solution, the distance the light must travel, and the probability that a photon will be absorbed by the material. Absorption assays are easier to perform than HPLC and gas chromatography but it is harder to control the variables in an absorption assay. Because absorption assays measure the difference in absorption of ultraviolet light, any impurities in the solution that can absorb UV light will skew the data, such as impurities in the water, or other molecules in the solution. However, it is still a fast, efficient way to measure the general concentration of molecules in a solution. UV spectrophotometry has also been applied to measure proteins of many sizes. These proteins include lysozyme, ovalbumin, and bovine serum albumin (BSA) [13].

2.4.2.2 Colorimetric Assays

Colorimetric assays depend on chemical changes and chemical interactions between the proteins and the solution used to measure their concentration. Colorimetric

assays use a solution and a spectrophotometer as well. The solution is used to either bind the proteins or uses the proteins to change a chemical in the solution. The chemical change in the solution normally leads to a change in color of the solution. There is a standard of known concentration that is used and tests are compared to that standard. An example of a colorimetric assay is the bicinchoninic acid (BCA) assay. This assay involves mixing the sample solution and the acid. In the acid solution, the copper molecules undergo an ionic change from Cu^{+2} to Cu^{+1} in the presence of proteins in an alkaline solution [20]. The Cu^{+1} ion in solution has a purple-bluish color in the bicinchoninic acid solution (Figure 2). The color absorbance maximum is 562 nm for this test. The change in absorbance is directly proportional to the concentration of protein in the sample. Pierce sells a BCA kit that has varying range of accuracy depending on the procedure used. The kit can measure protein concentrations of 20-2,000 µg/ml using its standard procedure. If a smaller concentration needs to be measured, they also include an enhanced procedure that measures protein concentrations of 5-250 µg/ml. The accuracy of these tests explains why this method is commonly used for the detection of proteins. One disadvantage of this assay is that it is non-protein specific, meaning that it will detect any protein in the solution. If a test is done using more than one protein, this assay will not be able to determine the concentration of just one of the proteins.

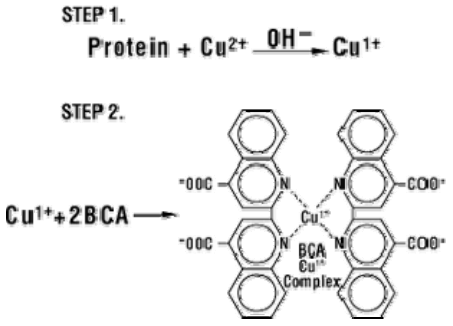


Figure 2: Schematic of BCA reaction in the presence of a protein[21]

Another example of a colorimetric assay is the use of Coomassie® Blue G-250 dye binding process, also known as the Bradford assay. The binding of the dye to the protein changes the absorption rate of the dye from 465 nm to 595 nm (Figure 3). The change in absorption rates can be measured using a spectrophotometer that measures the amount of light absorbed at the frequency of 595 nm. This method is quick, inexpensive, and very accurate. Its working range is from 1-20 µg/ml for the micro assay and 20-200 µg/ml for the macro assay. It also is easily used for different proteins. Leoni et al. used the Bradford assay to test for the concentration of albumin [16]. One of the disadvantages is that the curve of absorbance is non-linear over a large frequency range. This is due to how close the absorbance frequencies for the bound and unbound Coomassie® Blue G-250 dye are. Due to this, the standard curve is crucial. As with the BCA assay, the main disadvantage of this assay is that it is non-protein specific.

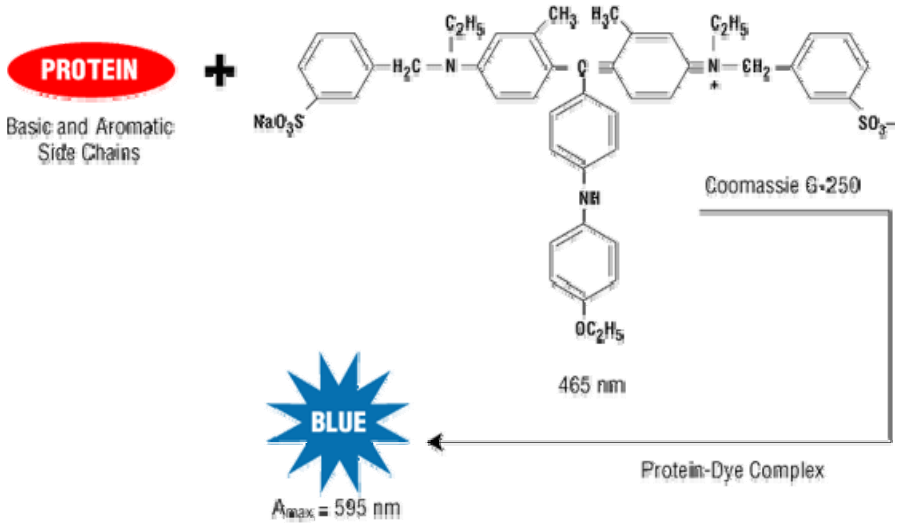


Figure 3: Schematic of Coomassie G-250 in the presence of a protein[21]

2.4.2.3 Enzyme-linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assays (ELISA) are a specific example of an assay that utilizes fluorogenic substrates. They are used to look for certain proteins using antibodies that are specifically tailored for a tested molecule. This means that the molecule being tested must be able to have an antibody attach to it. However not all molecules have antibodies made for them. This method is effective in isolating just one molecule by designing the antibody to attach specifically to the tested molecule.

A common practice is using indirect detection (Figure 4). This method is called the sandwich method. Indirect detection works through a several step process. This involves initially coating the plate with an antibody to the molecule being tested. This coating incubates overnight, and then the plate is washed to remove any excess antibodies that are not attached to the plate. The sample of a known or unknown concentration is then added. This sample is the molecule being tested. This is allowed to incubate for a while to allow binding of the sample and the antibody. The plate is then rinsed again with a solution such as phosphate buffer saline (PBS) or another buffer solution, in order to remove any of the molecules that did not bind to the antibody. Next, an antibody that is labeled with an alkaline phosphate conjugate is added to the plate, which binds to the top halves of the tested molecules. Again, the plate is rinsed to ensure no extra unbound antibodies are floating around. After the rinsing, a substrate solution is added, such as *p*-nitrophenyl phosphate (PNPP), which is activated by the alkaline phosphate group on the antibody. This results in the breaking of PNPP. One of the resulting structures of this breaking is a colorimetric chemical that turns the solution a color, in the case of PNPP, a yellow color. The intensity of the color is dependent on the amount of second labeled

antibody that is bound to the sample. The intensity of the color is measured at a specific frequency that is dependent of the colorimetric chemical resulting from the breaking of the substrate complex. The intensity is measured by putting the plate in an ELISA plate reader. This number is compared to standards that are made of the tested molecule. These standards are of known concentrations. The assay for the standards are performed as explained above. The absorption rates are charted on a graph and the unknown values are compared to the standards.

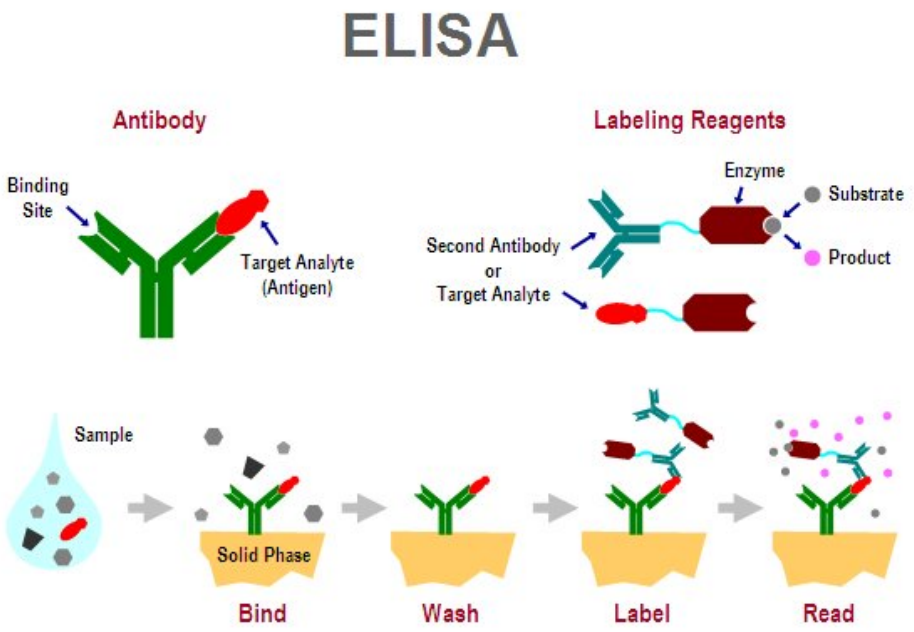


Figure 4: Schematic of an ELISA [22]

Some of the disadvantages of this method are the increased incubation time, as well as cross-reactivity with the second labeled antibody. The increase in incubation time could hinder how many tests that can be performed in a given time period. The high price of antibodies and other molecules required for the tests also limit the utility of this technique. However, there are several advantages to this method. Not only has it been

well documented and is well establish, but there are several secondary labeled antibodies commercially available. This technique is also very versatile. There are many labels for the secondary antibody, increasing the number of tests capable of determining the concentration of the protein.

2.4.2.4 High-performance Lipid Chromatography (HPLC)

High-performance lipid chromatography (HPLC) is a very versatile technique that is commonly used when determining the concentration of a molecule in a solution. The process is a complex one, as there are many ways to perform an HPLC test. The reversed-phase HPLC is the technique used to detect peptides and membrane proteins. HPLC has been well documented for the detection of hydrocortisone [23, 24] as well as for dopamine [25]. There are two phases used in an HPLC: a stationary phase, and a mobile phase. Different materials can be used for each phase, which increases the number of tests and molecules this technique can be applied to.

The reversed-phase chromatography operates by isolating the molecule based on the solution's hydrophilicity. A combination of resins is used to create the hydrophilic environment. Resins range in composition but an example would be the stationary phase being a silica-based packing with n-alkyl chains covalently bonded. This is hydrophobic, which the tested compound is attracted to. Hydrophilic compounds elude faster than hydrophobic compounds in this system. HPLC is a way of refining a solution. There is an absorption test after the refinement to acquire a concentration. This absorption assay is the same as the assay mentioned above but the spectrophotometer is normally built into the whole HPLC system.

One of the advantages of the HPLC is that it can be manipulated for different molecules that need to be tested. Different resins can be used to isolate different molecules based on their hydrophilicity. This is very useful when thinking about one device to test several molecules. However, due to the fact that different resins are needed for different molecules, this does increase the overall cost of the testing portion of the device.

2.4.2.5 Gas Chromatography (GC)

Gas chromatography mass spectrometry is a unique process to detect different molecules. It involves using an oven encased GC column, a mass spectrometer, and a computer to analyze the data [26, 27]. The computer presents the data as a measurement of the amount of an ion based on a specific mass. The separation that takes place in the column and the ionization that takes place in the mass spectrometer allows the detector to separate ions. The separation and ionization are the key factors in isolating the different ions.

The process involves heating the injection port up to about 300º C to cause the injected solution to vaporize. The solution is injected, vaporizes, and then travels 30 meters though a thin-walled tube that has been chemically treated on the inside. The chemical treatment on the lining of the tube separates the molecules based on the volatility. Essentially, the smaller molecules travel faster though the 30 meters of tubing. The oven is normally heated from 40º to 320ºC. The molecules then pass into a mass spectrometer. The mass spectrometer exposes the molecules to electrons, which breaks the molecules into ions. These ions pass through a filter, which is a magnetic field that slows down larger ions. After the filter, the ions finally hit a mass detector and a mass

spectrum is created. This information is sent to a computer, which records the data for future analysis.

One of the advantages is the sensitivity of the detector, whether it is a universal or selective detector [26]. It is also useful for many classes of organic compounds. Its strongest point is actually its high resolving power due to the number of stationary phase materials that can be used for any number of molecules. However, a system like this does not come without disadvantages. One of the biggest problems is that the compounds in the solution need to be sufficiently volatile [28]. This is essential for their separation. Due to the conditions in which they are injected, they also need to be thermally stable so they do not degrade when injected. This limits the range to non-polar or slightly polar molecules. Also, like the HPLC, the different resins needed to test each molecule would increase the cost of running the experiment.

Using these assays, the concentration of the molecules within the receiver chamber of the diffusion devices can be found. In order to carry out these diffusion studies, various devices have been developed over the years and are currently on the market to determine the parameters of diffusion for various membranes.

2.5 Devices

There are multiple devices on the market today that facilitate molecular transport through various membranes. There are currently three main types of molecular diffusion devices used for *in vitro* studies: the vertical type, horizontal type and flow-through type. The vertical type holds the membrane being studied horizontally but the direction of solution flow is perpendicular to the membrane, and vise versa for the horizontal.

All of the devices consist of two chambers; a donor and receiver. The donor chamber is where the tested molecules are put into the system, and the receptor chamber is where the samples are collected from. Both vertical and horizontal devices are static diffusion chambers, whereas the flow-through has a dynamic circulation of solution through the receiver chamber.

Each one of these devices has essential components that allow diffusion studies to be done *in vitro* and be compared to the actual functions of the basal lamina. Mixing is a key component of this device, due to the fact that it allows for solutions placed within the donor or receiver chamber to remain homogenous and prevent the formation of a boundary layer that can complicate diffusion. Temperature control is also a main function of these devices. By being able to control the temperature, the diffusion studies are run at different temperatures rather than just room temperature. For example, studies can be done at body temperature (37ºC) in order to simulate the diffusion of particular molecules at that temperature and see the effects of temperature on the specific membrane being studied. The placement of the membrane is also very crucial for the studies. Each of these devices optimizes the surface area of the membrane in contact with the solution. It is important to ensure that there is no other diffusion going on in the system. Each system should be in a closed system so that there are no outside influences that would interfere with passive diffusion. This is not always the case, but it does help when applying mass conservation laws. If it is an open system, there should be a way to calculate the amount of mass transfer coming in or going out of the system.

For the purposes of this project, horizontal and vertical diffusion cells were researched more in depth because the client informed us that the flow-through devices

were outside the realm of their interests. Our client's interest is in the interactions between the substrate and the cells on top of the membrane in a steady state environment. Furthermore, horizontal devices were of greatest interest because they provide a fluid to fluid phase system, whereas the vertical provides an air/gas to fluid phase system. Information researched includes the types of diffusion cells, the current usage of these devices, what is available to purchase today, and the advantages and disadvantages of the materials that make up these devices.

2.5.1 Vertical Type: Franz

The vertical diffusion cell is an air/gas to fluid orientated device. The most recognized and used type was developed by Dr. Thomas J. Franz. This device has a static receiver solution reservoir with a side-arm sampling port design as seen in Figure 5. The device can be used with the donor chamber cap, at the top of the system, open to allow ambient exposure or closed. The tested membrane determines whether the membrane in the donor chamber is exposed to the atmosphere or not. The bottom half of the cell is the receiver chamber where samples are removed. In the original Franz cell, the membrane was held in place by an o-ring between the two chambers and a magnetic stir bar was used in the receptor chamber for homogeneous mixing. A thermal jacket surrounded the receiving chamber so its temperature could be maintained by a circulating water bath. In 1975, Franz reported the comparison of his *in vitro* device measuring percutaneous absorption to results from studies which have been done *in vivo*. Quantitatively the permeability of the various molecules did not match exactly, but the results paralleled for which molecules had high versus low permeability. He found that

using this device allowed diffusion studies to be compared to natural diffusion within the body [29].

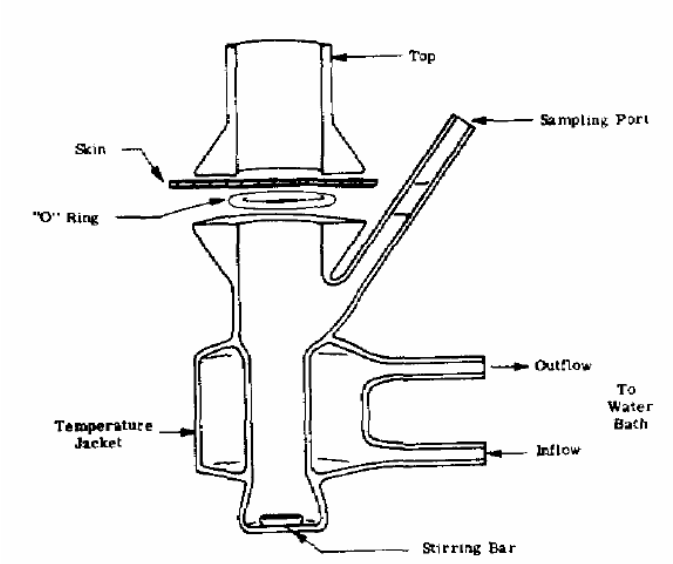


Figure 5: Original Franz Cell [29]

The original Franz cell has been modified for various applications of studying molecular behavior through membranes *in vitro*. This static, side-arm cell has been used to study skin permeability for drug delivery applications and also has been used to study enzymatic digestion product release.

A flat-bottom static diffusion cell, which can be purchased from Hanson Research [30], was used by sources to study the *in vitro* drug release from topical dermatological products that have corticosteroids [33]. The diffusion system included six cells in conjunction with an autosampler by Microette ® [31]. The cells were very similar to a Franz cell but maintained homogenous mixing in the receiver chamber with a stirring helix. The device setup was then modified slightly to use with the MicroettePlus® system. This modification occurred when researchers tried to establish a more in depth protocol for measuring *in vitro* release of semisolid preparations. The ultimate goal of

the work was to develop a standard test for quality control in industry. The modifications can be seen in Figure 6 [31].

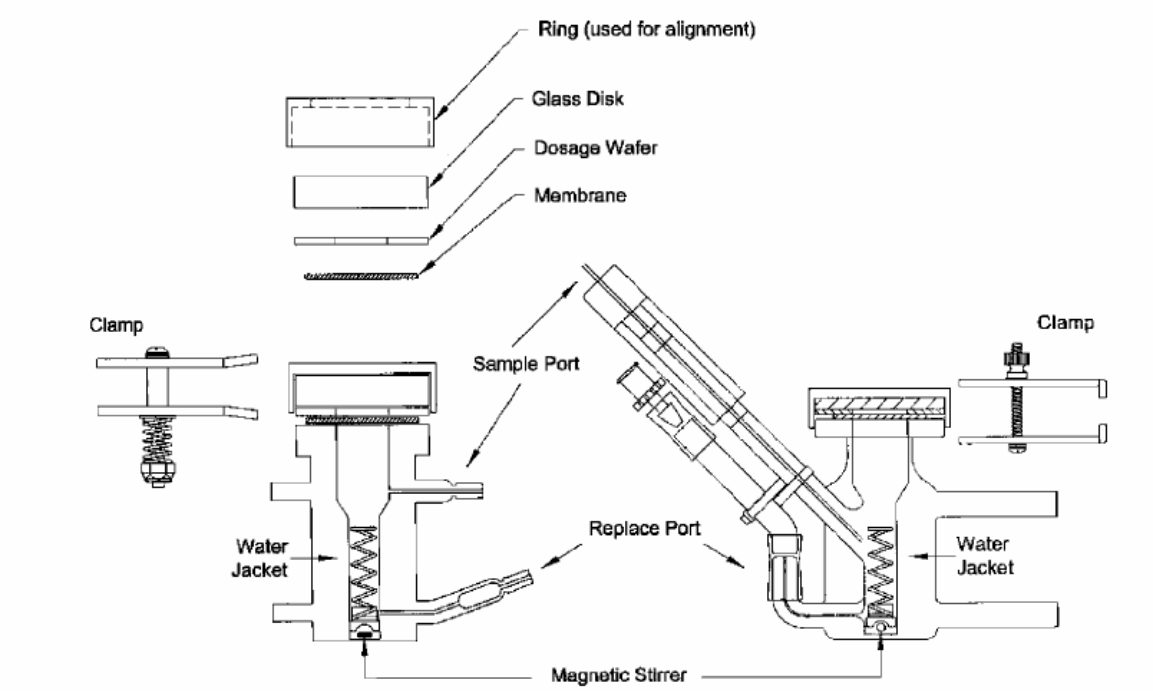


Figure 6: Design of the vertical diffusion cell with the MicroettePlus® system [31]

A quantitative analysis of enzymatic digestion product release was studied to improve the quality of debriding-agents used in the treatment of necrotic wounds [35]. The *in vitro* procedure proved to be useful in determining the efficiency of enzymatic debridement and provided well controlled pH and temperature, which were within physiological values. This *in vitro* method utilized a Franz diffusion chamber as well.

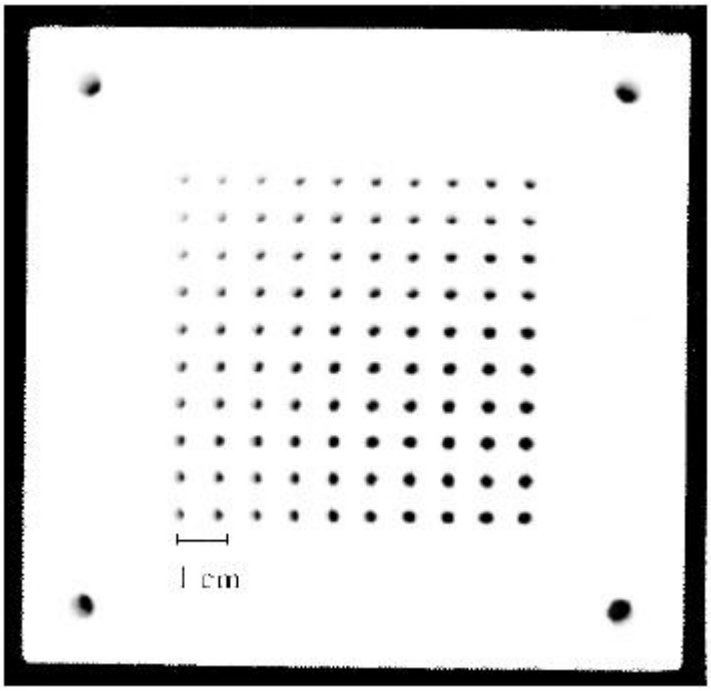


Figure 7: Cell used for High Throughput Screening [32]

A high-throughput device was designed and developed to see how chemicals affect skin permeability (Figure 7) [32]. It was predicted that combinations of these chemical "enhancers" may offer a safer formulation to increase skin permeability. The high-throughput screening method allowed researchers to test multiple different enhancers in a more efficient manner than the Franz diffusion cell allowed. It was stated by the authors that the disadvantages of the Franz diffusion chamber were that too large of a membrane sample needed to be used, the time it took to run the procedure was too long, and the time required to reach the steady-state was too long. Although in this article, the researchers are specifically interested in skin conductivity and measured it with electrodes; the high-throughput device exemplifies the fundamental purpose of efficiency. The device was created with Teflon and polycarbonate and the tests were performed with pigskin membranes and various enhancers. The conductivity was measured qualitatively with the high-throughput device, but it was concluded that the Franz cell was still

necessary to find the actual amount of drug delivered across the pigskin sample. The high-throughput device simply helped the researchers choose which enhancers should be tested with the Franz in a short amount of time; it avoided wasting time with unnecessary tests using the Franz cell [32].

Overall, the vertical diffusion cell is not desirable for the purposes of the studies being performed with our molecular diffusion system. The directional orientation of the device is incorrect for the desired applications of studying the transport rate of various molecules through membranes. The donor chamber is not equivalent in size to the receiver chamber, and cannot hold a homogenously mixed solution. One foreseeable problem is that there can be difficultly with air bubbles forming when placing the donor chamber on top of the receptor chamber. The proven reliability of the system to characterize *in vivo* application in an *in vitro* environment is desirable, as well as the system's ability to regulate temperature and mixing in the receiver chamber.

2.5.2 Horizontal

Horizontal chambers have a fluid to fluid phase system along with equivalent donor and receiver chamber sizes, which are both critical for the characterizing of different properties of a basal lamina. A fluid to fluid phase system is important because it mimics the environment in the body better than exposure to an open environment. Equal donor and receiver chambers are important because it allows for diffusion to be dependent on the diffusive properties of the membrane and not dependent on the size of the chambers.

In the previously discussed study by Gilbert, et al., a horizontal diffusion chamber was used to characterize porous random fibril collagen membranes crosslinked

with glutaraldehyde and PPE. Desai and Leoni also used a horizontal diffusion chamber to complete their studies in order to determine the diffusion parameters of both the membranes and biocapsules. Additionally, a polycarbonate horizontal apparatus was used to study diffusion through caco-2 cell monolayers. A diffusion device was developed specifically for this study and used a stir plate developed by PermeGear to run three of the chambers at the same time with magnetic stir bars [6]. A photo of a single diffusion chamber can be seen in Figure 8. Samples of 200μL of the solution were taken from the receiver chamber and tested with scintillation techniques for protein concentration. The device designed was also capable of holding Millicell® inserts and was durable enough to be used for four years as an *in vitro* model of intestinal drug absorption [33].

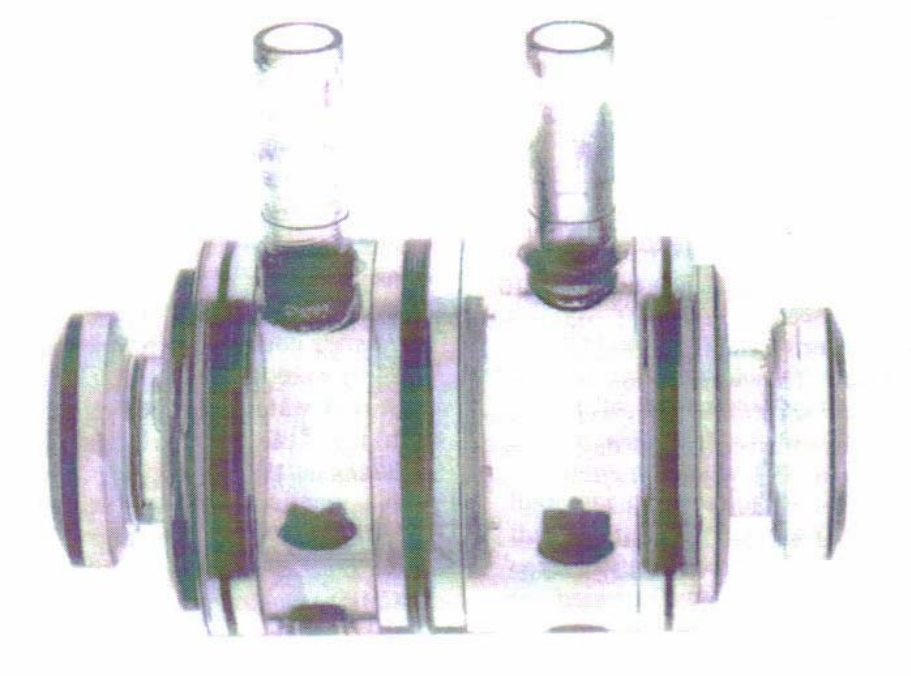


Figure 8: Diffusion chamber used in Kuhfeld and Stratford studies [33]

In a research project that studied protein transport and separation properties of poly(vinyl-acrylic) (PVA) gel membranes, a glass stirred diffusion cell was used, as seen

in Figure 9. To inhibit protein absorption, Sigmacote® was applied to the walls of the cell. Bovine serum albumin (BSA) and lysozyme were the molecules used in the study to show how surface modifications on the PVA membranes affected diffusion [34].

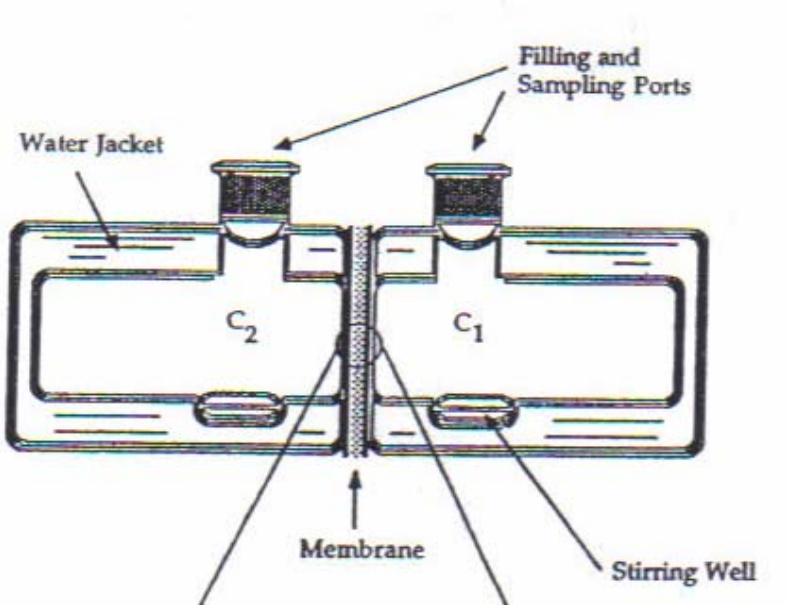


Figure 9: Glass stirred diffusion cell used by Li and Barbari [34]

2.5.3 Flow-through

Flow-through cells provide an automatic replenishment of receptor fluid. Sample collection is more uniform and the operation of these devices does not need to be attended with as much careful observation as the other devices previously mentioned. They were designed to be easy to use and maintain tissue vitality.

Flow-through devices are described in detail by Frantz in *Methods for Skin Application* [35]. He discusses how the flow rate affects the diffusion and how even though blood rates within an *in vitro* versus and *in vivo* study are different, diffusion devices help in approximating the true conditions of diffusion within the body [35]. It was also mentioned that the flow rate through the receptor does not ensure that the

chamber is being mixed well. The use of stirrers may be necessary in some systems, depending on the size of the chamber and the rate of flow.

An automated, dynamic diffusion cell was developed called the Kelder-cell, which was considered an alternative to the Franz cell and can be seen in Figure 10. These new cells were used in combination with the automatic sample preparation with an extraction columns (ASPEC) system. The system was developed for *in vitro* studies of transdermal permeation. The cell was designed to have automated sampling, provide a continuous replacement of receptor solution to mimic blood flow beneath the skin, and to have an unattended procedure for a period of 24 hours. The membrane tested was a non-reinforced silicone membrane, Silastic®. An anticholinergic, [^{3}H] dexetimide, which has been used as an internal standard to correct for variations in skin, was used in the experiments. The variables tested on the system included variability of injection height, the volume flowing through the receptor, and the temperature. An injection height which ensured air tightness was found; temperature increase was found to reduce the lag time and have no affect on flux; and the increase in collection volume was directly related to the increase of permeation. The Kelder cells were compared to the Franz diffusion chambers and it was found that data from each were comparable when the Kelder cells were in a pseudo-steady state [36, 37].

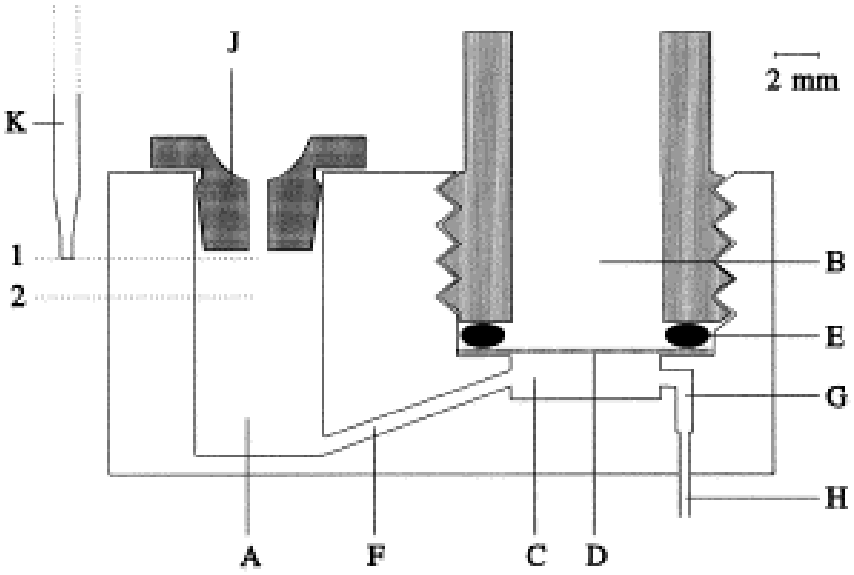


Figure 10: Cross-section of the Kelder-cell [36]
Key (A) inlet compartment; (B) donor compartment; (C) receptor compartment; (D) membrane; (E) O-ring; (F) inlet channel; (G) outlet channel; (H) outlet tube; (J) polypropylene cap; (K) needle; 1, injection height of −32 mm

Another flow-through device was developed by Bronaugh and Stewart. Their device used a minimal volume within the receiver chamber and allowed samples to be directly transferred to collecting tubes [38]. The cell was used for drug delivery applications. An automatic fraction collector was used to gather samples from the receptor chamber. A vehicle film was used with the membrane being studied in this experiment because the source was interested in aiding in the development of drug delivery via ointments and transdermal patches.

2.6 Current Devices Available to Purchase

There are many different diffusion cells and different approaches companies have taken to design diffusion devices. Several companies have modified the initial designs of diffusion cells, such as the Franz cell and the horizontal diffusion chamber, and created automated testing systems. A further step in the development of diffusion cell designs

was to incorporate measuring tools into the systems [39, 40]. All of the diffusion cells that were researched allow samples to manually be removed from the device. Information from websites for different devices currently available for purchase was also explored, as seen in Table 2. Companies that have developed these devices include Logan Instruments, Warner Instruments, PermeGear Inc., and Harvard Apparatus. Their devices are outlined in Table 2 and are further explained.

Currently, there are four varieties of joints for the Franz cell: flat ground, flat flange, O-ring, and spherical (Figure 11). Different joints are used to hold different materials for diffusion studies. For example, spherical joints are used for corneal work due to the concave shape of the tissue. However, the joint that is used most often in industry is flat ground joint that comes with an O-ring [41]. It is stronger and is used for membranes as thick as 4 mm. The spherical joint is used primarily for corneal work and comes in two sizes: 12 and 18 mm. PermeGear, Inc. also carries a nail adapter that allows for a fingernail or toenail sample to be tested.

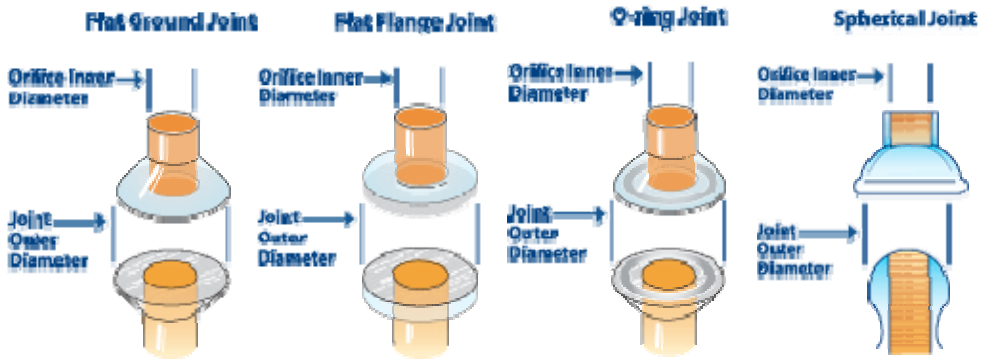


Figure 11: Various Joints for Franz Cell [37]

Table 2:Devices Currently on the Market

	Name of Device	Specs	Function/Usage	Company
1	Horizontal Diffusion Chamber System	6 tests can be run; heated; open/closed environment;	cells or tissues can be exposed to liquids, solids or gases while basolateral surface uses media	Harvard Aparatus
2	Franz Cell	4 Types: spherical, o-ring, flat ground, flat flange; 2 chambers (inlet and outlet)	membrane placed between top and chamber; sampling port; heating chamber surrounds	PermeGear, Inc.
3	Horizontal Cell	two chambers; two sampling ports; constant heat flow through each chamber, two stir bars	Liquid diffusion of media with cells to study	PermeGear, Inc.
4	Classic Ussing System	Two chambers; gas transport; condenser; acrylic	Ionic transport by diffusion or active transport	Warner Instruments
5	Easymount Multi-Chamber Vertical systems	2,4,6,or 8 chambers; needle valve gas control manager; electrodes for voltage measure and passiver current	High-throughput application for gases	Warner Instruments
6	Test cell	two smaller and two larger chambers; test window for membrane	Test CP diffusion through membrane	N/A (student thesis)
7	Vertical Diffusion Cell	3 sizes; receptor chamber, donor chamber , dosage wafer sampling and media replace ports, jacket for temperature control, Helix™ stirrer, occluded cell	skin permeation studies, including topical and transdermal drug delivery formulations	Hanson Research
8	Polycarbonate diffusion chamber	2 stirred chambers, Water tight seal for chambers, Liquid transport, 27 mL chambers, submersible	Isolate two sections in each chamber using a filter to test motion of the water on diffusivity	Lilly Research Laboratories

PermeGear, Inc. carries both Franz Cell and side-by-side (horizontal orientation) cell designs. All of their devices are hand blown glass. The joint sizes are also variable, as mentioned above. The side-by-side devices are water jacketed. The chambers are clamped together by a stationary vice that has rubber pads at the contact points of the chambers. The vice is tightened, via a screw, and uses pressure to seal the chambers with the membrane between them. PermeGear also sells devices that allow for multiple side-by-side chambers to be used at one time [42]. PermeGear's Franz Cells come jacketed or non-jacketed. Due to the fact that all of the devices are hand blow glass, the jackets are not interchangeable. The device is two pieces: the top (donor) chamber, and the bottom (receiver) chamber with or without the jacket. The size of the upper chamber of the Franz cell comes in diameters of 5 to 25 mm. The various tops are mentioned above. All of the jackets are 30 mm in diameter. The receptor volume varies from 5 to 20 ml.

Another company has taken a different approach. Logan Instruments Corporation has designed two separate systems using diffusion cells. The System-912 is an automated system that uses up to twelve Transdermal Diffusion Cells at one time [39] (Figure 12). The twelve cells allow for six experiments to be run at one time. The System-902 is also an automated system but it utilizes Franz Cells and is connected to an HPLC testing system [40]. The testing system reports for percentages of dissolved molecules or the amount in milligrams. The system is computer controlled.

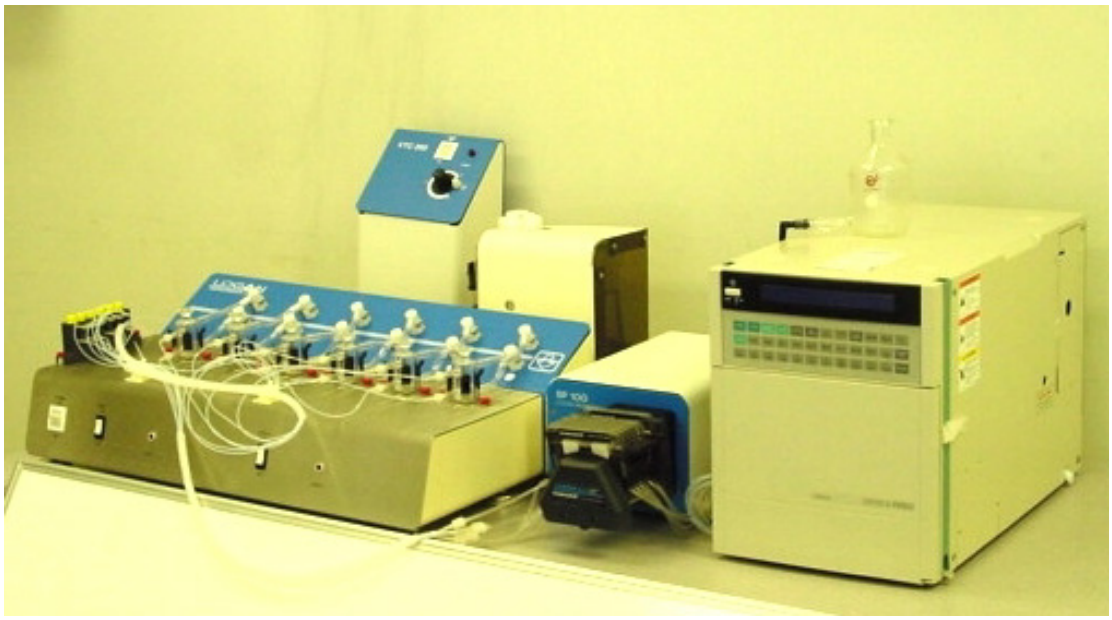


Figure 12: Logan Instruments System 912 [39]

Warner Instruments has many devices designed to measure diffusion of ions and molecules. The U2500 system is a side by side self-contained chamber system that has various inserts [43] (Figure 13). The round inserts with and without O-rings range from 3.8 to 13.5 mm. Warner Instruments also has another Ussing chamber, U9500 [44], that comes as part of a kit. The circulation reservoirs are between 4 and 30 ml. The chambers are machined, solid, clear acrylic. There are sharp pins that go from one chamber into the other in order to clamp down the membrane. Warner Instruments intended this product to be used for epithelium research. The U9500 and U2500 have holes drilled into the chambers that allow electrodes to take measurements. However, these holes could be used for sampling. Warner Instruments has also developed a multi-chamber vertical diffusion device. It holds 12 chambers, facilitating 6 experiments. This system is used primarily for gas transport. The 12 channel gas manifold distributes gas to each of the chambers. Electrode caps allow proper positioning of electrodes within the chambers.

The heat block provides precise heating or cooling for the chambers. The heat block is regulated using a circulating water bath.

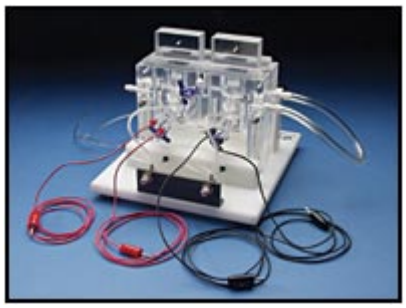


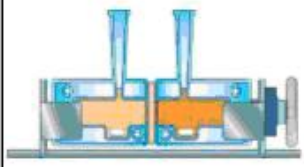
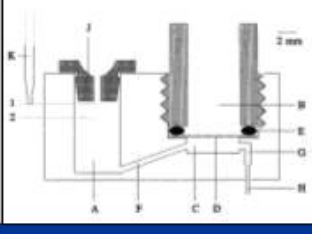
Figure 13: U2500 System by Warner Instruments [43]

Harvard Apparatus developed a two chamber, thermal controlled system that facilitates diffusion. The two Ussing chambers have an EasyMount insert slide for the membrane (Figure 14). The inserts are universal for the other devices Harvard Apparatus makes. The diffusion chambers vary extremely in size. The smallest is 2 mm in diameter and the largest is 12.7 mm diameter. Some of the diffusion chambers are designed specifically for a certain type of tissue [45]. The system also can be linked with several other chambers in parallel to accommodate high throughput applications. The tissues in the insert are easily placed in the chamber by loosening the thumbscrew between the chambers and then placing the insert with the tissue between the chambers and retightening the thumbscrew. This process helps in the high speed replacement of tissue, which makes this system an excellent high throughput device.

Figure 14: Diffusion Chamber of the EasyMount Diffusion System [45]

As stated in the previous sections, there are many types of devices on the market that are currently being used to study the diffusion parameters of various membranes. Each of these devices uses particular materials that have both advantages and disadvantages. Table 3 summarizes the pros and cons of each different types of devices discussed.

Table 3: Pros and Cons of the different devices discussed

Name	Device	Advantages	Disadvantages
Franz Cell		•Thermo-regulated water jacket •Receptor stirred to create homogeneous solution •Mimics dermal application •Skin may be left opened or closed	• No stirring in donor chamber •Air bubbles can form in donor chamber •Over-hydrating of membranes •Possible boundary layer development •Break down of barrier function of skin
Horizontal Cell		•Smaller donor and receiver chambers •Stirring within both chambers	• Difficult to change solvents •Air bubbles within chamber •Over-hydration of membranes •Difficult to set-up •Leaking problems
Flow-through cell		•Non-sink conditions •Automatic sampling •Continuous replacement of solution •Unattended operation	•Difficult to operate at times •Small sample

2.7 Device Materials

A component of designing a molecular transport device is to consider what materials would provide a transportable, transparent, water-tight, and cost-efficient system which was desired by the clients. The materials used in devices already available on the market and those used in studies previously mentioned were evaluated, including acrylic, glass, stainless steel, polystyrene, and polycarbonate. Other materials that have been used include Teflon and silicone, which were used for gaskets and other sealing purposes. There are advantages and disadvantages to all of the materials used for these devices.

The main advantages of using acrylic for a diffusion system are that the material's integrity will withstand long term use, is transparent, and light weight [46]. Acrylic has been compared to glass due to the common use of glass to create the chambers. Acrylic has a higher impact resistance than glass, which means it does not need to be handled as fragile as glassware. On the other hand, glass has more efficient heat transfer properties than acrylic. The transparency of acrylic is beneficial for being able to tell whether or not there are air bubbles forming in the system. It is a lightweight material, about half the weight of glass, which increases its portability, and acrylic can easily be machined. However, acrylic is non-resistant to chemicals such as ethyl alcohol, and therefore can only be cleaned with soap and water. However, it is able to be sterilized by gamma radiation, if necessary. It is compatible to use in conjunction with other materials and is overall very sturdy. As mentioned above, a horizontal device has been made from acrylic and performed well [16].

The devices offered by PermeGear® [6, 47], among others mentioned in the previous sections, are made from borosilicate glass. Those made by PermeGear® are hand blown and provide seamless joints, which avoids difficulties of having to use gaskets and other types of sealants to prevent leakage. Although heavier than acrylic, glass is still a fairly lightweight material. It is also a translucent material which is convenient for observation purposes, and is a material that can be sterilized either by chemicals or an autoclave.

In the past, stainless steel was used to create some diffusion devices. These had some obvious disadvantages; opaque, heavy, and expensive to create. Also, stainless steel is a bit of a misnomer for its properties because they also had a tendency to rust when used long term [29]. This material however is now used as fasteners for some existing devices such as those seen at warneronline.com [48].

In one study performed to determine the *in vitro* permeability of the sclera to high molecular weight compounds, polystyrene cuvettes were used to create a diffusion system. A window was carved into two cuvettes with a milling machine, and cyanoacrylate tissue adhesive was used to seal the sclera into place. The cuvettes were pressed together and served as a sufficient apparatus for diffusion [49] . An example of a polystyrene cuvette may be seen in Figure 15.

Figure 15: Polystyrene cuvette[50]

After examining all aspects of past devices, our group developed an approach and plan for our project in order to find out what the client needed and how our ideas could fit their needs.

3.0 PROJECT APPROACH

Once the background research was completed, the team brought focus towards the project deliverables. After clarifying this approach, the hypothesis, assumptions and aims of the project were defined.

3.1 Project Objective

The objective of this project was to design a molecular transport device to characterize membrane diffusion. This device must be capable of holding a variety of membranes and allowing the transport of molecules with varying molecular weights. Once the device is proven to function properly, it will be transformed into a high-throughput system. This system provides the user with a more efficient testing environment.

Currently, there are fluid-to-fluid phase devices on the market which allow passive molecular diffusion. The goal of our device is to mimic this fluid-to-fluid phase diffusion. The major limitations of this project are the time and budget constraints. However, when these obstacles are overcome, our device will be used to characterize diffusion through collagen membranes. Our final project will be able to guide any user from the production steps of the device to finally utilizing the device to carry out fluid-to-fluid diffusion studies through a membrane.

3.2 Project Assumptions

Some assumptions needed to be made in order to meet the previously stated objective:

- Device will allow controlled molecular transport to occur

- Collagen membranes being used have a uniform structure, such as porosity and cross-linking strength
- Device users know and understand basic laboratory safety, techniques, and equipment

3.3 Project Aims and Specifications

The overall goal of this project is to develop a high-throughput molecular transport system. The specific aims of this project to complete this goal include:

- To model *in vivo* transport in an in vitro environment
- To create a device with the appropriate materials that are non-porous and non-protein binding that develops a high-throughput system
- To develop strict protocols to assemble and operate the device
- To develop protocols for performing various experiments depending on transported molecules being tested
- To develop assays specific to each molecule to determine the concentration of the molecule within the receiver chamber of each study
- To generate and conduct analyses to assess the performance of the device for homogenous mixing, maintenance of constant temperature, and to not leak.
- To determine diffusion coefficients in membranes

3.4 Project Approach

In order to meet these goals, the group used previous research about the current devices on the market as their foundation. With this research of devices and materials,

the group used the engineering design process to define objectives for their own device. These objectives were weighted in order to determine the key factors that should be implemented to meet the user needs and requirements. The specific functions of the device were determined and means of implementing the functions were brainstormed. Comparisons of the various means were used to determine which mean was the best option for each function. Various design alternatives were defined as well, looking at which designs best met the weighted means. A final design was chosen for production. Working together, the team constructed a high throughput system which was then validated through testing temperature control, mixing, and leakage.

4.0 DESIGN

The use of molecular diffusion devices is of interest to many researchers within the field of tissue engineering. As previously stated, the basal lamina resides in many areas in the body. Scientists today are researching areas such as the skin, digestive system and respiratory tract to learn how the basal lamina affects bodily functions. The device desired by the client would allow researchers to characterize membranes that could be used in various areas of the body. This would assist in the research of possible substitutes for areas where the structure and function of the tissue is lost. The development of this *in vitro* device could advance medical research by assisting studies that are geared toward implementing a substitute within humans.

This project's device is being designed for specific users: Katie Bush, George Pins, Ph.D, and the MQP team. Bush is a graduate student at Worcester Polytechnic Institute and UMASS Medical School. She is conducting research on collagen membranes that are used for skin substitutes within the laboratory of Dr. Pins, an associate professor in the Biomedical Engineering Department at Worcester Polytechnic Institute. Bush's *in vitro* testing with the device would provide preliminary information about the effect of various membrane characteristics (i.e. pore size, density, thickness) on tissue regeneration. The device will provide a quantitative evaluation of how various sized molecules transport through the different membranes. This data will be analyzed and used to infer how cells, specifically keratinocytes and fibroblasts, within the skin substitutes, interact with each other via the diffusion of growth factors through the collagen membrane.

The original client statement was presented to the team on August 28, 2006 which read, “Design, develop, and validate a high-throughput device to measure molecular transport rates through self-assembled collagen membranes”. A brief background was provided with the statement, as seen in Appendix A, which demanded a need for a diffusion device and protocol of how to measure molecular transport rates. The results would be used to improve the development of scaffolds that promote tissue regeneration to assist Bush’s thesis work. The importance of making it a high-throughput system is to decrease the amount of time it takes to perform these diffusion studies.

4.1 Clarification of Design Goals

This section describes the course of action the team took to develop the Revised Client Statement. Overall project goals were identified and mapped out before the design process was initiated. Various methods were used to evaluate and establish the objectives of the design and formulate design specifications that shaped the Revised Client Statement.

4.1.1 Establishing Project Goals

Initially, the team began research on the topic and searched for information on different types of diffusion devices that have been used in the past and are being used currently. This research focused on the motivation of the diffusion studies, the devices used, molecules and membranes tested in the studies, and the validation techniques performed to produce quantified results. It was important to differentiate the studies by comparing and contrasting them to each other. Although scholarly articles were the main

resources for our research, information from websites for different devices currently available to purchase were also explored, as recorded in the background section.

Once the client's desires were better understood and enough information regarding the types of diffusion chambers was gathered and organized, a map of what needed to be completed for the project was created, as seen in Figure 16. This map details our design process. The base of this project began with the research, stated earlier in the background section. This section details the next step: the design of the device. The figure displays the overall steps of how the team planned to accomplish the task at hand.

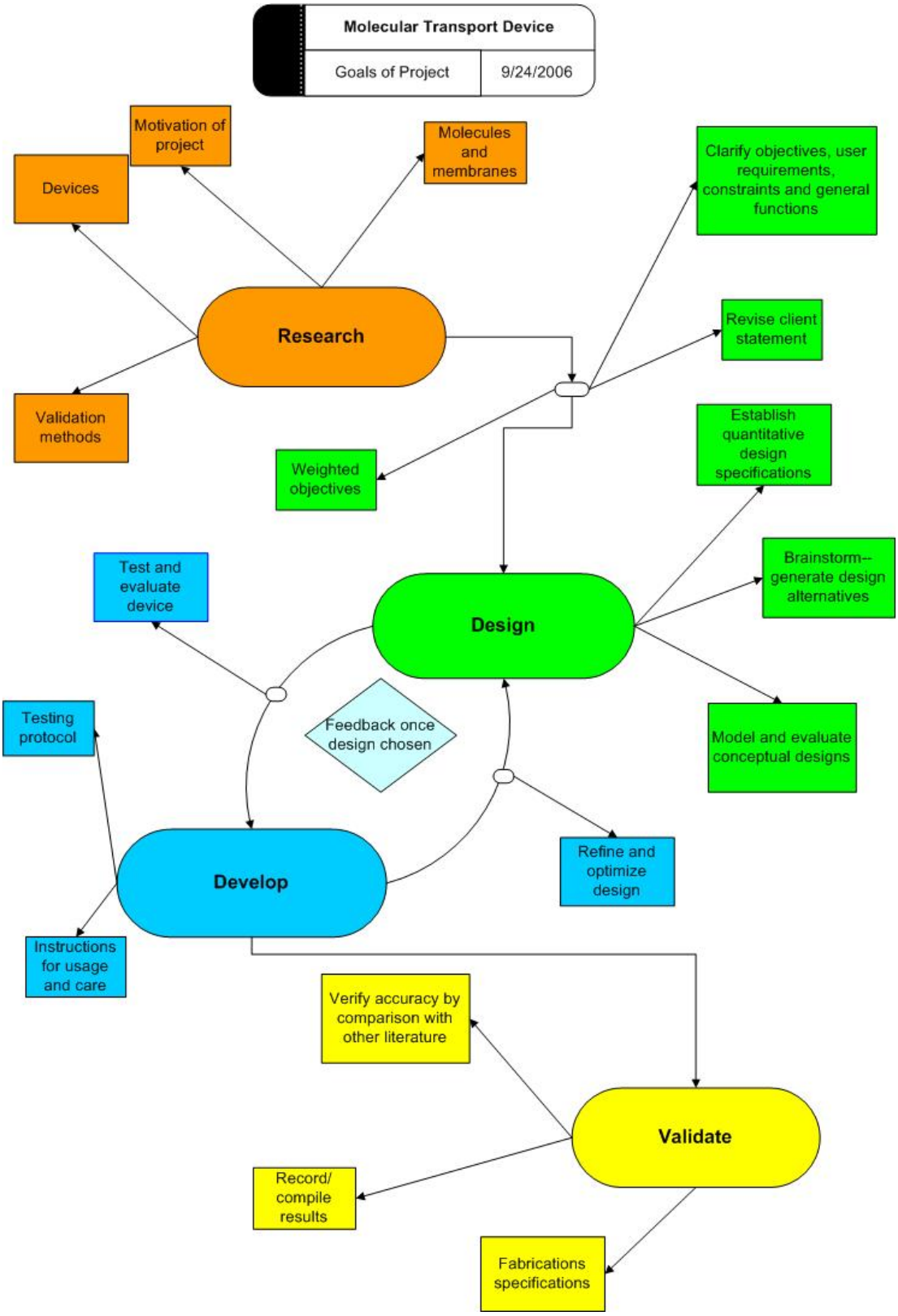


Figure 16: Map of Project

4.1.2 Constraints, User Requirements, and Functions

A meeting was set up with Katie Bush for the team to ask some client questions in order to grasp a better understanding of specific functions the device should be capable of. In this meeting, Katie and Dr. Pins explained the research that they are interested in. A detailed transcript of this meeting can be found in Appendix B. Also, equipment that is in Dr. Pins' laboratory that is available for the use of our project was shown to the team. This equipment included a magnetic stir plate, water bath, and shaker plate. A brief, general demonstration of the horizontal diffusion chamber from PermeGear® [6], seen in Figure 17 below, that Katie has been using in the laboratory was performed two days later to get a better understanding of what our device would need to be capable of.

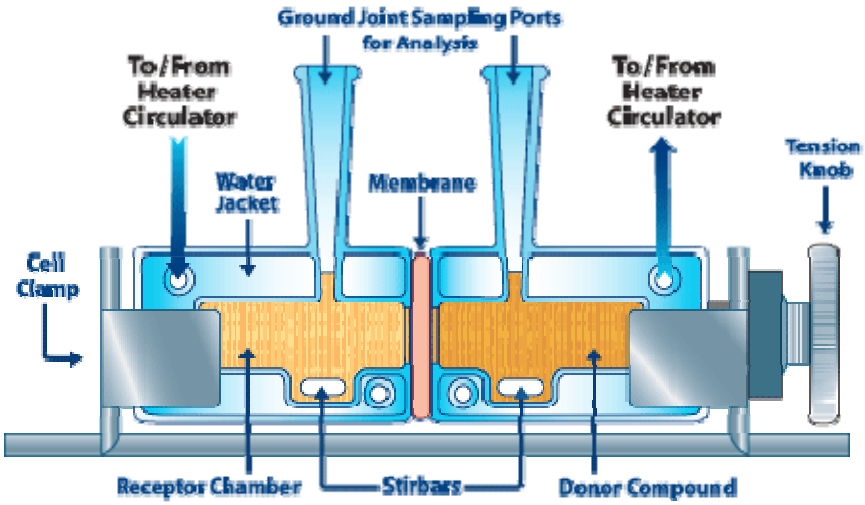


Figure 17: Side-Bi-Side Chamber

Katie's research was described to us in order to help us grasp the purpose for the device. Katie is currently working to fabricate collagen with topographical features similar to basal lamina in the skin. Keratinocytes would be seeded on this collagen. Below the collagen would be a dermal sponge with fibroblasts. The theory is that the fibroblasts send molecular signals to the keratinocytes that tells them to proliferate. Our

device will help Katie characterize the diffusion of molecular signals through the fabricated collagen that has been topographically modified to resemble the basal lamina in skin. Once the client's research was understood, the information pertaining to our project was extrapolated from the research materials gathered.

From the literature review, the advantages and disadvantages of the horizontal and vertical devices were established, as described in section 2.4 of the background. In order to determine and weigh the objectives of the project, the constraints, user-requirements, and general functions were established from this background research and the meeting with Katie. They can be seen in Table 4.

Table 4: User Needs, Wants and Constraints

User Needs	Withstand varying environments; control temperature (25-40°C)	Accessible for cleaning and sampling	High-throughput device	3-5 ml chambers homogenously mixed	Homogeneous mixing in chambers	Work with range of molecules
User Wants	Minimal use of materials	Translucent	Reproducible	Symmetric chambers	12 chamber pairs	Easily assembled
Constraints	$468 budget	Deadline April 17, 2007	Non-protein binding material	Non-porous	Bench-top fit	

Constraints of the overall project were defined, as well as constraints for the creation of the device. The project constraints included that the amount of money spent could not exceed what is reimbursed by the school, which is $468.00. Also, the project needed to be completed in time for Project Presentation Day at Worcester Polytechnic Institute, April 17, 2007. Additionally, the constraints surrounding the design of the device include the use of a material that is non-protein binding, non-porous and translucent. All areas of the device must be cleanable and therefore accessible, and the device's cost must not be excessive and it must be able to be reproducible.

User-requirements defined by the client were compiled. The laboratory that the device will be used in has a varying environment which this device must withstand. The client desires to use the minimal amount of materials, including molecules and membranes. The device must be transportable and must fit on a bench-top in the existing laboratory. The high-throughput device should have at least 12 chamber pairs, so the bench-top fit became a constraint. Also, it is preferred that the donor and receptor chambers are equivalent in size, which should be between 3-5 milliliters a piece. The size of the chambers was determined by the assays that would be used to detect the molecular concentration. The assays require approximately 100 µl to be run. The chambers should be translucent for clear observation, and the system must be easily assembled, operated and cleaned.

The device functions were then defined, and can be seen in Table 5. It was decided that the device must control a temperature of 37º C for the *in vitro* environment accurately and keep solutions in both the donor and receiver chambers homogenously mixed. The device must stand on its own and also secure a fixed position of the membrane between the chambers. Finally, the device must produce repeatable results (self- accuracy) and allow accessible extractions for sampling from the receiver chamber.

Table 5: Function Details

Function	Parameters
Molecular diffusion through membrane	2 symmetric 3-5 mL chambers that do not leak
	Means of holding membrane 50-200 µm thick between chambers
Temperature control of chambers	Chamber temperature achieves steady-state 25-40°C
Mixing of chambers	Solution and molecules homogenously mixed together
Chambers accessible for sampling	Port to each chamber large enough for 500µL pipette tip to fit

4.1.3 Weighing Objectives

From constraints, user-requirements, and basic functions the team began to formulate objectives for the design of the molecular transport device. The objectives created by the team originally are as follows:

Table 6: Objectives

■ Accurate	■ Versatile
■ Durable	■ Safe
■ Affordable	■ Efficient
■ Easily Used	■ High- throughput

These objectives were expanded upon and assigned into four tiers and then evaluated in a client meeting with Dr. Pins and Katie. Many of the team's original objectives were maintained, but were rearranged and clarified. Once the objective tree was finalized, a glossary of terms (Appendix C) was supplemented with definitions of any terms that could be misinterpreted. The glossary was especially helpful for Dr. Pins and katie to use when filling out the pair-wise comparison charts (PCCs) to quantitatively weigh the importance of the objectives against each other.

The PCCs are tables that are used to compare the objectives within each tier of the objectives tree against each other. The final PCCs can be found in Appendix D. In the

tables, a one assigned to a box means that the objective in that row was rated more important than the objective compared to it in the appropriate column. A zero means that the objective in the column was more important than that in the row, and a half of a point was used to establish that the objectives were equally important. The points for each were tallied, as seen in Appendix E, and then weighted. Katie Bush's input from the PCCs was weighted 100% because of her direct involvement with the device to be created and experience using her horizontal device from PermeGear [6]. The team's PCCs were weighted 75% total (25% each) because the team's understanding of the devices based on research, as seen in the previous sections. Finally, Dr. Pins' charts were weighted 50% because of his expertise in the studies carried out in his lab and knowledge of the device but limited experience directly using them. Once all of the PCCs were totaled and weighted, the objectives were weighted. Each objective was given two percentages, x | y, where x represents the percentage of the objective in relation to the other objectives within its tier and y is the percentage of the objective in relation to the preceding tier. The first tier of objectives rated in order from highest to lowest priority: effective, durable, easily used, safe, and practical to make. A tree with all of the objectives associated with these five and with their appropriate weights can be seen below in Figure 18. This figure also shows that versatility was a very important objective for us to meet. In its tier, leaking was found to be the most important objective to keep in mind. These objectives helped us when designing for the needs of our users. We also found from this weighted objective tree that it would not be as crucial for the device to be cell co-culture compatible, as this objective scored extremely low within its tier and as a whole. The most important objectives were to effectively carry out the desired functions,

for its durability to be reliable both long term and short term, and for its assembly, clean up and general size to be convenient. Table 7 below shows the first and second tier objectives which scored the highest in their comparison to the other objectives within their tier.

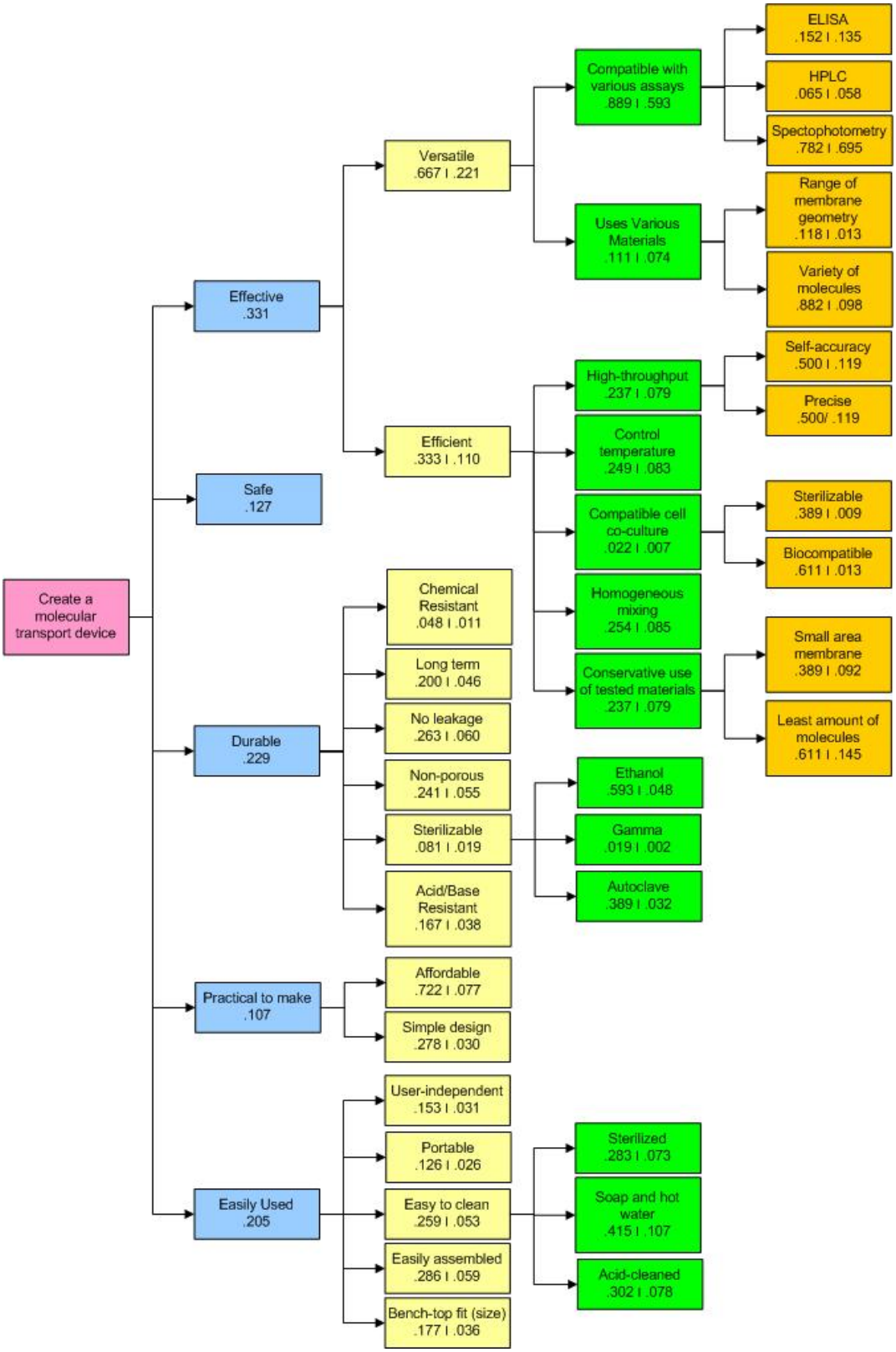


Figure 18: Weighted Objectives Tree

Table 7: Highest Ranked Objectives

First Tier		Second Tier	
Effective	0.331	Versatile	0.221
		Efficient	0.110
Durable	0.229	No Leakage	0.050
		Long Term	0.046
Easy to Use	0.205	Easy to Clean	0.053
		Easy to Assemble	0.059

4.1.4 Revised Client Statement

Developing design specifications was the next step in the design process in order to establish the final Revised Client Statement. These included prescriptive, procedural, and performance specifications.

The prescriptive specifications are those which specify values for attributes of what is being designed. These include that the chambers will be translucent, non-protein-binding, and each (donor and receiver) will be about 3-5 mL. The cost of designing and developing the device will be less than $468.00. The device will fit on a bench-top about 2 ft^2 and able to be cleaned.

The procedural specifications include that sample sizes extracted from the receiver chamber will be between 25- 500 µl. The buffer solution used in tests will be phosphate buffered saline (PBS). The device must be capable of maintaining temperature in the range of 25-40°C. Duration of experiments must be no longer than three days. The main assays being used will include the Bicinchoninic Acid (BCA) and Glucose Hexokinase assays, depending on the molecule.

The performance specifications include that the device should hold membranes of varying thickness, usually about 100 µm, but within the range of 50-200 µm. The receiver chamber must allow samples of 0.3 mL to be removed at a time. After researching the size of various molecules used within diffusion studies, the molecules for

our studies were chosen (molecule research see Appendix F). The molecules that can be tested include glucose, BSA, IgG, cytochrome C, and myoglobin. Finally, the device must homogenously mix the solutions in both the donor and receiver chamber and there is no specific stirring speed necessary.

Through the design techniques previously explained, the team was able to generate a revised client statement. The revised client statement:

Design a high-throughput device with 12 chambers to quantitatively characterize the rate of molecular transport via assays for glucose, equine myoglobin, BSA and IgG through self-assembled collagen membranes ranging from 50- 200 μm thick. The easily operated, assembled and cleaned device must have non-protein binding chambers, fit within one square foot area, and provide a controlled temperature environment (25-40°C) for testing. The developed device will have homogeneously mixed chambers between 3 and 5 mL, require a minimal use of materials of interest, and cost less than $468. The validation protocol of this device will follow a molecule dependent assay requiring a sample amount between 25 μl – 500 μl for maximum experiment duration of three days.

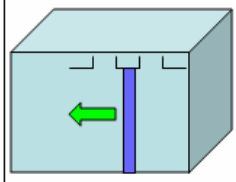
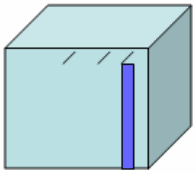
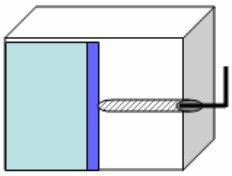
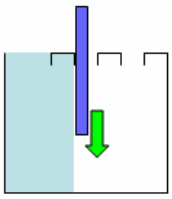
4.2 Brainstorming

After the Revised Client Statement was established, brainstorming sessions could begin for the design of the device. The purpose of the brainstorming sessions was to create a morphological chart of features and functions and the various means that could be used to implement them. From this chart, the pros and cons of each component could be evaluated to begin the conceptual design phase.

4.2.1 Brainstorming Sessions

There were three main brainstorming sessions organized by the team for the design of the device. The first was held on October 9, 2006 with Bush, Dr. Pins, and all three team members. At this session the features/ functions brainstormed on included temperature control, having an adjustable chamber size, homogeneous mixing, multiple chamber system (high-throughput), and materials for the chambers. The adjustable chamber size idea was very creative but not incorporated within the functions/ features that needed to be evaluated because it was outside the scope of what the device needed to perform. In Table 8 below, the possible means of the adjustable chamber that were brainstormed can be seen.

Table 8: Brainstorming Means of an Adjustable Chamber Size

Feature/ Function	Means			
Adjustable chamber size	 Spring loaded slide	 Spring in chamber	 Injection/Crank	 Slides and Slots

The second session was held among the three team members on October 27, 2006 and focused on the donor/ receptor chamber engagement, how the device may hold membranes of varying geometry, and the sampling ports. During this session all of the brainstorming ideas for the major features and functions thus far were complied into a morphological chart.

The final brainstorming session was held at Ximedica, a medical device design company in Providence, RI. Three designers/developers that work for the company brainstormed with the team on the design of the system using the functions means charts. From the brainstorming session the team decided to avoid having any extra areas that could leak. It was noted that motors would add many holes in the chambers of the system which would need a sealant and create more potential for leaking.

4.2.2 Morphological Chart

Upon completion of the brainstorming sessions, the means of each function/ feature that the molecular transport device should contain were put together as seen in Tables 9, 10, and 11 below.

Table 9: Functions Means Chart for Temperature Control, Engagement, and How to Hold Membranes

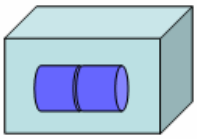
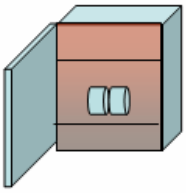
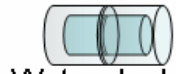
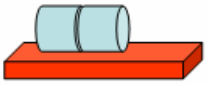
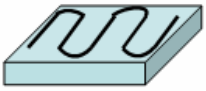
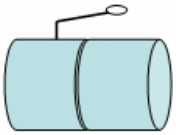
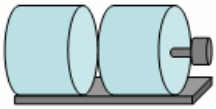
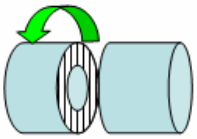
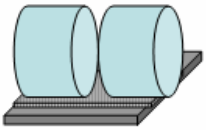
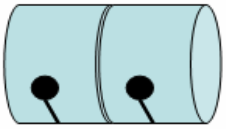
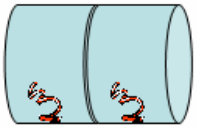
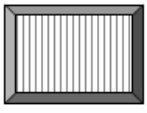
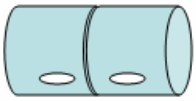
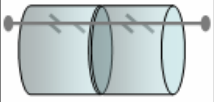
Feature/ Function	Means			
Control Temperature	 Bath	 Incubator	 Water Jacket  Heat Plate	 Snake
Donor/ Receptor Chamber Engagement	 Latch	 Manual Screw	 Screw Lock	 Slide
Homogeneous mixing	 Rocking Stirrers	 Small Mechanical Stirrers	 Shaker Plate  Stir bars	 Retractable fan stirrer

Table 10: Function Means Chart for Multiple Chamber System and Mixing

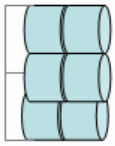
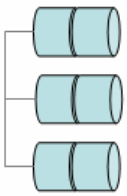
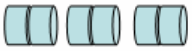
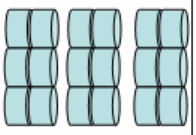
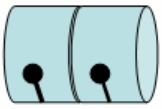
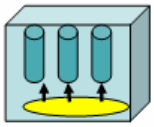
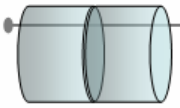
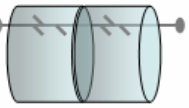
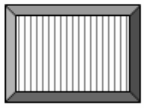
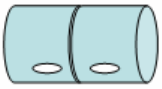
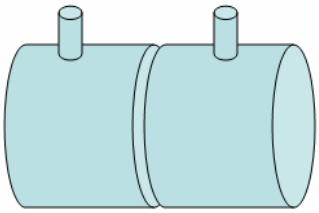
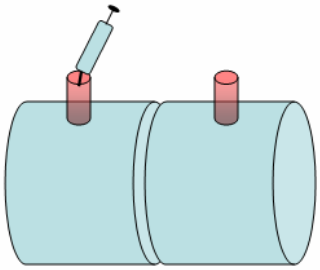
Feature/ Function	*Means*				
Multiple chamber system	 Parallel vertical	 Parallel horizontal	 Series	 Parallel and Series	
Homogeneous mixing	 Rocking Stirrers	 Inflatable Balloon	 Small mechanical stirrers	 Large mechanical stirrers	 Stirrers with retractable fans
	 Shaker table	 Stir bars			

Table 11: Function Means Chart for Materials and Sampling Port

Feature/ Function	*Means*	
Material for chambers	Acrylic	Polycarbonate
Sampling Port	 Open slot	 Rubber Syringe

4.2.3 Pros and Cons of Means for Each Function/ Feature

Each of the possible means for each feature/ function was evaluated and a detailed list of each of their advantages and disadvantages was recorded which helped to determine the best possible means to accomplish the desired functions of the device. Listed below are the seven features/ functions that were identified during brainstorming, a brief description of each mean possible to implement them and summaries of the pros and cons of each (Tables 12-17).

1. Temperature Control (means within Table 9)

Temperature can be controlled through two mediums: water and air. There are various ways these mediums can be applied to a molecular transport device.

A. *Snake* – A solid block of material drilled out to allow water to flow through tube on each side of the chambers with use of a water bath and peristaltic pump. The flowing water will keep the chambers at the desired temperature by thermal diffusion.

B. *Plate* - A heating plate will be placed beneath the system to control the temperature of the chambers.

C. *Water Bath* – The chambers would be submerged in a temperature controlled water bath
D. *Water Jacket* – A sleeve will be built around the chambers to hold flowing water to control the temperature within the chambers—used with peristaltic pump and water bath.
E. *Incubator* - The device will be placed in an incubator to keep it at a constant temperature.

Table 12: Temperature Control Pros/ Cons

1. Temperature Control	Pros	Cons
Snake	▪ Controlled use of water ▪ Simple design ▪ Adjustable temperature	▪ Does not heat from all sides
Plate	▪ Adjustable temperature ▪ Available in laboratory	▪ Heats from one direction ▪ Cannot work in conjunction with some mixing means
Water Bath	▪ Heating from all sides ▪ Available in laboratory ▪ Adjustable temperature	▪ Higher potential of leakage ▪ Contamination of tests ▪ Cannot work in conjunction with multiple mixing means
Water Jacket	▪ Constant temperature control ▪ Heating from all sides ▪ Adjustable temperature	▪ Manufacturing difficult — keeping jacket stationary around chamber ▪ Needs more ports to seal ▪ Possible contamination
Incubator	▪ Available in laboratory ▪ Adjustable temperature ▪ Accurate temperature settings	▪ Time to normalize each time incubator opened ▪ Door must be opened each time samples taken ▪ Cannot work in conjunction with multiple mixing means

2. <u>Donor and Receptor Engagement (means within Table 9)</u>

The two chambers need to be fitted together water tight in order for controlled transport to take place.

A. *Draw Latch* - A latch with tension on top of one chamber that hooks into the other chamber.
B. *Screw- lock* - Each chamber machined to have an interlocking component that, when the chambers are twisted, will pull the two chambers together.
C. *Manual Screw* - One of the chambers will be stationary and the other chamber will be pushed against it and screwed into compression—a vice system.
D. *Slide* – One chamber is stationary, the second is in a groove and slides up into a fixed position against the stationary chamber.

Table 13: Donor and Receptor Engagement Pros and Cons

2. Donor and Receptor Engagement	Pros	Cons
Draw Latch	▪ Easy/ quick to use ▪ Simple design ▪ Usable in conjunction with gaskets/ o-rings for sealing	▪ Bottom half bulging open ▪ Cannot be put on either side if parallel arrangement ▪ Additional part to purchase and attach
Screw- lock	▪ Locking system built in – no extra parts	▪ Difficult to machine ▪ Requires twisting of chambers
Manual screw	▪ Easy/ quick to use ▪ Usable in conjunction with gaskets/ o-rings for sealing ▪ Even compressive force ▪ Good alignment	▪ Extra component to design or purchase
Slide	▪ Good alignment	▪ Weak compressive force

3. Holds Membranes of Varying Geometry (means within Table 9)

The device must hold the membrane stationary and secure during testing. Also, membranes can be created in various shapes and sizes. The device could allow various sized membranes to be used.

A. *Teeth Cartridge* – A system in which all membranes being tested could be lined up at once and pressed between two slabs; this entire system could slide into place with each membrane between chamber pairs.

B. *O-Ring* – This piece could be used to press the membrane into place on the face between chamber pairs – currently how PermeGear side-bi-side system works.

C. *Individual Cartridge* – Similar to teeth cartridge, but placed in between chambers separately—not all chambers connected.

D. *Press-fit* – Similar to individual cartridge except that the hole on each side of the two pieces is what seals the membrane and presses it into place.

E. *Multi-shape Cartridge* – Similar to "teeth cartridge" but would have two different pieces available; one to hold a larger membrane and one to hold multiple smaller membranes.

Table 14: Holds Membrane of Varying Geometry Pros and Cons

3. Holds Membrane of Varying Geometry	Pros	Cons
Teeth Cartridge	▪ Membranes all set up in one system ▪ Seal around membranes to prevent leakage	▪ No size variability ▪ Takes up whole system
O-ring	▪ Proven to work ▪ Guarantees seal	▪ Material degradation ▪ Strain on membrane when pushed into place – could tear when putting in place
Individual Cartridge	▪ Easy to slide membrane into place ▪ Seal around membrane to prevent leakage	▪ Need more components made ▪ No size variability
Press-fit	▪ Seal around membrane ▪ Easy to assemble	▪ Could tear membrane ▪ No size variability ▪ Needs to be slid between chamber pair carefully to maintain alignment
Multi-shape Cartridge	▪ Size variability	▪ One large membrane could have leaking ▪ Takes up whole system

4. Multiple Chamber System (means within Table 10)

   The chamber pairs can be aligned in different orientations in order to make this a full high-throughput system.

   A. *Parallel-Vertical* – Stacking parallel chambers on top of one another
   B. *Parallel-Horizontal* – Lying chamber pairs next to one another
   C. *Series* – Aligning chamber pairs end to end
   D. *Parallel and Series* – Aligning chamber pairs in grid-like fashion

Table 15: Multiple Chamber System Pros and Cons

4. Multiple Chamber System	Pros	Cons
Parallel-Vertical	▪ Takes up less bench-top space	▪ Need to sample from the side ▪ Need a fixture to balance
Parallel-Horizontal	▪ Chamber pairs can be more independent from one another ▪ Easily accessed ports	▪ Consumes more bench-top space
Series	▪ May be easier to create temperature control water flow system ▪ Easy access to all areas of chambers	▪ Not an efficient use of space ▪ May limit what temp. control may be used due to space consumption
Parallel and Series	▪ Efficient use of space	▪ Efficient use of space ▪ Chambers in center difficult to access and observe ▪ Chambers would be as independent

5. <u>Homogenous Mixing (means within Table 10)</u>

Both the donor and receiver chambers will hold a solution that need to maintain homogenously mixed in order to reduce boundary layer formation.

A. *Shaker Plate* – Entire system can be placed on shaker plate and set at constant rate to agitate solution.

B. *Rocking Stirrers* - Simple hinge flapping back and forth with shaker plate that would increase solution movement.

C. *Inflatable Balloon* – If the chambers are in a water bath, a balloon could be placed in the bottom that inflates and deflates to push each chamber pair up and down to agitate the fluid.

D. *Stir Bars* – Magnetic stir bars are placed in each chamber and system put over a magnetic plate.

E. *Small Mechanical Stirrers* – Each chamber gets a motorized stirrer that can be attached to the sampling port cap.

F. *Large Mechanical Stirrers* – Motorized stirrers go through all receptor chambers/ all donor chambers — a long shaft that rotates.

G. *Stirrers with Retractable Fans* – It's the same concept as large mechanical stirrers but have fans on the shaft that go into each chamber and retract when shaft is removed from system for cleaning.

Table 16: Homogeneous Mixing Pros and Cons

5. Homogenous Mixing	Pros	Cons
Shaker Plate	▪ Available in laboratory	▪ Securing system
Rocking Stirrers	▪ Easy to manufacture	▪ Difficult to clean ▪ Securing system
Inflatable Balloon	▪ Rate of bobbing easily controlled	▪ Chambers need to be submergible ▪ Major space consumption I water bath
Stir Bars	▪ Easily used	▪ High- throughput system cannot be placed on a single magnetic plate and ensure controlled stirring
Small Mechanical Stirrers	▪ Easily cleaned	▪ Taken out when samples extracted ▪ Motors could get wet
Large Mechanical Stirrers	▪ Few motors to control system	▪ Need multiple sealing points ▪ Contamination from sealant debris
Stirrers with Retractable Fans	▪ Few motors to control system ▪ More surface area stirring chambers to ensure homogeneous	▪ Need multiple sealing points ▪ Contamination from sealant debris

6. Material for Chambers (means within Table11)

Acrylic, or Plexiglas, is a good material due to its availability. It does meet the transparent requirements. However, it's not known how well proteins don't bind to it.

Polycarbonate was found to be more expensive than acrylic. It also has been used in previous devices. Polycarbonate is also available.

7. Sampling Ports (means within Table 11)

The device can either have open ports that would need to have a removable cover, or a rubber stopper to use with a syringe.

Table 17: Sampling Ports Pros and Cons

4. Sampling Ports	Pros	Cons
Open Slot	▪ No obstruction during sampling ▪ Can use with pipette tips ▪ No extra parts	▪ Splashing ▪ Need to remove and replace cover for each sample extracted ▪ Splashing may cause some solution to be lost
Rubber Syringe	▪ No splashing ▪ No cover	▪ Must use needle syringe rather than pipette ▪ Degradation of material

4.3 Preliminary Design

Once the team had an idea of the various means to carry out specific functions of the device, each previously defined objective was reviewed in order to create a ranking system metric. This system would be used to rank how each means of carrying out a specific function would meet each objective. Every objective was looked at individually in order to develop a quantified ranking system. Due to the fact that some of the first tier objectives were very broad, the second or third tier of the objectives tree was used to develop the metrics. The metrics were based on qualitative and quantitative measurements of the objectives for the device. They allowed the team to see which means are most favorable for the final design proposal or at least narrow down choices to bring focus to what should be prototyped. The development of the metrics is also what led to the generation of full conceptual design alternatives, which consisted of putting together of various means for the different components to see how an entire system could work. These conceptual designs can be found in Figures 20-25 below, along with the pros and cons of each system in the corresponding tables (Tables 18-23). The pros were based on whether or not the design would satisfy the objectives of the first tier.

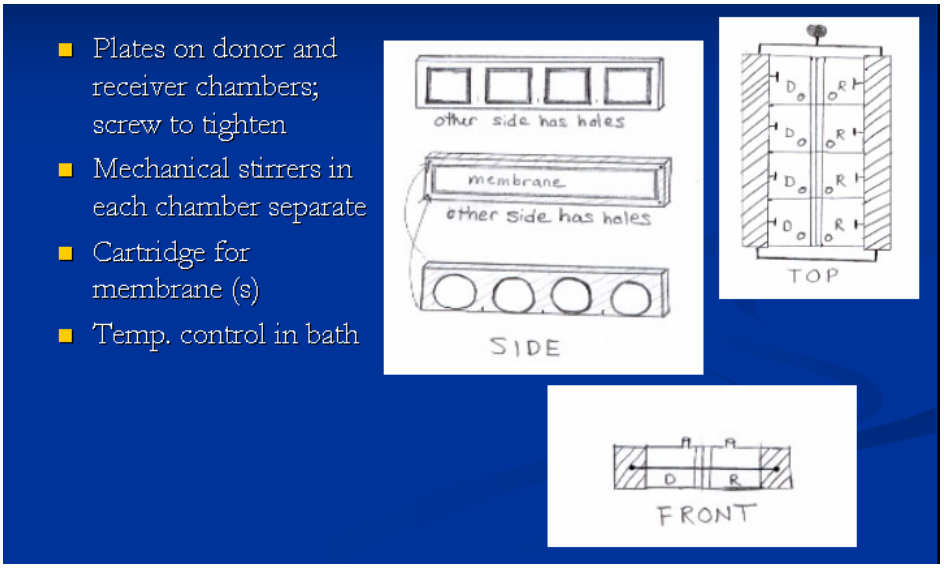


Figure 19: Conceptual Design I

Table 18: Pros and Cons of Conceptual Design I

Conceptual Design I	
Pros	*Cons*
▪ Effective ▪ Practical to make ▪ Easy to use	▪ Larger membrane may not be conservative use of materials ▪ Motors in water ▪ Possibility of leaking ▪ Cartridge implementation may be hard to seal

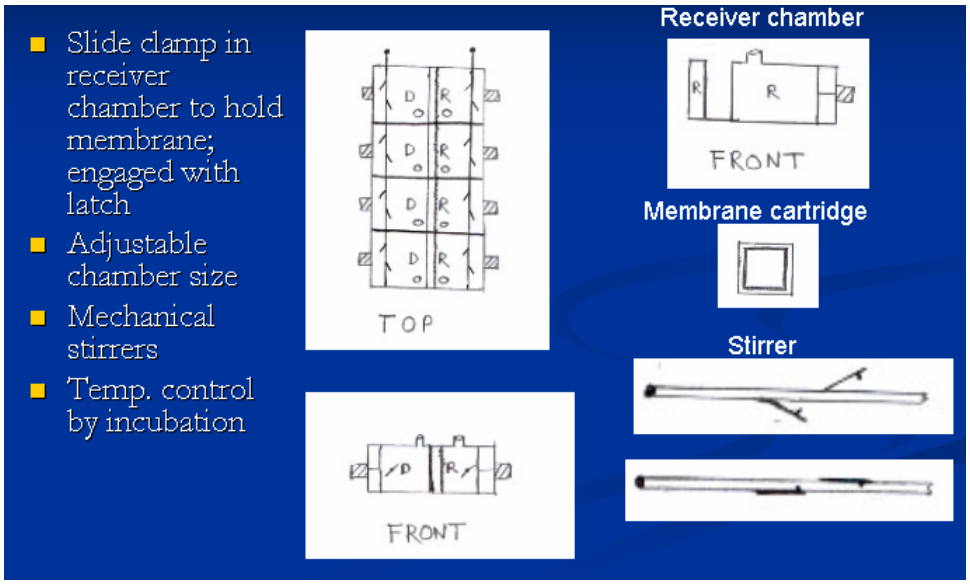


Figure 20: Conceptual Design II

Table 19: Pros and Cons of Conceptual Design II

Conceptual Design II	
Pros	*Cons*
▪ Safe ▪ Practical to make ▪ Durable	▪ Variation in temperature ▪ No change in membrane size ▪ Possible leakage due to improper engagement or weak compression ▪ Necessary for user to hold donor and cartridge while trying to latch receptor

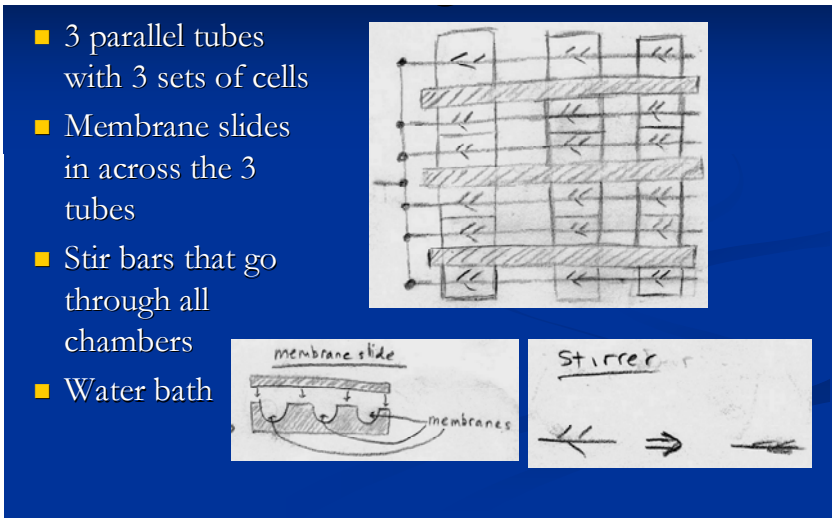


Figure 21: Conceptual Design III

Table 20: Pros and Cons of Conceptual Design III

Conceptual Design III	
Pros	*Cons*
▪ Effective ▪ Easy to clean ▪ Easy to use	▪ No change in membrane size ▪ Many parts ▪ Motor in water ▪ Many openings to seal ▪ User required to assembly and clean many pieces

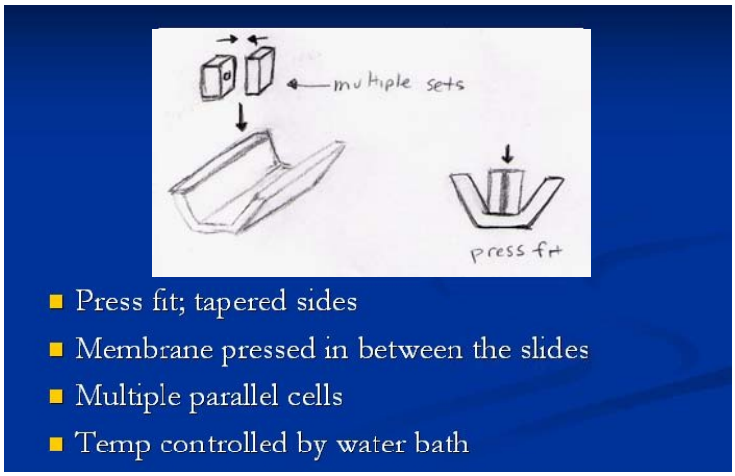


Figure 22: Conceptual Design IV

Table 21: Pros and Cons of Conceptual Design IV

Conceptual Design IV	
Pros	*Cons*
▪ Safe ▪ Easily Used ▪ One base can fit several sets of chambers	▪ No mixing ▪ No varying membrane size ▪ Possible material damage due to press fit ▪ Hard to bend material to make press fit

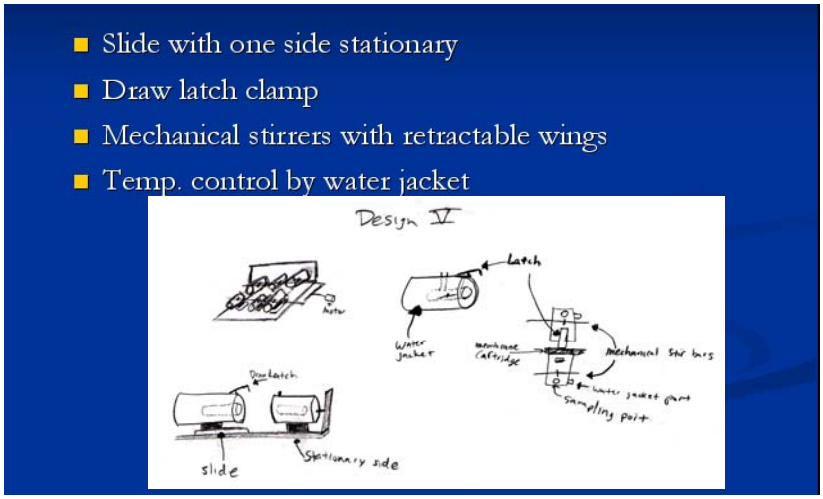


Figure 23: Conceptual Design V

Table 22: Pros and Cons of Conceptual Design V

Conceptual Design V	
Pros	*Cons*
▪ Effective ▪ Safe ▪ Durable ▪ Practical to make ▪ Easy to use	▪ Various membranes ▪ Various number of tests ▪ Minimal leakage ▪ Stirrer inserts ▪ Easy for user to slide/manipulate

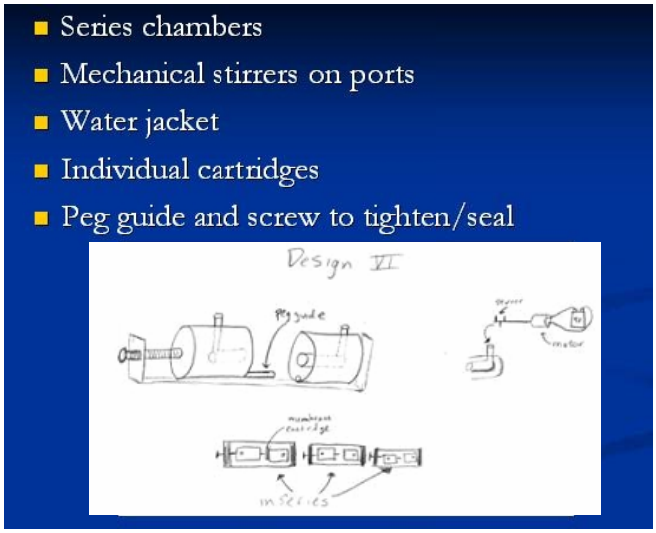


Figure 24: Conceptual Design VI

Table 23: Pros and Cons of Conceptual Design VI

Conceptual Design VI	
Pros	*Cons*
▪ Effective ▪ Safe ▪ Durable ▪ Practical to make	▪ One membrane size ▪ Very safe for user ▪ Possible leakage ▪ Not as easy for user to assemble/clean

4.3.1 Metrics

In creating the metrics, we further analyzed and defined the objectives we had identified earlier in the design process. Metrics were scored on a variety of scales; each scale has the lowest number denoted as being the worst and the highest as being the most favorable. The rankings led to the determination of the most favorable means of the features and functions desired for the design of the device. The best design was formulated from the implementation of these metrics to the means because it incorporated the components that score the highest in the rankings.

For two main objectives, controlling temperature and homogeneously mixing, sub-objectives/metrics needed to be defined due to the fact that each function (temperature control and mixing) could not be solely weighted against the general

objective. For example, to meet the specific function of controlling the device's temperature, more variables needed to be taken into account such as the ability to evenly distribute heat, not allow infiltration of water, maintain temperature within two degrees of the actual setting, and allow the use of a range of temperatures. All metrics that were developed and justified can be found in Appendix G and H, respectively.

4.3.2 Decision Matrices

Once all of the metrics were developed, each possible mean previously defined for each specific function was weighted using the metrics (Appendix I). First, the weight for each objective was obtained from the weighted objectives tree. Next, this weight was multiplied by the highest possible ranking that the objective could obtain. For example, the high throughput objective was weighted 7.9 percent. It could receive the highest ranking for 4, on a 1 through 4 scale. The weighted percentage was then multiplied by 4 to calculate the highest ranked weighted percentage (7.9% x 4 = 31.6%). For each objective, the highest ranked weighted percentage possible was computed to find the total possible weighted percentage an ideal device's function could have. Each means for a particular function was first ranked based on the metrics. These rankings were then weighted and summed for all of the objectives for each means. The sums of each mean's weighted total percentages were then compared to the total possible weighted percentage that a mean in that category (function) could obtain. The mean with the highest summed weighted percentage was defined as the best mean to carry out that particular function. This process was carried out for each function.

Particularly for two functions, extra information needed to be compiled. In order to actually weigh each mean that affordability was applicable to, outside research needed

to be compiled. Various prices were found for each mean, and an average price was calculated which was used in the affordability ranking (see Appendix J). Also, for the ability to control temperature for the two materials that were compared (acrylic and polycarbonate), crude temperature calculations were carried out to see which material was a better conductor of heat (see Appendix K).

4.4 Proposed Final Design

From the evaluation of the various means for each feature and function, the final components desired for our device were established and can be seen in Table 24. The next step was to itemize how each one of these components would be implemented in the design.

Table 24: Results of Rankings

Function/ Feature	Means
Temperature Control	**Snake**
Engagement	**Screw**
Hold Membranes	**Single Cartridge**
Mixing	**Shaker Plate**
Multi- Chamber	**Parallel Chambers**
Materials	**Polycarbonate**
Sampling	**Open ports**

The first feature the team focused on was the snake for controlling temperature. Originally the team thought that all of the chambers would be cut out of a single block and there would be a passage for water flow carved through around the chambers. This concept changed slightly when the client desired to have each experiment being tested independent from one another. For example, if one diffusion experiment was being carried out and there was a problem with bubbles or leakage, the client would not want to

have to interrupt all of the other tests in progress. If the chambers and temperature control system were all connected this would create an issue for being able to control each experiment independently. Therefore the team discussed how water from a bath could be run through the entire system while keeping each donor/receiver pair independent from one another. From this, the team decided to have each chamber pair separate from one another and use tubing to connect the water bath together. There would need to be further research on various means to connect tubing from each water flow path carved, which is detailed in the following Section 4.4.2.

The engagement of the two chambers together with a screw mechanism as described earlier essentially calls out for some type of vice or clamping system. The group decided to explore various products available on the market in order to implement this component.

The single cartridge to hold each membrane would need to serve two purposes. Each side of the cartridge would have to come together tightly to hold the membrane securely in place and there would need to be a way for it to connect to the donor/receiver system. The team decided there would also need to be some type of sealant between the cartridge sides and where piece of the device would be against the donor and receiver parts.

From this simple analysis of how each feature and function would be implemented, the team began to compile research on ways to implement tubing for the temperature control, various sealants, and ways to clamp the system together. First, a simple model of the main components of the design was created in order to provide the team and clients with a hand on visual aid of the concepts discussed above.

4.4.1 Model

Before creating the model, CAD models were created for dimensions. The drawings were completed with metric units and did not include any tolerances. The purpose of this model was purely for a better conceptual understanding of each component. The specific sizes of each part were negligible. The dimension of the two blocks clamped together was 50 X 60 X 50 mm (1.97 X 2.36 X 1.97 in).

A model of the donor and receiver portions of the device was constructed from rigid polyurethane foam. The pieces used were from a scrap bin. Scrap pieces of unknown plastic were used to implement the portion of the donor chamber to hold the membrane cartridge. Scrap pieces of wood and a bolt were used to display a clamping mechanism. Figure 26 shows a schematic of the components. The bottom of both chambers also featured a thin, rectangular extrusion through the middle that fit into a trough of the clamp which was intended to help guide the two blocks together with proper alignment (Figure 27). Also, tabs on each side of the front face of the receiver blocks matched with two cuts holes on the donor block to be sure there was proper alignment when they clamped together.

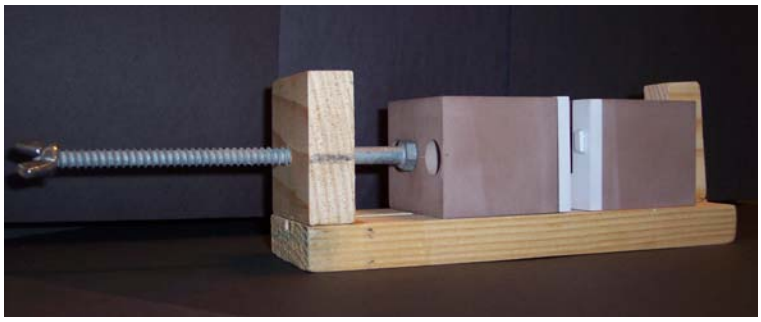


Figure 25: Model constructed from rigid foam, plastic, and wood

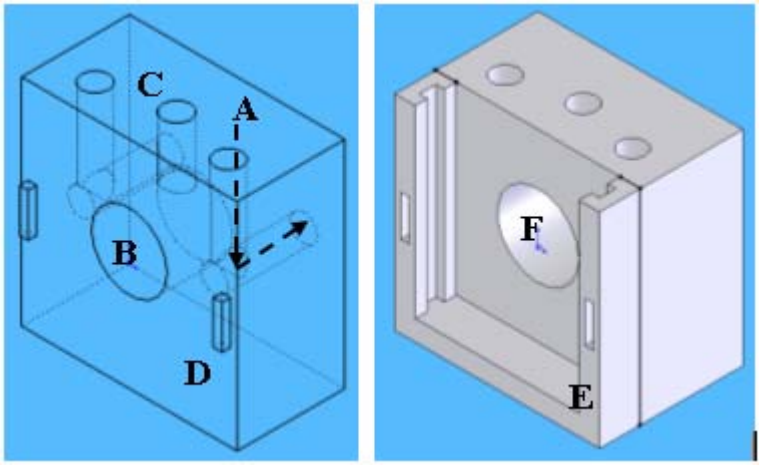


Figure 26: Receiver (left) and donor (right) models: A. flow path for water bath, B. receiver chamber, C. sample port, D. tabs for fitting together, E. cartridge holder, F. donor chamber

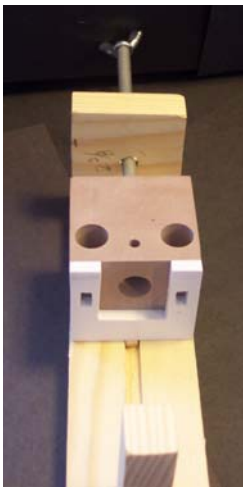


Figure 27: Trough of clamping system for model

Once the model was created, it was decided that flexible tubing would be used to connect the snake temperature control system. In order for this to occur, connectors were needed for each of the inlet and outlet sites on each block. The team decided that the inlets and outlets of each end of the path for water could be tapped and luers could be fixed into place permanently. Silicone would be used in the threads of the luers in order to prevent leakage in the temperature control snake. The water would run through the system as seen in the schematic below (Figure 28).

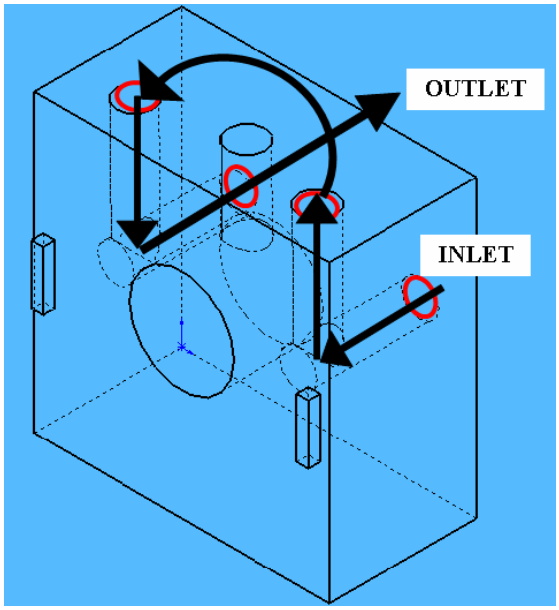


Figure 28: Arrows denote the flow from water bath to control temperature; red oval indicate where tubing connectors would be fixed into block

It was also decided that the connectors on the top of the block should be angled so that the tubing between them could be fed around the sample port. If straight connectors were used, such as that in Figure 29, the tubing would run orthogonal to the block and possibly obstruct the sample port. The team looked various at companies that sold such luers including Qosina, Value Plastics, Cole Parmer, McMasterCarr and Home Depot.

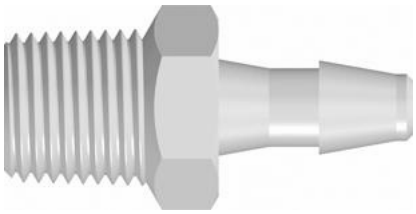


Figure 29: Example of the luer fitting[51]

The two types of flexible tubing that the team desired to use were latex and polyvinyl chloride (PVC). The PVC had slightly more rigid properties, so the team decided it should be used to connect the two tubing connectors on the top of the block,

because this tubing would be permanently fixed. It was desired that the tubing in between blocks could be disconnected to make cleaning purposes easier for the user.

The next component of the system the team evaluated from the model was how to prevent leakage between the cartridge pieces and the interface between the two blocks. The system would need some type of sealant. O-rings were chosen as an appropriate component to do this. The team decided that a series of three o-rings would effectively seal the system; between the sides of the cartridge, and between the cartridge and blocks on each side. In Figure 30 of the cartridge, the grooves around the hole in the middle where the membrane is exposed between the chambers are where the o-ring between the two cartridge sides would be placed. These drawings of the cartridge were made to fit the model, although a physical representation of them was not created.

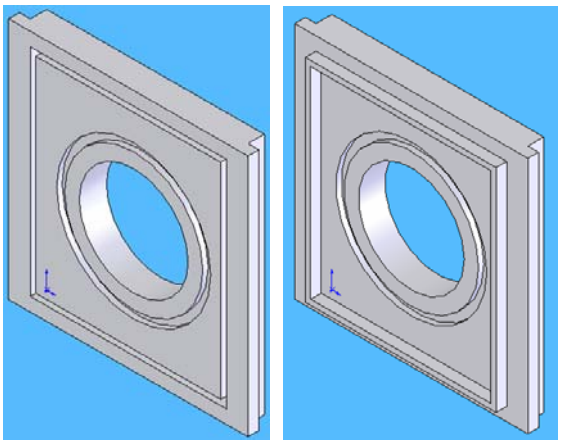


Figure 30: Cartridge design for model with o-ring grooves

4.4.2 Additional Constraints

The team became aware that the weight tolerance and size of the pad of the shaker plate in Dr. Pins' laboratory must be known before specific dimensions of the device could be made. The VWR DS-500 Orbital Shaker Plate present in the laboratory (Figure 31) has a platform area of 10 ½ X 12 ½ in (27 X 32 cm) and a weight capacity of 16kg (35lbs.).

Figure 31: VWR DS-500 Orbital Shaker Plate

These constraints had the most effect on the final component of the system that needed to be explored which was how the two blocks and membrane would be clamped together. The team made the decision that it would be best to modify an existing clamp or vice on the market for the system. From basic searches through catalogs, it was identified that vices are rather heavy, expensive tools because many are made from steel or thick aluminum. These tools are also bulky and would use up too much of the limited space on the shaker plate. The team therefore began to look into multiple different types of clamps and vices including bar clamps, C-clamps, and a variety of vices. The team decided to use an aluminum corner clamp from Home Depot. The corner clamp features two simple clamps to set pieces of wood orthogonal to one another in order to create a frame. The team decided that this clamp could be modified to use with the design system and that each clamp could be cut in half and one side could be used for each set of donor/ receiver blocks (Figure 32).

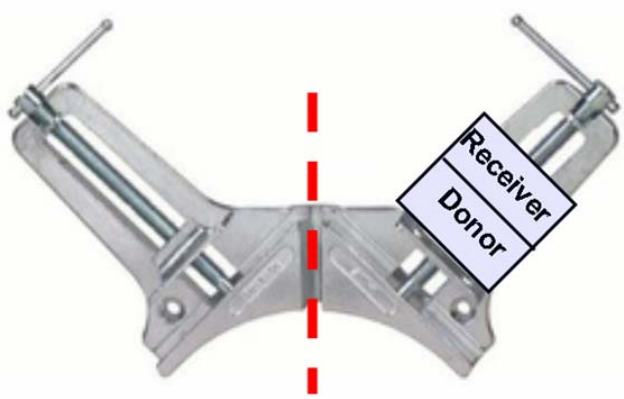


Figure 32: Top view of how corner clamp would be split in half

The limited space of the shaker plate platform for the system also required the team to evaluate the best way the chambers should be lined up. The team decided that there would be two columns of chambers parallel to each other. A schematic of this layout can be seen in Figure 33 below.

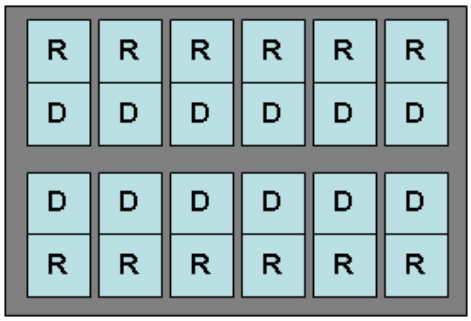


Figure 33: Layout of chamber pairs on shaker plate; R is receiver chamber, D is donor chamber

4.5 Development

Once each component of the device was discussed, the team decided to consult, Mike O'Donnell, head of the WPI manufacturing laboratory. The first suggestion that was given to the team was to make all parts with English units because the machine laboratory had limited tools and hardware in metric sizes. Also, the team was informed that scrap materials in the stock rooms and/or hardware within the shop was available for

se. More specific design modifications from this consultation are described in Section 4.5.1.1.

A major constraint arose from this first meeting with O'Donnell. He was informed that the team's goal was to produce twelve copies of each part in the system; he replied that there were limited amounts of people working on parts for projects on campus. He estimated that that volume of parts that the team demanded would take until about May to produce because there were many steps involved in the process of creating them and their small size and tolerances would require careful attention that would be very time consuming.

At this point in the design process, the team decided it would be necessary to appoint each member to specific portions of the project. Christina and Tom would work together on the experimental design of the project, which included the development of protocols for how the system would be operated, tested, and evaluated. Rachael's task was to oversee the development of the device, which included learning how to operate the appropriate machinery to create the parts needed.

4.5.1 Prototypes

The next step of the development process of the device was to construct a prototype of the design. This would give the team feedback on which components would function appropriately. The prototype would be tested for its ability to homogenously mix the solutions in the chambers, maintain a controlled temperature in the chambers, and to establish any leakage issues in both the snake system and chambers.

4.5.1.1 Initial Design Modifications

The first manufacturing issue that the team needed to adjust the design for was on the donor chamber. After the consultation with O'Donnell, the team was informed that the current donor design would be impossible to create. The block would have to be made in three pieces and fixed together (Figure 34). It was decided that the best way to do this would to have the sides of the block which hold the cartridge for the membrane made into separate pieces from the chamber itself and then screwed into place. The screws would have to be stainless steel to avoid corrosion and their heads would be countersunk into the sides of the donor in order to save on space.

The next grouping of changes that needed to be made were to add a radius to multiple corners of the design. The most important radius was for the o-ring groove. When an o-ring is used to seal similar to our application, the entire outside surface area of the o-ring needs to be touching the surface it is sealing. Without a radius, this would not occur. A detailed explanation of how the o-ring should fit into the groove can be found in Appendix L. Other radii needed to be added where the milling tool would be unable to make a sharp edge. For example, the holes on the donor block for the tabs on the receiver to fit into could not be made with sharp edges. Therefore, a radius was added the corners of tabs themselves so they would fit into the holes appropriately.

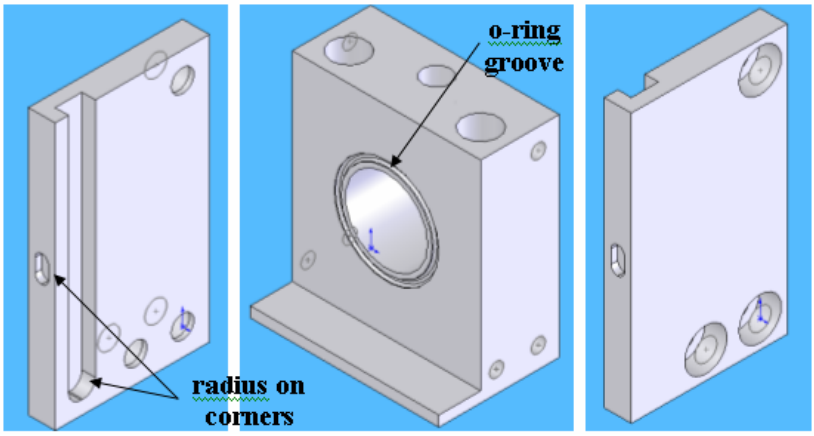


Figure 34: Modifications of the donor block for prototype

Specific modifications of the corner clamps being used had to be made at this time. The major change of the clamps was to add a piece of aluminum to the sides of the clamp because they were not the same height as the blocks. It was feared that the sides would only apply pressure to the bottom point of the two blocks. The pressure needed to be applied in the middle of the block, specifically where series of o-rings are in place to seal the membrane between the chambers. Therefore, taller pieces of aluminum were screwed onto the sides of the clamp. This piece could not be a solid block, because there needed to be clearance for the luers to be exposed. Even with this additional piece of aluminum, each clamp for the blocks was weighed at only about 188.5g (0.42lbs).

During the design of the prototype, final dimensions of the system were decided upon. The factors that affected these decisions were the orientation of the devices on the shaker plate and the sizes of tubing, luers, and o-rings could be purchased. The layout of all devices was the first consideration. The optimal goal was to create a system with twelve pairs of donor/ receiver chambers. The excess space being occupied by the clamping system adapted would not allow the team to fit the twelve chamber pair system on the shaker plate platform. With this system, the team would only be able to fit a ten

chamber pair system on the platform, even with making the dimensions of the blocks as small as possible.

The two dimensions that drove all other dimensions were the sizes of o-rings available and the need for a 5mL chamber. It was found that 1" outer diameter (OD), 7/8 inner diameter (ID) with thickness of 1/16" were the best o-rings to use for the 5mL chamber. This would shorten the length of the blocks so their dimension together was 2.2 X 1.9 X 2in.

4.5.1.2 Prototype I

During the creation of the first prototype, machining the parts became a challenge. The main issue when milling was with the plastic being too pliable for the power of the machine. The milling machines being used are not specialized to cut plastic, they are meant to cut metals. Problems with plastic deformation occurred only with the cartridge pieces and the sides of the donor due to their thickness (0.20in). When the parts were loaded in the vice, they to be held tight enough so that the milling bits did not just toss the part from the jaws, but loose enough that they did not warp the shapes desired. It was found that as material was removed from a face of the part, the grip of the vice would begin to deform the plastic because it was loosing material to withstand the stress of being held. It was decided that all of the cartridge pieces and donor sides would have to be stuck down directly to the flat surface of the vice in the machine with double-sided tape. This would add a great deal of time to the process of machining because each part would have to be stuck down, then the tape residue would have to be cleaned thoroughly from the vice and each part. Although it would make the task of creating the parts more time consuming, it was necessary to avoid deformation.

When the prototype was complete, it was tested for leakage in the temperature system and between the chambers. A silicone in Pins' laboratory, Dow Corning Silastic Medical Adhesive Silicone Type A, had been used on the luers and was only given about 30 minutes to cure. The team was unaware that recommended cure time for the amount of silicone used is approximately 24 hours.

It was found that the chamber system leaked immediately. There was a significant gap between the cartridge and blocks near the top as you can see in Figure 35 by the paper that could be slid in between this interface. Also, the addition piece of aluminum added to the clamping system can be seen in this figure. The point of pressure was still too low on the system. When the temperature system was tested there was a small volume of leakage at the seams of the luers and face of the block. The team realized soon after testing that this was due to the fact that the silicone was not given enough time to vulcanize. Due to the fact that the chambers could not hold liquid, the team was unable to test the ability of the snake to control the temperature within the chambers.

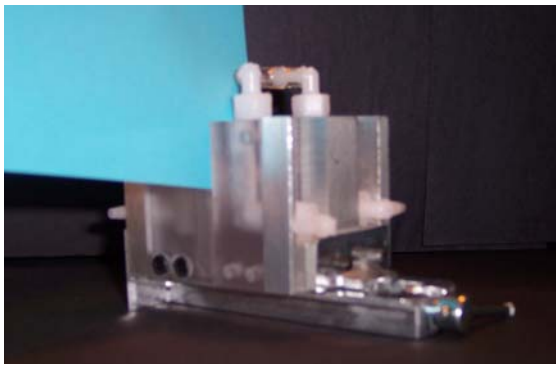


Figure 35: Prototype I - gap between cartridge and faces of receiver chamber

Another reason that the chamber system was unable to hold liquid was due to the fact that an o-ring could not be placed between the cartridge and donor block. The design intended the cartridge to slide down into the slots in the block, flush to the surface of the

donor block. The team did not realize until the prototype was created that this would not allow an o-ring to be put in between. See Figure 36 for a visual of how the o-ring cannot be placed at the appropriate interface.

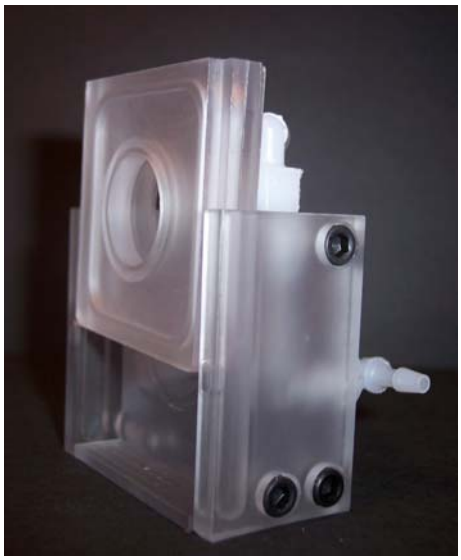


Figure 36: Prototype I - the cartridge slides into place and an o-ring cannot be placed in between donor block and cartridge

Another issue that occurred during these preliminary tests was the lack of tubing reducers available in the laboratory. The luers purchased required the use of 1/8" inner diameter tubing for the snake system. The tubes connected to the water bath were both 7/16" inner diameter. After a vast amount of searching, it was established that there is not a tubing reducer on the market that tailors a 7/16" diameter to a 1/8" diameter. Luckily, the team was able to use an outside resource to have a part turned for no cost. A drawing of this piece can be found in Appendix M.

4.5.1.2 Prototype II

Due to the unfortunate results of the first prototype tests, the team needed to proceed with a second prototype phase. The main components that were changed in the design were the way the blocks were clamped together and its cartridge holder on the

donor chamber. These were both considered the key features that hindered the function of the first prototype.

The first component of how the blocks clamped together that was changed was the tabs. It was found that these tabs did not align themselves as well as the team had hoped. This was mostly due to the fact that there were problems with plastic deformation for the sides of the donor. The tabs were so small that the slight bend in the plastic did not allow them to align properly. Although there was going to be the new technique of double sided tape used to machine the parts, the team was still skeptical about any deformation ruining the purpose of the tabs. Therefore, the shape of the receiver chamber was changed to fit into the donor block and push the cartridge into place (Figure 37). This also took down the length of the two chambers together.

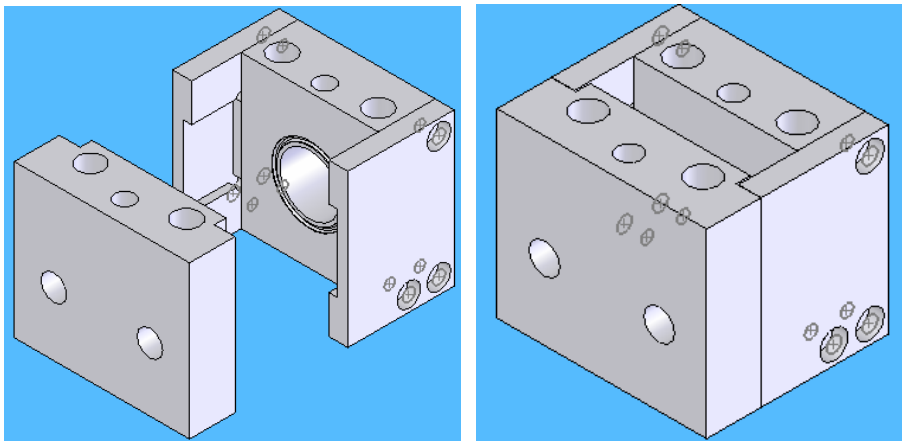


Figure 37: Donor/ Receiver block engagement without cartridge

Not only did this design change provide a proper alignment between the two chambers, it also improved the design of the cartridge holder. The cartridge would now be positioned between the two chambers when they were apart and pushed back into place by the two chambers being clamped together. This would allow an o-ring to be

placed between the cartridge and donor face. The image on the left in Figure 37 depicts the channel in the donor block where the cartridge would slide into place.

The next element of design that had to be changed was the clamping system. The team still desired to use the corner clamps due to their low cost and light weight. It was decided that two clamps should be used for each block pair; one on each side. This would provide uniform pressure across the system to prevent leakage. The downside of this design was that each clamp would have to be modified a great deal to allow clearance for the luers. Also, since the clamps would have to lie on their side for this to occur, then brackets would be needed for the clamps to stand vertically on either side of the blocks. It was decided that corner braces (Figure 38) would be fixed to the clamp in order to give them something to stand on. It was also decided at this time that some type of fixture would have to be developed for each of the clamps to stay permanently in place on. Details of the fixture were not decided upon because the team wanted to see if the prototype would pass testing. The modified clamping system for the second prototype can be seen in Figure 39.

Figure 38: Corner brace needed for clamping

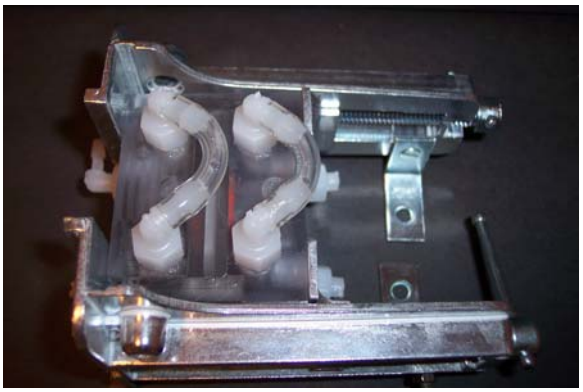


Figure 39: Clamping on either side of the blocks with corner braces- Prototype II

A few minor changes were made to the cartridges during this phase of development. A chamfer was added to the pieces that mate the sides of the cartridge together around the perimeter of the membrane. This was due to the fact that the sharp edge was creating interference when the two were put together. A cut was made at the top of the cartridge on the side that would be pressed against the donor face. This was so the user would be able to easily pull the cartridge away from the face once an experiment was complete. Finally, the sides of the cartridges were altered so they would fit into the new channels on the donor sides. One side of the cartridge was made so that there were two tabs on each piece that could be used to pull apart the sides of the cartridge. A visual of these modifications is provided in Figure 40. An example of how the membrane fits between the two sides of the cartridge can be seen below this figure.

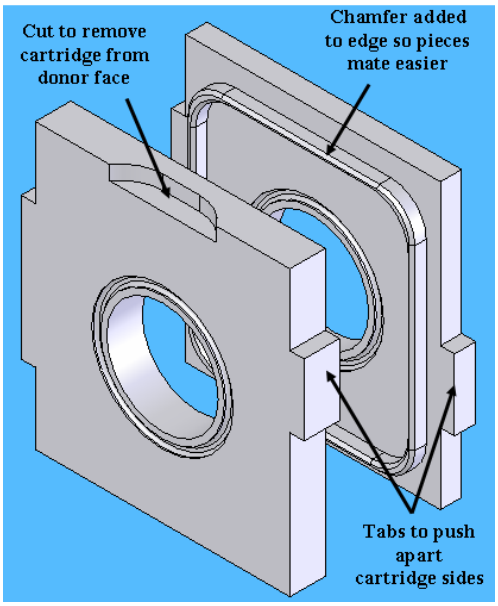


Figure 40: Cartridge modifications

Figure 41: Example of how membrane fits into cartridge

The second prototype testing produced positive results. Both the chamber system and temperature system did not leak. A new silicone, GE II from Home Depot, was used on the luers and was cured for 24 hours before testing this time. This silicone is much less expensive than the medically regulated silicone used on the first prototype. This prototype was used to calculate the time the system takes to achieve a steady-state temperature within the chambers also. See section 5.0 for details on testing this system.

4.5.2 Budget

The team had to be very careful with the budget during the development phase because it was one of the major constraints of the project. The Department of Biomedical

Engineering only provides the team $468.00, which is very little when it is considered that the team must purchase polycarbonate and testing materials.

During the development stage, the team was resourceful and gathered scrap materials from Ximedica, as described in the creation of the model. Also, the prototypes brought forth changes in the manufacturing design. The team knew very little about manufacturing parts before undertaking the development of the project. It was not realized until the first prototype was created that it would be easier to make the dimensions of the part in available stock sizes when possible. During the design changes for prototype II, the thickness of the cartridges and donor sides were made so ¼" polycarbonate stock could be used. Also, when additional polycarbonate was purchased for the donor and receiver blocks, the thickness of the stock was less than the original purchase for the first prototype. This is because thicker stock was found to be significantly more expensive.

Another decision during the development stage that saved money was for all of the luers to be elbowed. The luers could only be purchased in large packages, so the team decided it would be best to simply make all of the luers used one type to save on additional purchases. The budget during the development stage can be found in Appendix N. This budget displays all materials purchased for the final design also which totals $568.81. This total is just over one hundred dollars of the given budget by the school, which means the team must contribute out-of-pocket to the project.

4.6 Final Design

4.6.1 Modifications/ Improvements

The last stage of design refinement was during the creation of the final product. The only two components that changed were the cartridge engagement with the donor block and the clamping system. These changes were made to improve the ease of operation for the user.

During the development of Prototype I, there were issues with the cartridge engagement not allowing an o-ring between the face of the cartridge and the donor block. In the prototype II design this was changed so that the cartridge slid back into place. It was found that it was difficult to slide the cartridge into place because in the clamping system the receiver and donor blocks were too close together before being clamped. The team decided that the cartridge would need to slide into place from the top as they originally planned. The team re-designed the cartridge so that it could slide down from the top and then be pushed back into place by clamping the two blocks together. Figure 42 shows how the sides of the donor are milled differently, and a simple step by step process of how the cartridge is aligned. The figure also depicts where the o-ring is in place for the system and a removable stopper in the sample port that was used to occlude the chambers during testing.

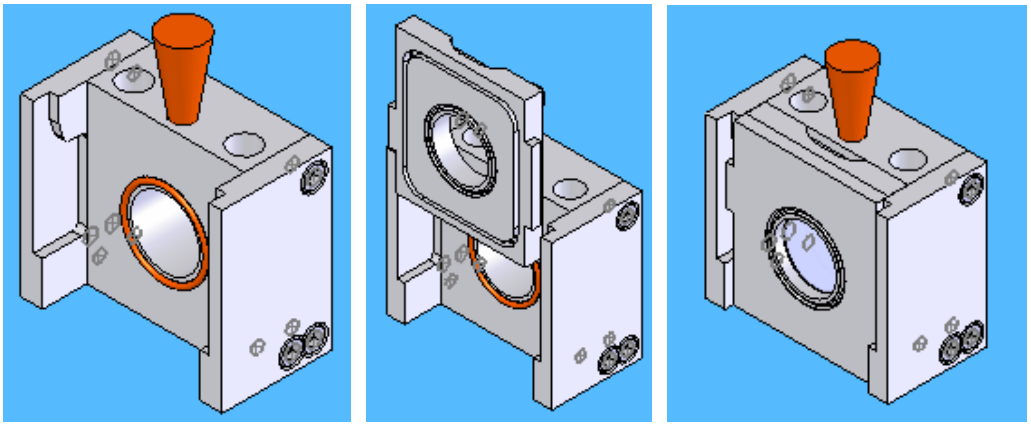


Figure 42: Donor block with sides [left]; donor block with one side of cartridge sliding down [middle]; donor block with both sides of cartridge in place [right]

The clamping system was re-designed based on the decision that the development for the second prototype was not user friendly. It would require the user to spin two screws to clamp the system together for each pair of blocks. This would be time consuming. Also, the clamping system would require more space, which with the constraint of the shaker plate the team did not have. Finally, the clamping system was not aesthetically pleasing, which was not a directly an objective of the project, but relates having a simple design.

The team decided to design a custom clamping system for all ten of the chamber pairs. It was verified that if a single point of pressure was applied directly in line with the chambers, then the system would not leak. This was done by a simple test with a C-clamp as seen in Figure 43.

Figure 43: Prototype with C-Clamp

Once this was verified, a design was developed and machined that required the purchase of bolts and ergonomically styled handles to tighten them. Also, a type of rubber cap, neoprene, was purchased to fit on the end of the bolt that would touch the blocks. The final system with this clamping device can be seen in Figure 44. The decision to not use the corner clamps also decreased the final budget as seen in Appendix O.

A final view of the donor and receiver chambers along with the cartridge to hold the membrane can be seen in Figure 45. Also featured in this picture are the rubber stoppers purchased for the sampling ports and the o-rings used in the system. The sides of the cartridge are open in front of the two chambers.

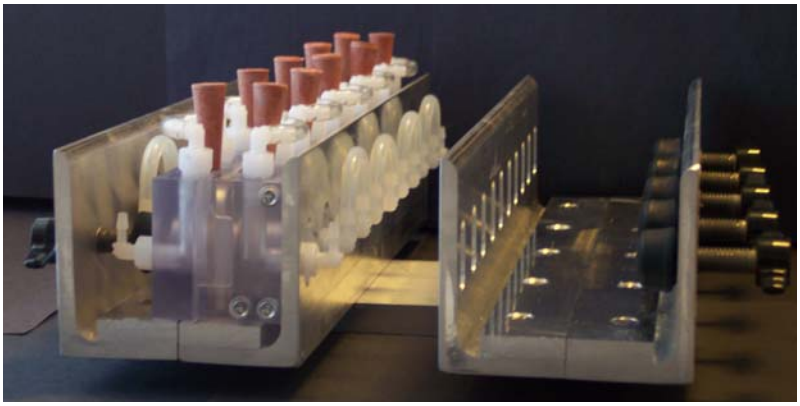


Figure 44: System featuring 5 out of the 10 chamber pairs and clamping system

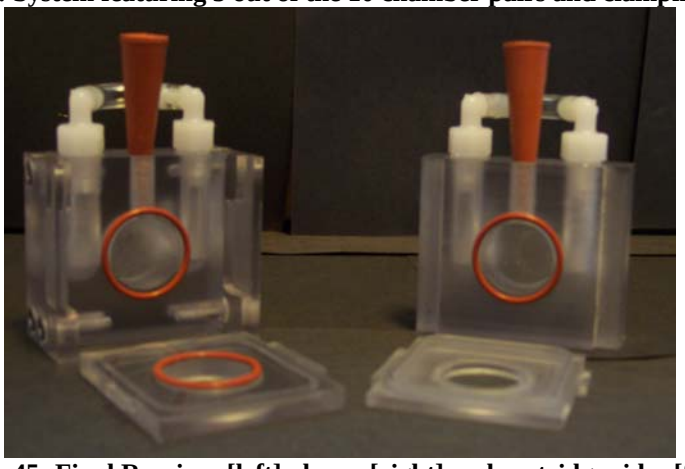


Figure 45: Final Receiver [left], donor [right] and cartridge sides [front]

4.6.2 Components of System

The final system comprised of multiple components which are detailed in Table 25. Each component contributed to the integrity of the system. The components of the system that were machined are detailed in Computer Aided Design drawings as seen in Appendices P-AC. These drawings include all tolerances and specifications necessary for reproducing their fabrication.

Table 25: Final System Components

Product	Use
Polycarbonate	Milled into 10 sets of donor and receiver blocks, both sides of cartridge, and sides of donor
Rubber Stoppers	Occluded chamber during testing and allow samples to be taken from chamber; removable piece in sample port hole
Elbow Luers	Connects temperature control snake system to the blocks; 4 luers on each donor and receiver block.
Silicone	Sealed luers into place on blocks
O-rings	Series of three on interfaces of cartridge and blocks for each system pair
Anti-seize	Prevented the bolts of clamping system
Bolts with handle	Clamped the blocks together
Fasteners	Screws for clamping system, donor sides, and buts to fit cap on bolts for clamping system
Neoprene caps	Caps on the end of bolts for clamping system
Aluminum	Milled into clamping system
Tubing	PVC between luers on top of blocks; silicone between block pairs and to water bath
Water Bath	Provided controlled temperature of water flow through snake system
Shaker Plate	Homogenously mix solutions in chambers

5.0 METHODOLOGY

Once the high-throughput system was completed, various types of testing needed to be conducted in order to validate our device and characterize the collagen membranes. Molecules for the diffusion studies needed to be chosen and preliminary validation testing needed to be completed on the high-throughput system. These preliminary studies were used to make sure it could fully function for testing. Experiments were then conducted on both the PermeGear® device along with the high-throughput system and samples were collected and analyzed to determine the diffusion coefficients of each type of membrane and molecule studied.

5.1 Molecules for Studies

The team compiled a list of molecules to decide which molecules could be used in the studies to characterize membrane transport. From the literature gathered, a list of all the types of molecules tested was compiled. This list includes the molecule, molecular weight, and molecular radius (as previously described in Appendix F). From this table, the following molecules were selected to be used within our diffusion studies: glucose, cytochrome c, myoglobin, bovine serum albumin (BSA), and gamma globulin which have molecular weights of about 180, 13000, 18000, 50000, 150000 Daltons, respectively. These molecules were chosen due to their large range of molecular weights, which would allow for better characterization of the membranes.

The cost of the molecules, however, changed the desire for cytochrome c. From Table 26 below, one may see that the cost of myoglobin is significantly lower than that of

cytochrome c. Since they are similar in molecular weight, it was decided that it was unnecessary to use cytochrome c, and that equine myoglobin would be sufficient.

Table 26: Comparison of Cytochrome C to Myoglobin

Molecule	Price	Type	Company
Cytochrome C	$89.50/ 100mg $285.00/500mg	horse ~90%	Sigma-Aldrich, St. Louis, IL
	$77.20/100mg $134.00/250mg	bovine ≥60%	
	$425.50/ 250µg	human -- lyophilized powder	
	$139.00/ kit $84.00/kit	250tube/ 5000plate 250tube/2500plate	Pierce, Rockford, IL
	$279.00/ 150 µg	human 85%	Calbiochem La Jolla, CA
	$360.00/ 10 µg	human	R&D Systems Minneapolis, MN
	$185/ 250mg	purified horse	Biogenesis Raleigh, NC
Myoglobin	$139.00/ kit $84.00/kit	250tube/ 5000plate 250tube/2500plate	Pierce Rockford, IL
	$120.00/ 1mg	> 60% human	Fitzgerald Industries International Concord, MA
	$37.50/ 250mg $109.00/ 1g	equine 95%	Sigma- Aldrich St. Louis, IL
	$41.50/ 250mg $122.00/ 1g	equine 90%	
	$178.00/ 250µg	human 95%	
	$62.00/ 250mg $172.00/ 1g	equine 90%	USB Corp. Cleveland, Ohio
	$218.00/ 1mg	Recombinant human	

After looking at the duration of time left to complete this project, it was then decided that the group would be unable to complete all of the appropriate testing for all four molecules that were previously chosen. Myoglobin was eliminated as a molecule that would be used for testing. This still gave the team three molecules to study and keep a large range of molecular radii to better characterize the membranes. Also, glucose,

BSA and gamma globulin have been used as three main characterization membranes in previous studies, as stated in the background section.

Validation methods for each of the desired molecules were also researched. It was found that the Bradford or BCA protein assay can be used to determine concentration of bovine serum albumin (BSA), and gamma globulin. Glucose HK assay can be used to detect glucose which was acceptable[52].

5.2 Assay for testing

Various assays needed to be developed in order to determine the concentration of the molecules with the receiver chamber during the experiments conducted on the device. Two assays were acquired and adapted: the BCA assay and the Glucose HK assay. Each of these assays work with specific molecules to not only read the concentration of the experimental samples collected but also to create a standard concentration curve to use as a comparison to the concentration of the collected samples.

5.2.1 BCA Assay Protocol

The BCA assay was used for both BSA and gamma globulin samples. It requires a mixing of two reagents (A and B) in order to make the working reagent. Reagent A is a solution containing sodium carbonate, sodium bicarbonate, bicinchoninic acid, and sodium tartrate in 0.1 M sodium hydroxyl (Pierce Biotechnologies Product # 23227). Reagent B is a solution of 4% cupric sulfate (Pierce Biotechnologies Product # 23227). The two need to be mixed in a ratio of 50 parts A to 1 part B. This mixture can remain viable at room temperature for several days in a closed container. Even though it can be stored, there should only be enough reagent mixture made for the tests that need to be

completed within a day. The equation to calculate how much reagent to make is as follows:

(# standards + # unknowns) x (# of replicates) x (200 µl of Reagent per sample) = Total volume of Reagent needed

It is very important to ensure accurate pipetting, so a 20-200 µl pipette should be used for the sampling from the receiver chamber. Only 25 µl of a sample taken during an experiment is used to run the BCA assay. The sample, however, needs to be large enough to run the sample in triplicate for the assay. In total, the net volume of sample should be 150 µl.

From the 150 µl sample, 25 µl is removed placed in triplicate in a 96-well plate. Each well with a sample should be filled with 200 µl of the reagent mix previously described. The 96-well plate should be covered, stirred on a shaker plate for 30 seconds, and incubated for 30 minutes at 37º C. The plate is removed from the incubator and cooled to room temperature. This is then placed in the plate reader (Molecular Devices, Spectra Max 250, serial # S01539) (see plate reader protocol Appendix AO) and read at 562 nm.

5.2.2 Glucose HK Assay Protocol

In order to determine the concentration of glucose within a solution, the Glucose HK Assay (Sigma-Aldrich, Product GAHK20) was used. Within this assay, a standard is created using the specific protocol and the sample reading is compared to this standard. The original protocol was altered based on our design and also to use the resources of the kit in a resourceful manner.

To create the standard, dilutions are made using the glucose standard solution and PBS within epindorph tubes (A-E). The concentration of glucose within each test tube increases from A to E. Next, an equal amount of the assay reagent was added to each test tube. The test tubes are then incubated for fifteen minutes at room temperature. Samples were taken from each epindorph tube and place within a plastic cuvette and read using a plate reader program within a UV spectrophotometer (Beckman DU® 640 spectrophotometer, serial # 4314297) to determine the absorbance value within each cuvette(see standards results Appendix AP).

Once all the samples have been taken throughout the testing period, 100 µl of the diluted sample is then placed within a new epindorph tube and used to determine the concentration of the receiver chamber throughout the experiment. Again, 1 mL of the HK assay reagent is added to each epindorph tube, which is then vortexed. All of the tubes are incubated at room temperature for fifteen minutes. 1 mL is then removed from the epindorph tube and place within a plastic cuvette. The plate was then read using the UV spectrophotometer and compared to the standard results.

This assay's results were given in the form of absorbance values. These values needed to be converted into a concentration using the conversion equation provided within assay instructions. All data was converted before using it to determine the diffusion coefficient for glucose through either of the devices.

Before conducting any tests on our device, it was important to complete some preliminary tests using the device to test for any problems regarding leakage, mixing and temperature control. Also, preliminary testing was done using the previously described

PermeGear® device. These studies would be used as a comparison for our studies conducted on both previously characterized membranes and collagen membranes.

5.3 Preparation of Self-Assembled Collagen (SAC) Membranes

Collagen membranes were prepared from RTT collagen using a concentration of collagen of 10 mg/ml and went through a self-assembly process [53] (See Appendix AD). These membranes were stored in desiccators and were hydrated in PBS before being used for an experiment.

5.3.1 Membrane Pore Size

The collagen membrane pore size needed to be determined in order to properly characterize the membranes. SEM images of the membranes were taken at a very high resolution. These images were then analyzed with image software (ImageJ, 1.37v). Using this software, the area of the individual pores could be determined. The area of 50 pores were determined within the picture and reported in pixels. This number was then converted to the correct units (nanometers) based on the scale of the picture. The amount of pixels of the scale bar represented the conversion factor (ex. 126 pixels = 5 μm). This conversion factor was used to obtain the radius of the average pore size, which could be used to determine the pore diameter. This allowed the average pore size to be determined to better characterize the membranes.

5.4 Device Assembly

This section describes the steps to assemble each system. The PermeGear® is a simple system due to its use of only one chamber set. The high-throughput system uses a

multi-chamber set-up to conduct multiple experiments simultaneously. Protocols were developed for each system's assembly.

5.4.1 PermeGear®

A protocol for setting up the PermeGear® diffusion chamber was created for the preliminary diffusion studies (see Appendix AE). Initially, a SAC membrane was hydrated in phosphate buffer solution for thirty minutes while the user was setting up the diffusion chambers. A water bath was connected via a connector and silicone tubing to the PermeGear® device to create a closed system for water flow. The water bath was turned on and set to a temperature of 37ºC. The chamber set was then allowed to heat for 30 minutes. The stir bars were placed within each side of the chambers. Once the membrane was fully hydrated after 30 minutes, the membrane was placed between the donor and receiver chambers and was sealed by tightening the fixture around the two chambers. Liquid could now be added to each of the chambers. The proper fluids (molecular solution and PBS) were heated for 30 minutes in a heated water bath (set at 37ºC). The molecular solution was placed within the donor chamber of the device (3.4 mL) and PBS was added to the receiver chamber of the device (3.4 mL) when ready to carry out the experiment. Both chambers ports were closed with plugs once the solutions were placed within them.

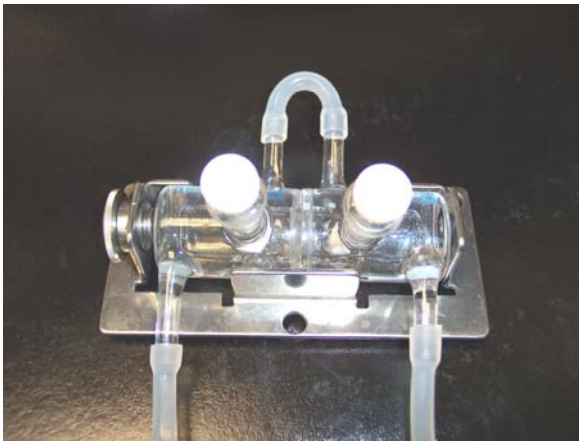


Figure 46: PermeGear® Device Set-up

5.4.2 High-throughput System

A protocol was also developed for the high-throughput system (see Appendix AF). Ten SAC membranes were hydrated in PBS for 30 minutes. Using specialized connectors, the laboratory water bath was connected to the system and set to 45ºC. The system was allowed to heat for the specific amount of time in order for it to reach 37 ºC. Also, the system was placed on a VWR 5000 Orbital Shaker for mixing purposes. Once the membranes were fully hydrated and the system was at the correct temperature, the membranes were placed within cartridges. These cartridges were then placed within the proper donor-receiver chamber set and clamped shut with each set's individual clamping system. The molecular solution (5 mL) was placed within the donor chamber and PBS (5 mL) was placed within the receiver chamber when the testing was ready to be started. Silicone stoppers were placed within the sampling ports during testing to prevent any other materials from entering the solution.

Figure 47: High-throughput System Set-up

5.5 Preliminary Validation

Various types of validation were completed before the device was actually used for experimentation. The device was tested for the following: leakage, control of temperature, and ability to mix and keep chamber solutions homogenous. All tests were completed on the prototype. In addition to these tests, a temperature test was completed using the completed high-throughput system due to multiple chambers within the system.

5.5.1 Leakage

In order to make sure our device did not leak, both the water system and the chamber system needed to be analyzed. The heated water bath system located within the laboratory was connected to our prototype to test the water system used to heat the prototype and entire high-throughput system. The device was connected to the water system for three hours, and was checked each half hour for any leakage that might have occurred from any of the luer connectors.

Our device was also tested for leakage between the chambers. For this test, a fully hydrated membrane was placed within the cartridge and the device was completely assembled without being connected to a water bath. This would show any leakage between the chambers that was independent of the water bath. The device was press-fit

using the clamps of the device system and 5 mL of PBS was placed within each chamber. The device was then inspected over 2 hours to see if any leakage occurred.

5.5.2 Temperature Control

The ability of our device to control and maintain the temperature of the inner chambers was also tested. The chamber system was assembled (as explained in the assembly protocol) and 5 mL of PBS was placed within the donor and receiver chamber. The water bath was turned on, set at 37ºC and allowed to flow through the prototype. A thermometer was placed within the first, third, and fifth receiver chambers of the system and paraffin was used to cover the receiver sampling port with the thermometer submerged within the PBS inside. The temperatures were recorded every 10 minutes. Once the chambers reached a steady-state temperature lower than the needed temperature, the duration of time it remained at the steady state was recorded.

Once having this information, another temperature test was completed with the believed set temperature of 47ºC that would produce a temperature of 37ºC within the chamber of the device. Again, the water bath temperature was set at 47ºC and a temperature probe was placed within one chamber of the device containing PBS solution. The water bath was allowed to circulate through the device in order to heat it. Temperature readings were taken every 10 minutes until the temperature of the device reached a steady state. From this data, a temperature test needed to be completed at the 45 ºC. This temperature was used to conduct a temperature test on the high-throughput system.

A temperature study was also completed using the PermeGear® device. This study was used to compare our temperature studies to a device already on the market. It

would be used to compare the time it took for our prototype to reach a steady state and compare the steady-state temperature between the prototype and this device. It was carried out using the same previous steps described for the high-throughput system temperature test.

5.5.3 Mixing

In order to determine if the high-throughput system would remain homogenously mixed, a mixing study needed to be completed with the prototype as well. For this test, phenol red (Sigma Aldrich, P4633) was used in order to show that the liquid within the system was being completely mixed when on the shaker plate. The prototype device was assembled as if a normal diffusion study was being carried out; however, 5 mL of water was placed within the donor and receiver chambers. Water turns yellow when phenol red is completely dissolved within it. A small amount of phenol red was added into each chamber. The prototype was then placed on the VWR orbital shaker, which was set to 150. An initial sample was taken from the stationary chamber, using a capillary tube, and then the shaker plate was turned on to allow the liquid within the system to be mixed. Samples were taken every 30 seconds until the solution turned completed yellow, stopping the shaker plate each time a sample was taken. Pictures were taken of both the sample capillary tubes and the chamber of the device each time the shaker plate was stopped to sample.

5.6 Experiments using PermeGear® and the High-throughput System

Testing was completed on the previously described PermeGear® device to use as a control for the experiments using the high-throughput system. Tests were completed on

the PermeGear® using the same protocols used for testing the high-throughput system. All three molecules were tested using the PermeGear® and the BCA or glucose assay was used to analyze the results. Collagen membranes were used in these studies.

Testing of the high-throughput system was completed using all three molecules as well. Dialysis and collagen membranes were studied using this device. Tests were completed for each molecule based on each molecule's protocols.

5.6.1 Experiment Preparation

Before beginning an experiment, the proper solutions were made with the particular molecule of choice for the study. Each solution was made at a concentration of 5 mg/mL of the molecules (see protocol about how to make solutions in Appendix AG). These solutions were made in bulk and either stored within glass containers or made into aliquots and frozen until they were ready to be used.

When beginning the experiment once the assembly was complete, whether for PermeGear® or the high-throughput system, the solution of molecules needed to be heated to 37ºC before being placed within the chambers of the respective devices. The molecular solution and PBS were heated in a water bath to have the temperature of the liquids at the same temperature as the device. Also during this preliminary period, microcentrifuge tubes were labeled with the appropriate times on the top of the tubes signifying the time at which the sample was taken (ex. 30 on top of the tube means sample was taken 30 minutes into the study)

Once the solutions were heated, they could be added to their proper chamber within either device. For the PermeGear® device, 3.4 mL of the molecular solution was placed within the donor chamber and 3.4 mL of PBS was placed within the receiver

chamber. For the high-throughput system, 5 mL of the molecular solution was placed within the donor chamber and 5 mL of PBS was placed within the receiver chamber. 200 μL samples were removed from the receiver chamber at the proper sampling time for the specified experiment. The volume of the receiver chamber was then stored with 200 μL of PBS. Each diffusion study had a proper protocol that was followed to carry out the experiment (see Appendix AH - AJ). Samples were taken every minute for the first 10 minutes and at 30 minutes for glucose studies. For BSA studies, samples were taken every half hour over the course of four hours. For the gamma globulin studies, samples were taken every two hours over the course of eight hours. A longer period was necessary due to the increase in the size of the molecules being used, which lengthens the time it takes the molecules to travel through the membrane. Each sample was kept within a freezer until ready to be used to carry out the analysis (BCA or glucose HK assay depending on the molecule of the study).

5.6.2 PermeGear® Trials

Trials were conducted on the PermeGear® device using collagen membranes made of rat tail tendon collagen at a concentration of 10 mg/mL. Glucose, BSA and gamma globulin diffusion studies were carried out using this device. A standard curve was also created for each molecule which could be used for both the PermeGear® and the high-throughput trials. The data from the trials was necessary in order to compare the performance of the high-throughput system to that of the PermeGear® device. The samples taken from these studies were then analyzed using the proper molecular assay previously described.

5.6.5 High-Throughput System Trials

Once the high-throughput system validation was completed, various tests were conducted. Diffusion studies were carried out using a dialysis membrane as a standard for diffusion rates. A dialysis membrane is a characterized membrane that would be used as a standard for the diffusion rates of molecules within our device system. Once these studies were completed, diffusion studies were carried out using SAC collagen membranes. Multiple chamber sets were assembled, as previously described, to carry out more studies over a shorter time period. For one experiment, three sets of chambers were assembled for each molecule that was studied. During each experiment, samples were taken from the receiver chambers of the devices at the necessary times dictated within the protocol. These samples were then analyzed once the tests were completed using the previously described assays (BCA and glucose HK).

5.6.2 Determining Sample Concentrations

Once the diffusion study was completed, samples were frozen until needed for the assays previously discussed. Samples were thawed in a water bath when the assays were ready to be carried out. The BCA protocol (Appendix AK and AL) was followed to determine the concentration of BSA and gamma globulin within the receiver chamber of both the PermeGear and high-throughput system. The glucose HK assay (Appendix AM) was used to determine the concentration of glucose. These protocols were followed and a plate reader was used to measure the absorbance values of the samples using the BCA assay (see Appendix AN for plate reader protocol). A UV spectrometer was used to analyze the samples using the HK assay. The trial results were then compared to each other for a particular molecule and device. Average trial results between the

PermeGear® and high-throughput system were also compared. Using these sets of data, the diffusion coefficients were calculated for each molecule.

5.6.3 Calculations for Diffusion Coefficient

In the PermeGear® and high-throughput system studies, there are a few assumptions that needed to be made in order to calculate the diffusion coefficient. The diffusion can be considered steady state diffusion due to the negligible concentration change within the donor chamber; the donor chamber acts as an infinite source of solute. It is also assumed that the mixing in the chambers prevents any boundary layer from forming, therefore making the concentration of the solution at the membrane surface equal to the concentration of the solution in the bulk chamber. This assumption also makes the samples removed from the receiver chamber a good representation of the concentration in the receiver chamber. For our studies, the process of diffusion of a molecule in solution across a collagen membrane between the two chambers (A and B) can be described by Fick's first law for diffusion across a thin film:

$$J = \frac{D_{eff} \times A_{eff}\left(C_a - C_b\right)}{L} \qquad \text{[Eq.1]}$$

where J is the molar flux, D_{eff} is the effective diffusion coefficient, A_{eff} is the membrane cross sectional area that is in contact within the liquid of the chambers, L is the membrane thickness (assumed 100 μm), and C is the measured concentration in the chambers. There also needs to be a mass balance equation for this problem. Using the molar flux, this is found to be:

$$J = \frac{d(V_b \times C_b)}{dt} = \frac{-d(V_a \times C_a)}{dt} \qquad \text{[Eq. 2]}$$

where V_A and V_B are the volumes of the donor and receiver chambers, respectively. The volumes in this experiment are kept constant so, the only change is the concentration. The diffusivity can be calculated using Fick's Law and then applying a mass conservation equation to Fick's Law to come up with the equation [16]:

$$\frac{\left(-L \times V \times \ln\left(\frac{(C_a - 2C_b)}{C_a}\right)\right)}{2A_{eff}} = D_{eff} \times t \qquad \text{[Eq. 3]}$$

where C_{A0} is the initial concentration in the donor chamber (5 mg/ml) and C_B is the measured concentration in the receiver chamber at time *t*. By plotting the combined Fick's Law and mass balance against time, the diffusion coefficient can be determined. This is a linear curve for the measured values over time *t*. A linear trendline is applied to this curve and the slope of this line is the effective diffusivity. This method was used for determining the diffusion coefficient for each molecule tested.

6.0 RESULTS

After completing all the necessary preliminary and trial studies, all data was collected and analyzed. The preliminary trials were able to prove that our device functioned properly. The trials completed on the PermeGear® and high-throughput systems were then each used to determine the diffusion coefficients for each molecule studied.

6.1 Specifying Membrane Porosity

An important factor to consider when characterizing the rate of molecular diffusion through self-assembled collagen membranes is the membrane porosity, which can be defined by the size of pores and the fraction of void space of the material. Scanning electron microscopy (SEM) is a common technique that is utilized to determine the pore size of materials because of its ability to capture high resolution images under high magnification. SEM was performed and using Image J, we analyzed the pore size of a self-assembled collagen membrane at 5000x magnification (n=1) (Appendix AO). This image can be seen in Figure 47. The areas of 50 pores within the membrane were analyzed to determine the average pore diameter of the pores within the membrane assuming a circular area. The average pore diameter was calculated to be 344 nm.

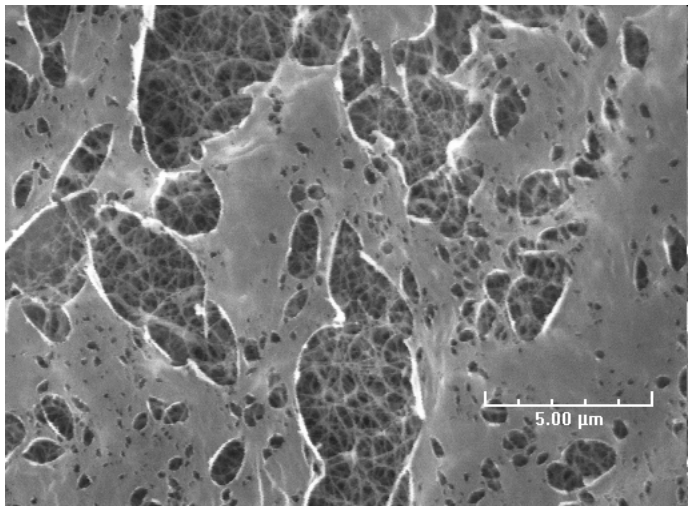


Figure 48: SEM of Collagen Membranes (5000x)

6.2 Preliminary Validation Results

Before testing our high-throughput system, a variety of preliminary studies were necessary to complete. The prototype needed to initially be tested before mass producing the device to ensure: 1) no leakage, 2) ability to control and maintain temperature at 37ºC, and 3) homogenously mix chambers. Also, using the PermeGear® side-bi-side device, diffusion studies needed to be completed to act as a standard which the high-throughput system could be measured against. These studies and their results would be used as the benchmark for the diffusion studies carried out using the high-throughput system.

6.2.1 Leakage

In order to make sure that our device did not leak, the water bath was connected to the heating system of our device to check for any leakage around the luers. After allowing the water system to run for an hour, no leakage was seen from any of the luers after inspection. The system was allowed to continue to run for another hour. The luers still did not show any sign of leakage.

Figure 49: Example of Luers Checked During Leakage Study

Our device was also tested for leakage by adding liquid into the donor and receiver chamber once the full device was assembled with a hydrated membrane. This test was used to see if the chambers within the device had trouble holding the necessary 5 mLs of liquid. Five mLs of phosphate buffer solution (PBS) were placed within the donor and receiver chambers of the assembled device. The height of the solution within

the chambers was then watched over time. The prototype was shown to hold and maintain the 5 mLs of PBS within each chamber.

6.2.2 Temperature Control

For the first initial temperature test for the prototype, the water bath was set at a temperature of 37ºC. Temperature recordings were taken every 10 minutes over the course of 100 minutes. By the end of 60 minutes, the temperature had equilibrated and reached a temperature of 26.9 ºC. It was believed that there was a 10° difference between the temperature setting on the water bath and that of the actual prototype.

Another temperature test was completed, after reviewing this data, using a water bath temperature of 47 ºC. The previous steps were followed again and the temperature was recorded for 100 minutes. The temperature was seen to remain constant again for 40 minutes. This temperature was shown to be 39ºC. A temperature study was also performed with the PermeGear® device for comparison purposes. The PermeGear was seen to reach its steady state temperature in approximately 25 minutes. Figure 49 shows the graph of all tests completed (prototype and PermeGear®), displaying the temperature plotted against time (see Figure 50). This graph also shows the required temperature line of 37 ºC (plotted in green).

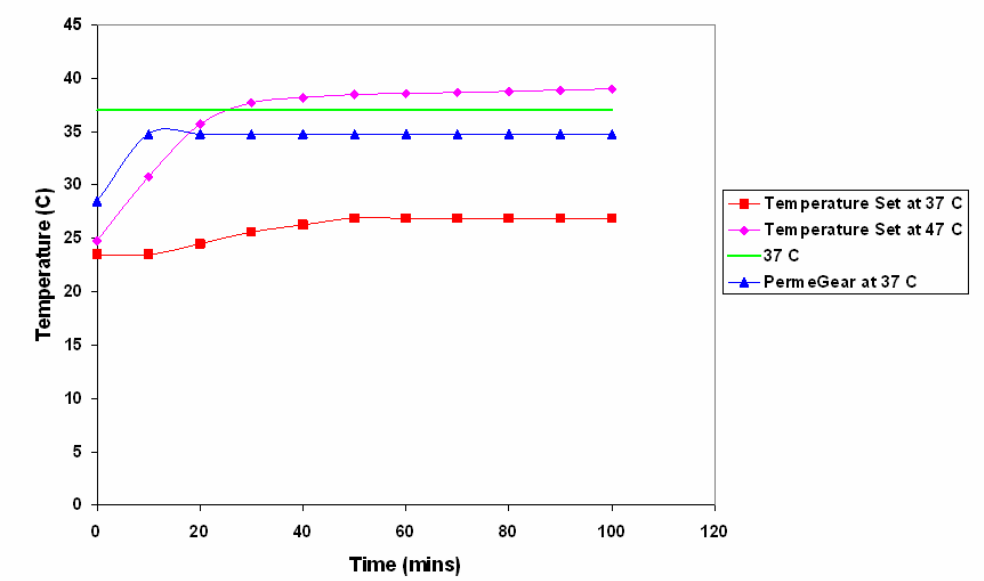


Figure 50: Temperature Validation Test

After having both data sets, a linear interpolation was used to calculate the specific temperature that the bath needed to be set at in order to have the temperature of the chamber remain at 37 ºC. The following equation was used:

$$y = y_a + [(x - x_a)(y_b - y_a)]/(x_b - x_a) \quad \text{[Eq. 4]}$$

where *y* is the needed temperature (37ºC), y_a represents the temperature within the chamber at equilibrium when the bath was set at 37ºC, *x* represents the unknown water bath temperature, *xa* is the set temperature of water bath for first experiment (37ºC), *xb* is the set temperature of water bath for second experiment (47ºC), *ya* is the final temperature at equilibrium of first experiment (26.9ºC), and *yb* is the final temperature at equilibrium of second experiment (39ºC). When solving for *x*, the temperature calculated was found to be 45.35 ºC. This temperature represents the temperature the bath needs to be set at in order to reach a final equilibrium temperature of 37 ºC.

A test was completed with the high-throughput system at this temperature in order to check this interpolation and determine the time it took for the system to reach 37 ºC

(see Figure 50). It was found that it took the system took approximately 40 minutes to reach 37ºC. Considering this is 10 chambers heating up in comparison to just one device, these temperature results are comparable to that of PermeGear ®.

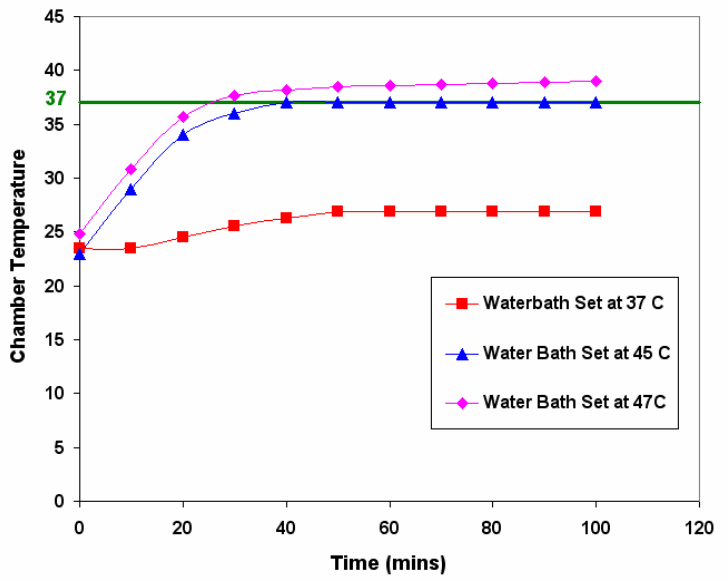


Figure 51: Determination of Water Bath Temperature

6.2.3 Mixing

Phenol red was used to determine if the solution within the high-throughput chambers was being mixed properly. Phenol red is a chemical commonly used to test pH in pools. It turns a yellowish color when mixed in a solution with a pH of 6.5. The results of the mixing test using the prototype can be seen below. Figure 51 shows the capillary tube samples, with the first tube representing water without any phenol red added and the other tubes representing the samples taken at each time point. Figure 52 shows the pictures of the chamber taken at each sample time. It was found that using the shaker plate set at 150 RPM the solution was completely mixed which can be seen by the yellow color of the solution in the sample.

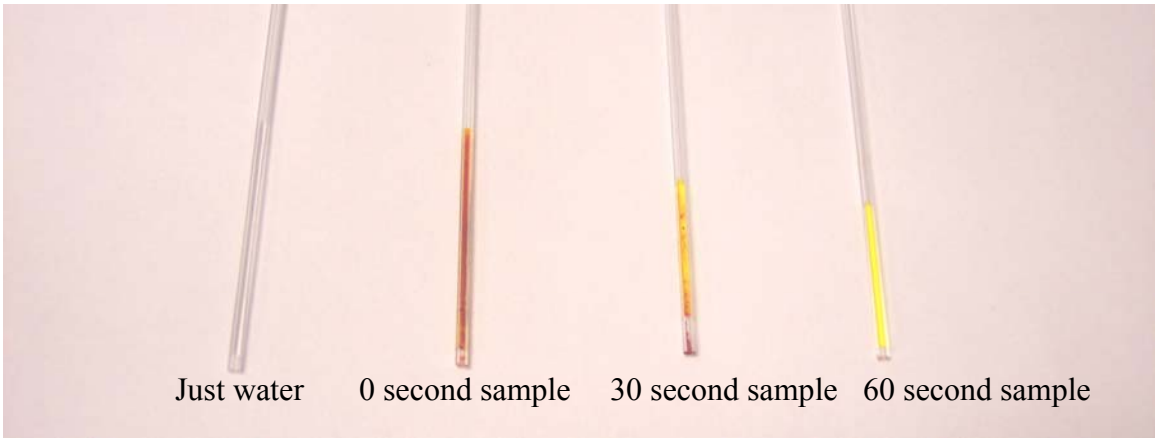


Figure 52: Capillary Tube Samples for Mixing Study

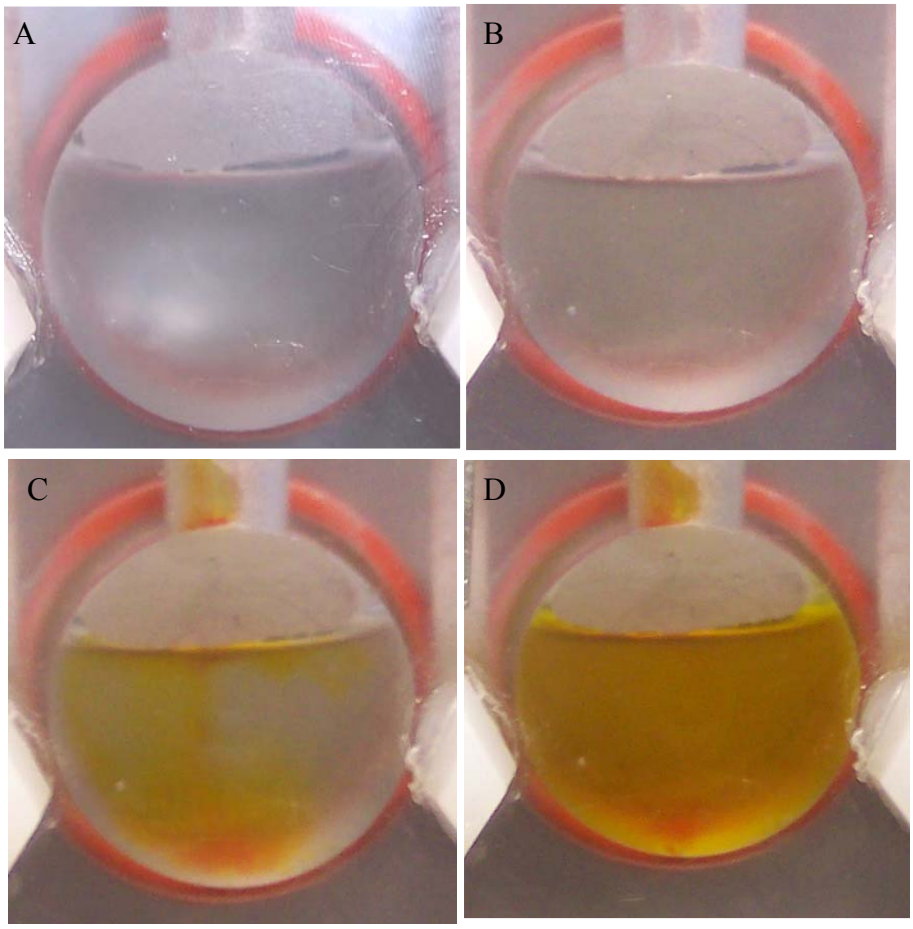


Figure 53: Mixing Test - Liquid in Chambers. A) Water only, B) Water and Phenol Red, C) Chamber at 30 seconds, D) Chamber at 1 minute

6.3 PermeGear® Studies

Trials were completed using the PermeGear® Device using self-assembled collagen membranes. Each molecule was tested using this device. The samples were collected and frozen at -20ºC until ready to be analyzed. Each specific molecule was analyzed with its applicable assay (glucose – glucose HK assay, BSA and gamma globulin – BCA assay). Raw data for each molecule tested can be found within Appendices AQ-AS. This data is then manipulated in order to determine the diffusion coefficient.

6.4 High-throughput Experimental Results

Trials were also completed using the high-throughput system. Trials were performed using both dialysis membrane and self-assembled collagen membranes. The Specta/Por® Dialysis Membrane (MWCO: 14,000) was used as the standard for diffusion; however, due to its molecular weight cut off, data could only be collected for glucose using this membrane. All three molecules were tested using the self-assembled collagen membranes. The raw data from these collected samples can be seen in Appendix AT-AW for both the dialysis and SAC membrane trials.

6.5 Graphical Representation of Diffusion Studies

The raw data previously collected using the PermeGear® and high-throughput system was used to calculate the diffusion coefficient for each particular molecule through a specific type of membrane.

6.5.1 Glucose

Once all of the data was collected and converted from absorbance values to concentrations using the conversion equation provided by the assay and a standard curve, the glucose data could be used to determine the diffusion coefficient. Each concentration value found for each specific sample time point was substituted within the Eq. IV(see Appendices AX-BA) previously discussed. These calculated values were then plotted against time, producing a linear curve. This was completed for each trial (n=3) for each device. All three trials per device were plotted on the same graph for comparison purposes but remain distinguishable due to difference in line color (see Figures 53 and 54).

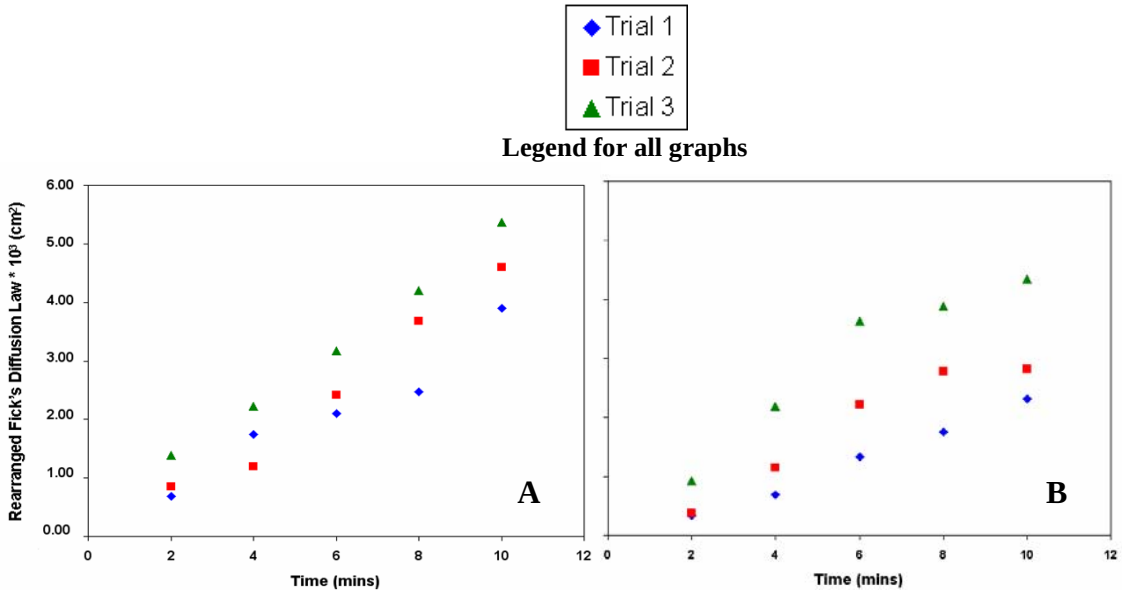


Figure 54: A) PermeGear® Glucose Diffusion Results using SAC membranes B) High-throughput System Glucose Diffusion Results using SAC Membranes

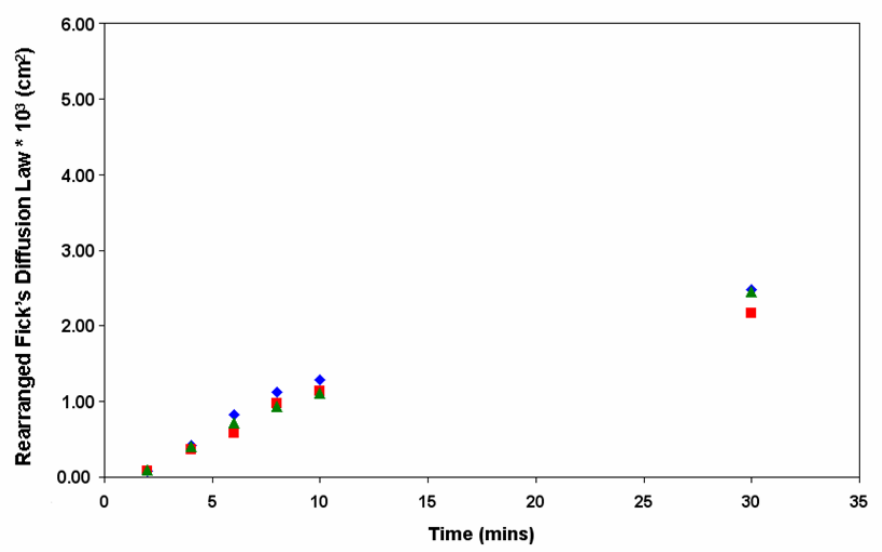


Figure 55: High-throughput System Glucose Diffusion Results Using Dialysis Membranes

Once plotting this data, a linear trend line was added to each trial's plot. This trend line's slope is defined as the diffusion coefficient for that trial. This was completed for every trial for both devices used within these studies (see Figures 55 and 56)

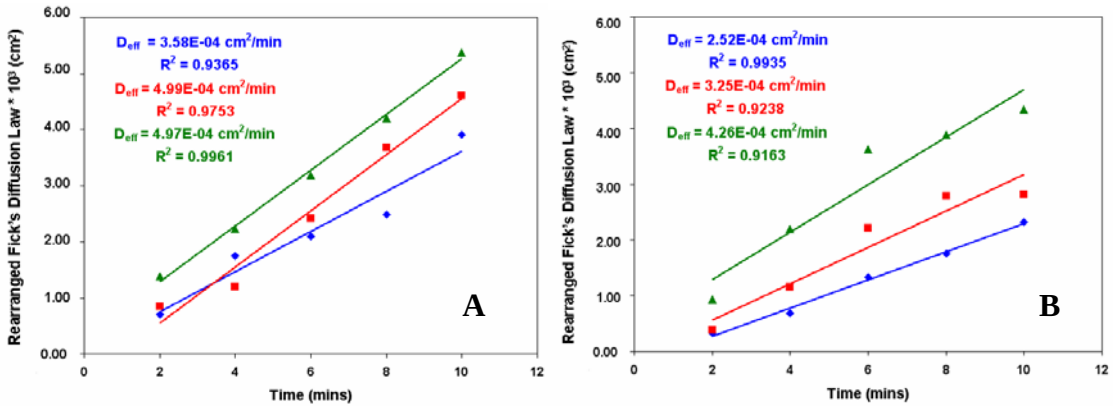


Figure 56: A) PermeGear® Diffusion Coefficients for Glucose Trials using SAC Membranes B) High-throughput System Diffusion Coefficients for Glucose using SAC Membranes

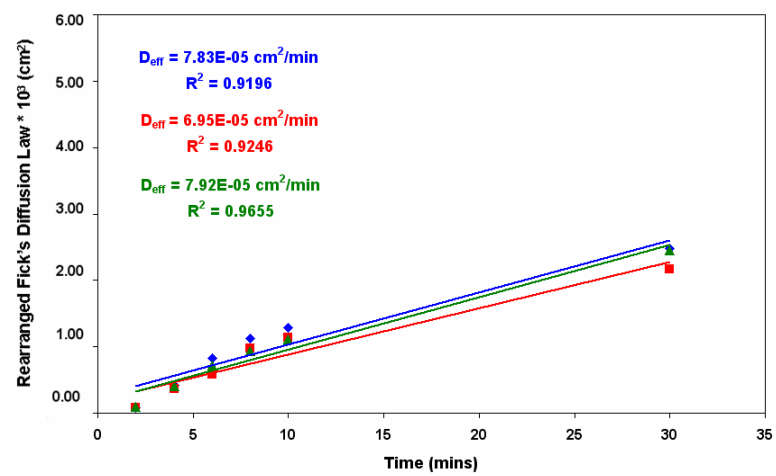


Figure 57: High-throughput System Diffusion Coefficients for Glucose Using Dialysis Membranes

6.5.2 BSA

Each trial carried out using BSA was graphed for both the PermeGear® device as well as the high-throughput system after substituting and manipulating the data using the previously described Eq. IV. Only collagen membranes were studied within these experiments due to the molecular cut-off of the dialysis membrane being smaller than the size of BSA. A graphical representation of each trial can be seen in Figure 57. A linear trend line was then added to each trial order to determine the diffusion coefficient for each. Figure 58 show the diffusion coefficients for each trial using the PermeGear® device and those using the high-throughput system.

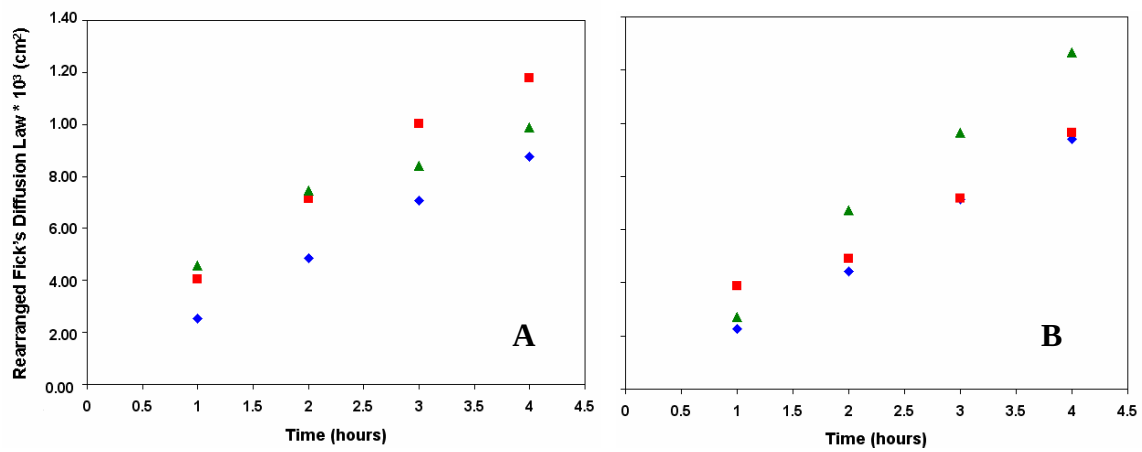


Figure 58: A) PermeGear® Diffusion Results for BSA B) High-throughput Diffusion Results for BSA

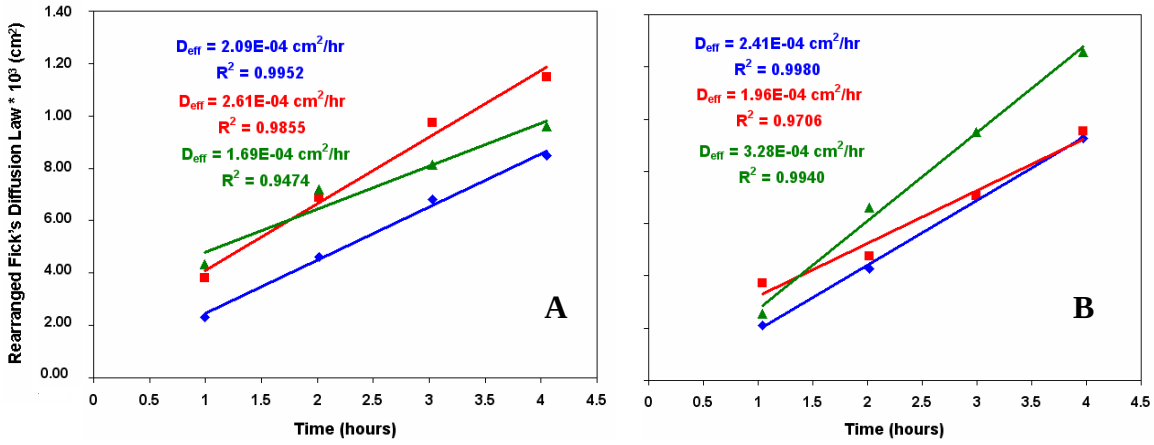


Figure 59: A) PermeGear® Diffusion Coefficients for BSA Trials B) High-throughput System Diffusion Coefficients for BSA Trials

6.5.3 Gamma Globulin

The steps previously described to obtain the graphical results for BSA were used for gamma globulin. All data was graphed for each trial carried out on each specific device. Only two trials could be completed on the PermeGear® device using gamma globulin due to time constraints. Linear trend lines were added to each of these graphs in order to determine the diffusion coefficients for each trial. Figure 59 show the graphical results while Figure 60 contains the trend lines used to determine the diffusion coefficients for each trial.

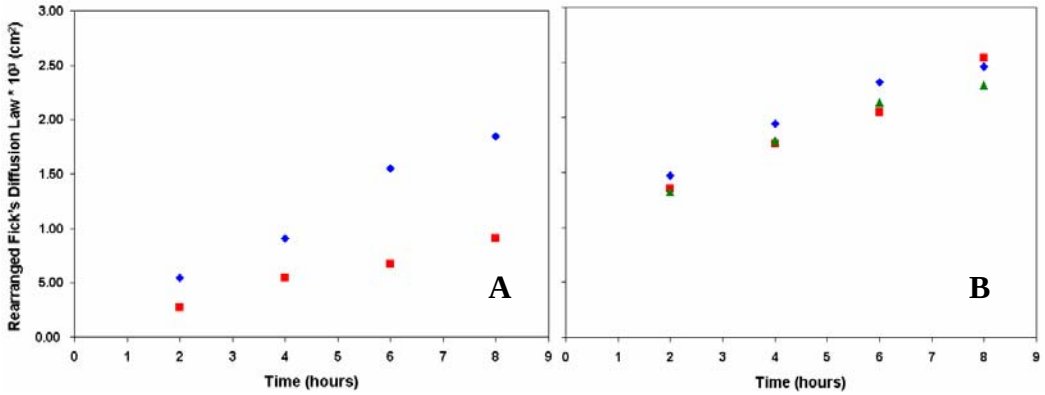


Figure 60: A) PermeGear® Diffusion Results for Gamma Globulin B) High-throughput Diffusion Results for Gamma Globulin

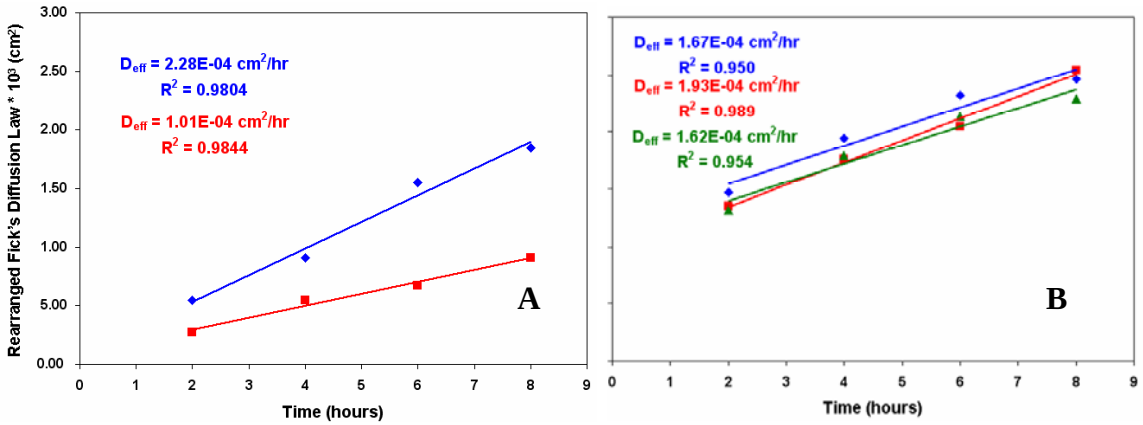


Figure 61: A) PermeGear® Diffusion Coefficients for Gamma Globulin Trials B) High-throughput System Diffusion Coefficients for Gamma Globulin Trials

6.6 Calculated Diffusion Coefficients

Using all of the previously analyzed data, the average diffusion coefficient for each type of molecule tested using the high-throughput system was calculated. These can be found in Table 27.

Table 27: Calculated Diffusion Coefficients

Molecule	Membrane	Diffusion Coefficient (cm^2/s)
Glucose	*Dialysis*	1.26×10^{-6}
	Collagen	5.58×10^{-6}
BSA	*Dialysis*	No Diffusion
	Collagen	7.08×10^{-8}
Gamma Globulin	*Dialysis*	No Diffusion
	Collagen Membrane	4.83×10^{-8}

Further data analyses were completed to determine if there was a statistical difference between the PermeGear® and high-throughput system trials. Also, these calculated diffusion coefficients were compared to previous diffusion studies using collagen membranes to see if previous results were comparable to the high-throughput system findings.

7.0 ANALYSIS AND DISCUSSION

An analysis needed to be completed for all of the experimental trials using both of the devices. The preliminary studies needed to be reviewed in order to make sure the high-throughput system functioned properly. The diffusion trials completed using either device needed to be compared in order to prove the functionality of the high-throughput system and the reproducibility of the trials. Lastly, the calculated diffusion coefficients needed to be compared to previous research using the same molecules of study.

7.1 Preliminary Trials

All preliminary trials were successfully completed to prove that the high-throughput system could be for molecular diffusion experiments. After visual inspection, no leakage was found around any of the components of the device when water was placed within the snake system, which controls temperature; or the chambers, which hold molecular solution. The snake temperature system was able to produce and maintain a necessary temperature of 37ºC for testing. Also, the solution within the chambers was found to be homogenously mixed after placing the system on a shaker plate. These findings all supported the reasoning that this device would function properly during the diffusion studies using the three molecules of choice.

7.2 Diffusion Trial Comparisons

After determining the diffusion coefficient for each trial carried out using the PermeGear® or the high-throughput system, the trials for a specific device were graphically and numerically compared. It was found that each trial produced a similar diffusion coefficient that was carried out on a specific device. For example, all three

trials carried out on the high-throughput device using gamma globulin produced similar diffusion coefficients as seen in Table 28. These trials are all comparable to one another. This similarity was found for each of the trials carried out on the PermeGear® or high-throughput system using one of the specified molecular solutions. This proved the reproducibility of the high-throughput system by producing similar results for each trial using a particular molecule. It was also important to test the functionality of one device to another. A statistical analysis was completed to compare the trials carried out with the PermeGear® device and those carried out with the high-throughput system for a specific molecule in order to try and prove that the devices were comparable to each other.

Table 28: Diffusion Coefficients Calculated from Trials for Gamma Globulin

Experiment	Calculated Diffusion Coefficient (cm^2/hour)
1st experiment	1.67×10^{-4}
2nd experiment	1.93×10^{-4}
3rd experiment	1.62×10^{-4}

7.3 PermeGear® vs. High-throughput System

A statistical analysis was completed in order to compare the trials, and overall performance, of the high-throughput system to that of the PermeGear®. A student's t-test was used to compare the average diffusion coefficient for a specified molecule found from the PermeGear® trials to that of the average diffusion coefficient calculated from the high-throughput trials (n=3 for each device). These trials used collagen membranes for their studies. This was completed for each molecule tested (glucose, BSA, and gamma globulin). No statistical test was completed using the dialysis membranes due to the fact that dialysis membrane is a previously characterized membrane and did not need to be tested with the already marketed PermeGear® device. Appendices BB-BD have the

calculated student's t-test results for each molecule tested using collagen membranes. It was found, for all three molecules, that there was no statistical difference ($p<0.05$) between the diffusion coefficients calculated from the PermeGear® trials and the diffusion coefficients calculated from the high-throughput system trials. These findings statistically prove that our device produces statistically similar diffusion results to that of the PermeGear® diffusion trials. It also supports that our device can be comparable to the PermeGear® due to the similarity in the produced results. These results are also graphically shown in the comparisons found in Figures 61 - 63 for each type of molecule used within these studies.

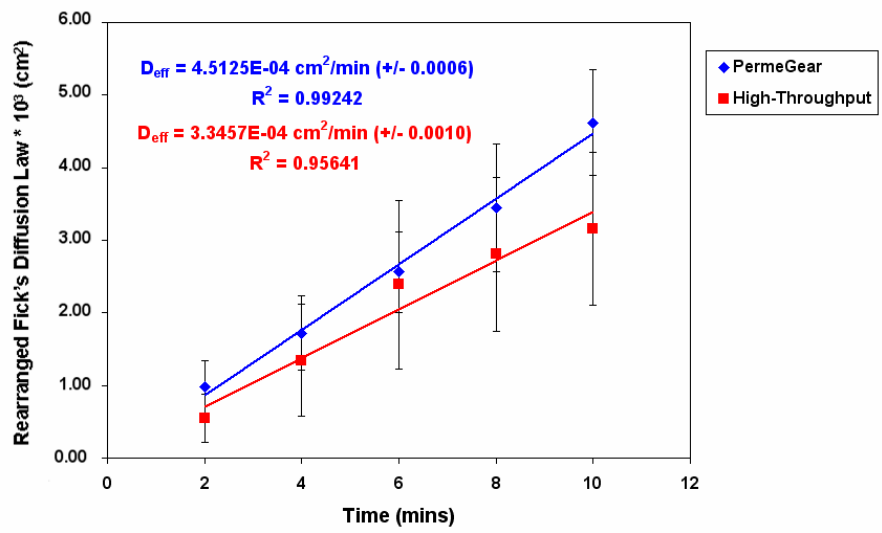


Figure 62: Comparison of Glucose Diffusion Coefficients

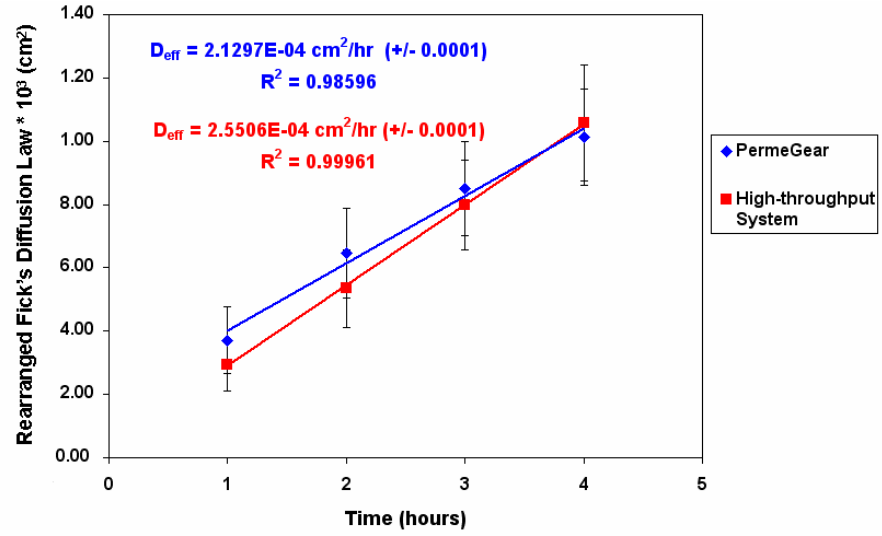


Figure 63: Comparison of BSA Diffusion Coefficients

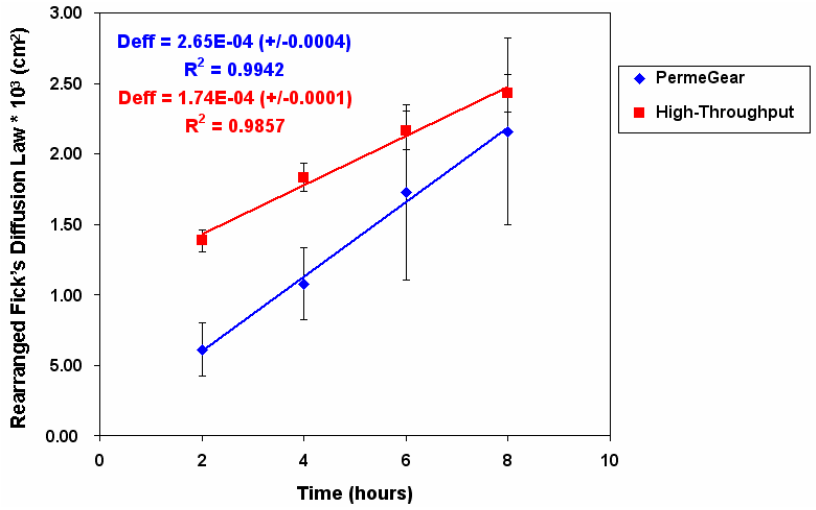


Figure 64: Comparison of Gamma Globulin Diffusion Coefficients

By comparing the PermeGear® device and the high-throughput system, it was found that the device could produce similar results. This proves that the high-throughput system is comparable to the PermeGear® device. After proving the high-throughput system's performance, it was necessary to compare the calculated data found for collagen membranes to data from previous studies. This evaluation will determine whether the studies of glucose, BSA and gamma globulin through collagen membranes would

compare to previous studies, proving the success of the experiments using the high-throughput system.

7.4 Comparison to Previous Research

Previous studies were found for each type of diffusion study and a numerical comparison was used to see how well our studies compared to previous research. By comparing the calculated results to the previous research, we could determine if our results were comparable to previous studies. If the results were found to be similar, we could assure these findings would be helpful in characterizing these membranes.

7.4.1 Glucose

Glucose was the only molecule in which the diffusion studies could be compared to previous research for both the dialysis and collagen membranes. Myung et al, previously studied the diffusion of glucose through dialysis membrane (MWCO: 14 kD), finding a diffusion coefficient of 3.4×10^{-7} cm^2/s[17]. The calculated value using the high-throughput system was 1.26×10^{-6} cm^2/s. These values are comparable to each other but not exactly the same due to the type/brand of dialysis membrane used for each of these studies.

Glucose diffusion studies have also been completed previously on collagen membranes. Within the study performed by Liu, a glucose diffusion coefficient was found to be 2.70×10^{-6} cm^2/s[18]. The calculated value using the high-throughput system was 5.58×10^{-6} cm^2/s. In these results the orders of magnitude are the same, showing similarity. One reason there is a difference in the coefficients could be due to the fact that Liu cross-linked the membranes using an EDC/NHS treatment before performing the

diffusion studies. Cross-linking can affect many parameters such as membrane thickness and porosity [54, 55]. Changing these parameters can also change the diffusion coefficient for the membrane. This is can be shown by directly looking at the variables at the rearranged Fick's law previously described in Section 5.

The diffusion of glucose was also previously studied within native skin. Khalil et al, studied the diffusion of glucose through various parts, such as the dermis and epidermis, using the PermeGear® device used within our studies[19]. A diffusion coefficient of 2.98 x 10^{-6} cm^2/s was found for cadaver dermis. This number is extremely close to the number calculated (5.58 x10^{-6} cm^2/s) using the high-throughput system through collagen membranes. Although these collagen membranes are trying to be used to mimic the basal lamina of skin, the diffusion coefficient calculated within the high-throughput system experiments were compared to that of the cadaver dermis due to the thickness of the collagen membrane actually being tested (100 μm). The basal lamina of native skin is much thinner then the tested collagen membranes; therefore, the diffusion coefficient of the dermis was a better comparison to our studies. The slight difference between the calculated number and that found in Khalil's study may be due to the fact that many components of native skin, such as glycoproteins and glycoaminoglycans, can hinder the diffusion of polar molecules. These components are space filters which is why smaller molecules may have a harder time diffusing through the interstitial matrix of native skin.

7.4.2 BSA

BSA could only be studied through collagen membranes due to its large molecular size. The diffusion coefficient calculated using the high-throughput system

trials is 7.08 x10^{-8} cm^2/s. Previous studies have found the diffusion of BSA through collagen membranes to have been 1.48 X 10^{-7} cm^2/s and 1.60 x 10^{-7} cm^2/s after cross-linking these membranes with glutaraldehyde and EDC/NHS respectively [13, 18]. As previously stated, cross-linking can directly effect the diffusion coefficient of a membrane. This treatment may account for the difference between the calculated diffusion coefficient and that found in previous studies. The orders of magnitude of the calculated diffusion coefficient and the diffusion coefficients of the previous studies are very similar, thereby, supporting that the high-throughput system's functionality and ability to help in characterizing the collagen membranes.

7.4.2 Gamma Globulin

Although a diffusion coefficient was able to be calculated for gamma globulin using the high-throughput system, many previous studies focus on inhibiting the transport of gamma globulin through collagen membranes. Within many of these studies, diffusion coefficients are calculated but represent only a small amount of gamma globulin that was transported because these studies were preventing its diffusion through the membrane. Due to this preventative theme throughout previous studies, the calculated diffusion coefficient using the high-throughput data could not be compared to previous studies.

8.0 CONCLUSIONS

Using engineering design, a high-throughput system was created. This device was able to meet the needs, wants and constraints of the clients and was able to perform the necessary functions previously discussed within the design section. This device and all of its main functions were evaluated through preliminary studies which proved that the device functioned properly.

Experimental trials were carried out using the device to study the molecular diffusion of glucose, BSA, and gamma globulin through both dialysis and collagen membranes. The samples taken from these trials were used to analyze the diffusion coefficients of each particular molecule through the tested membrane. When these trials were compared to one another, it was found that each trial produced similar results, supporting the reproducibility of studies using the high-throughput system.

The diffusion coefficients calculated for collagen membranes using the high-throughput system were compared to those calculated using the Side-Bi-Side PermeGear® device. Using a Student's t-test, no statistical difference was found between the results produced from using the two different devices. These findings prove that the high-throughput system is comparable to the PermeGear® device already available on the market. The high-throughput system, however, allows multiple studies to be run at one time and decreases the experimental time needed.

Previous molecular diffusion research completed using collagen membranes was compared to the calculated diffusion coefficients using the high-throughput system trial results. Although the diffusion coefficients were not exactly the same due to differences in membrane treatment, the diffusion coefficients were comparable to one another. These

findings helped validate the diffusion studies completed with the designed device and meet the ultimate goal of characterizing self-assembled collagen membranes.

9.0 FUTURE RECOMMENDATIONS

The diffusion coefficients calculated within this project help to quantitatively define self-assembled collagen membranes. However, these studies are only the initial steps to characterize them. There are many other studies that can be carried out to better define the parameters of diffusion for these membranes.

For example, as previously discussed, some studies have looked at the diffusion of molecules through cross-linked membranes. Cross-linking can effect many variables of collagen membranes which dictate molecular diffusivity, such as membrane thickness and pore size. The team suggests using self-assembled collagen membranes that have been crosslinked using a variety of established techniques to understand the effects of crosslinking on the diffusion rates of the molecules studied within this project. These studies can help better characterize these membranes and may determine how crosslinking effects molecular diffusion.

The team also suggests possibly using a wider variety of molecules within its diffusion studies. By understanding the diffusion properties of more molecules, the more specific a collagen membrane can be defined. For example, the skin allows many different types of molecules to diffuse between the epidermal and dermal layer via the selective-permeable barrier known as the basal lamina. By studying the diffusion rates of more molecules through collagen membranes, a tissue-engineered basal lamina may be produced sooner that will better mimic that of natural skin. This substitute will be able to control paracrine signaling between the dermal and epidermal layer of the skin, while also providing structural support for tissue re-growth.

References

1. **Alberts, B.,** ***Chapter 19: Cell Junctions, Cell Adhesion, and The Extracellular Matrix,*** **in** ***Molecular Biology of the Cell.*** **2002, Garland Science: New York.**

2. **Pins, G.D., Toner, M., and Morgan, J. R.,** ***Microfabrication of an analog of the basal lamina: biocompatible membranes with complex topographies.*** **Faseb J, 2000. 14(3): p. 593-602.**

3. **Burgeson, R.E.,** ***Chapter 24: Basement Membranes,*** **in** ***Dermatology in General Medicine*****. 1987, McGraw-Hill, Inc. p. 288-303.**

4. **Vracko, R.,** ***Basal lamina scaffold-anatomy and significance for maintenance of orderly tissue structure.*** **Am J Pathol, 1974. 77(2): p. 314-46.**

5. **Langer, R., and Vacanti, J,** ***Tissue engineering,*** **in** ***Science*****. 1993. p. 920-926.**

6. **PermeGear.** ***Side-by-Side Cell*** **(2005). PermeGear, Inc. Accessed:August 30, 2006. <http://www.permegear.com/sbs.htm >**

7. **Chamberlain, L., and Yannas, I.,** ***Preparation of Collagen-Gycosaminoglycan Copolymers for Tissue Regeneration,*** **in** ***Tissue Engineering Methods and protocols,*** **J.R. Morgan, Editor. 1998, Humana Press: Totowa, NJ. p. 3-17.**

8. **Lavker RM, S.T.,** ***Heterogeneity in epidermal basal keratinocytes: Morphological and functional correlations.*** **Science, 1982. 215: p. 1239-1241.**

9. **Lavker RM, S.T.,** ***Epidermal Stem Cells.*** **Journal of Investigative Dermatology, 1983. 81: p. 121s-127s.**

10. **Smola, H., G. Thiekotter, and N.E. Fusenig,** ***Mutual induction of growth factor gene expression by epidermal-dermal cell interaction.*** **J Cell Biol, 1993. 122(2): p. 417-29.**

11. **Boyce, S.T., et al.,** ***Comparative assessment of cultured skin substitutes and native skin autograft for treatment of full-thickness burns.*** **Ann Surg, 1995. 222(6): p. 743-52.**

12. **Falanga, V.e.a.,** ***Rapid healing of venous ulcers and lack of clinical rejection with an allogeneic cultured human skin equivalent.*** **Arch. Dermatol., 1998. 134: p. 293-300.**

13. **Gilbert, D., et. al,** ***Macromolecular Diffusion Through Collagen Membranes.*** **International Journal of Pharmaceutics, 1988. 47: p. 79-88.**

14. **Desai T.; D.Hansford, M.F., *Characterizatoin of micromachined silicon membranes for immunoisolation and bioseparation applications.* Journal of Membrane Science, 1998. 159: p. 221-231.**

15. **Leoni, L., Desai, T., *Nanoporous Biocapsules for the Encapsulation of Insulinoma Cells: Biotransport and Biocompatibility Considerations.* IEEE Transactions of Biomedical Engineering, 2001. 48(11): p. 1335-1341.**

16. **Leoni, L., Boiarski, A., Desai, T., *Characterization of Nanoporous Membranes for Immunoisolation: Diffusion Properties and Tissue Effects.* Biomedical Microdevices, 2002. 4(2): p. 131-139.**

17. **Myung, D., et al, *Glucose Permeability of Human, Bovine, and Porcine Corneas in vitro.* Ophthalmic Research, 2006. 38: p. 158-163.**

18. **Liu, Y., et al, *A Simple, Cross-linked Collagen Tissue Substitute for Corneal Implantation.* Investigative Ophthalmology & Visual Science, 2006. 47(5): p. 1869-1875.**

19. **Khalil, E., et al, *Glucose Partition Coefficient and Diffusivity in the Lower Skin Layers.* Pharmaceutical Research, 2006. 23(6): p. 1227-1234.**

20. ***Bicinchoninic Acid Kit: Protein Quantitation* (2005). Sigma-Aldrich. Accessed:September 2006. <http://www.sigmaaldrich.com/Area_of_Interest/Life_Science/Proteomics_and_Protein_Expr_/Protein_Analysis/Protein_Quantitation/Bicinchoninic_Acid_Kit.html>**

21. ***Pierce* (2007). <http://www.piercenet.com/>**

22. ***BioSystem Development* (2007). < www.biosystemdevelopment.com>**

23. **Asbill C., N.K., A. El-kattan, K. Creek, P. Wertz and B. Michniak, *Evaluation of a Human Bio-Engineered Skin Equivalent for Drug Permeation Studies.* Pharmaceutical Research, 2000. 17(9): p. 1092-1098.**

24. **Shah Vinood; Jerome Elkins, S.S.R.H., *In Vitro Release: Comparative Evaluation of Vertical Diffusion Cell System and Automated Procedure.* Pharmaceutical Developmend and Technology, 2003. 8(1): p. 97-102.**

25. **Broadhead, K., Roy, B., and , Tresco, P., *Hollow fiber membrane diffusive permeability regulates encapsulated cell line biomass, proliferation, and small molecule release.* Biomaterials, 2002. 23: p. 42689-4699.**

26. **Advanced Analytical Center for Environmental Sciences. *Gas Chromatography Advantages* (2005). University of Georgia: Savannah River Ecology Laboratory. Accessed:October 10, 2006. <http://www.uga.edu/srel/AACES/GCtutorial/page6.html>**

27. **Oregon State University. *GCMS: How Does It Work?* (2006). Accessed:October 10, 2006. <http://www.unsolvedmysteries.oregonstate.edu/GCMS_05.shtml>**

28. **Advanced Analytical Center for Environmental Sciences. *Gas Chromatography Disadvantages* (2005). University of Georgia: Savannha River Ecology Laboratory. Accessed:October 10, 2006. <http://www.uga.edu/srel/AACES/GCtutorial/page6.html>**

29. **Franz, T., *On the relevance of in vitro data.* Journal of Investigative Dermatology, 1975. 64: p. 190-195.**

30. **Hanson Research. *Hanson Research Dissolution Test Systems for Pharmaceutical Quality Assurance* (2006). Topical and Transdermal Diffusion Cell Systems: The Vertical Diffusion Cell. Accessed:September 6, 2006. <http://www.hansonresearch.com/vert-diffusion-cell.htm>**

31. **Hanson Research. *Hanson MicroettePlus™ Autosampling System* (2006). Topical and Transdermal Diffusion Cell Systems. <http://www.hansonresearch.com/microetteplus.htm>**

32. **Karande, P., Mitragotri, S., *High Throughput Screening of Transdermal Formualtions.* Pharmaceutical Research, 2002. 19(5).**

33. **Kuhfeld, M., and Stratford, R., *In vitro measurement of drug transport using a new diffusion chamber compatible with Millicell® culture supports: performance with caco-2 monolayers.* International Journal of Pharmaceutics, 1996. 133: p. 47-58.**

34. **Li, R.H., *Protein transport through membranes based on toluene diisocyanate surface-modified poly(vinyl alcohol) gels.* Journal of Membrane Science, 1994(88): p. 115-125.**

35. **Frantz, S., *Instrumentation and Methodology for in vitro Skin Diffusion Cells*, in *Methods for Skin Absorption*, R.W. Kemppainen B, Editor. 1990, CRC Press: Boca Raton, FL. p. 35-59.**

36. **Bosman, I.J., *A novel diffusion cell for in vitro transdermal permeation compatible with automated dynamic sampling*, in *Journal of Pharmaceutical and Biomedical Analysis*. 1996, University of Groningen. p. Chapter 5.**

37. **Bosman, I.J., Avegaart, S.R., Lawant, A.L., et. al., *Evaluation of a novel diffusion cell for in vitro transdermal permeation: effects of injection height, volume and tempearture.* Journal of Pharmaceutical and Biomedical Analysis, 1998. 17: p. 493-499.**

38. **Addicks, W., et. al.,** ***Topical drug delivery from thin applications: theoretical predictions and experimental results.*** **Pharmaceutical Research, 1990. 7(10): p. 1048-1054.**

39. **Logan Instruments Corp.** ***System-912 Automated Transdermal Sampling System*** **(2003). Transdermal Products. Accessed:September 2006. <http://www.loganinstruments.com/trans-sys912.html>**

40. **Logan Instruments Corp.** ***System-902 Automated Franz Diffusion Cell HPLC System*** **(2003). Trandermal Products. Accessed:September 2006. <http://www.loganinstruments.com/trans-sys902.html>**

41. **PermeGear, Inc.** ***Franz Cells - Answers to Frequently Asked Questions*** **(2005). Accessed:September 2006. <http://www.permegear.com/franzatfaqs.htm>**

42. **PermeGear, Inc.** ***H3 Stirrer with Cells and Cell Clamps*** **(2005). Accessed:September 2006. <http://www.permegear.com/h3.htm>**

43. **Warner Instruments.** ***U2500 Dual-Channel Self Contained Chamber*** **(2006). Epithelial Research. Accessed:September 2006. <http://www.warneronline.com/product_info.cfm?id=970&name=U2500%20Dual-Channel%20Self%20Contained%20Chamber>**

44. **Warner Instruments.** ***U9500 Classic Ussing System*** **(2006). Epithelial Research. Accessed:September 2006. <http://www.warneronline.com/product_info.cfm?name=U9500%20Classic%20Ussing%20System&id=88>**

45. **Harvard Apparatus.** ***EasyMount Vertical Diffusion/Ussing Chamber Systems*** **(2006). Tissue/Cell Culture. Accessed:September 2006. <https://www.harvardapparatus.com/wcsstore/ConsumerDirect/images/site/hai/techdocs/BS4_M_183.pdf>**

46. **United States Plastic Organization.** ***Acryllics*** **(2006). Accessed:October 2006. <www.usplastic.com>**

47. **PermeGear.** ***Franz Cell*** **(2005). PermeGear, Inc. Accessed:August 30, 2006. <http://www.permegear.com/franz.htm >**

48. **Warner Instruments.** ***Warner Online*** **(2006). Accessed:October 2006. <http://warneronline.com/>**

49. **Ambati, J., et. al. ,** ***Diffusion of High Molecular Weight Compounds Through Sclera.*** **Investigative Ophthalmology and Visual Science, 2000. 41: p. 1181-1185.**

50. ***Polystyrene Cuvette*** **(2007). BrandTech Scientific, Inc. <http://www.brandtech.com >**

51. ***ValuePlastics*** **(2006). <www.valueplastics.com>**

52. **Sigma-Aldrich*. Glucose HK Assay* (2006). Sigma-Aldrich Product Catalog: Glucose (HK) Assay Kit Technical Bulletin. Accessed:October 2006. <http://www.sigmaaldrich.com/sigma/bulletin/gahk20bul.pdf>**

53. **Elsdale, T., et al, *Collagen substrata for studies on cell behavior.* Journal of Cell Biology, 1972. 54: p. 626-637.**

54. **McKegney, M., et al, *The influence of crosslinking agents and diamines in the pore size, morphology and the biological stability of collagen sponges and their effect on cells penetration through the sponge matrix.* J Mater Sci: Mater Med, 2001. 23: p. 833-844.**

55. **Chen, D., et al, *Osteoblastic response to collagen scaffolds varied in freezing temperature and glutaraldehyde crosslinking.* .Journal of Biomedical Materials Research, 2006. 80A(2): p. 399-409.**

Appendix A – Original Client Statement

Designing a High-Throughput Device to Characterize Molecular Transport through Collagen Membranes *(GXP-0702)*

Date: August 28, 2006

Client Statement:
Design, develop and validate a high-throughput device to measure molecular transport rates through self-assembled collagen membranes.

Background

Characterizing molecular transport through membranes with precisely designed porosities (e.g. pore size, density, thickness) is essential for the development of scaffolds that promote tissue regeneration. Membrane porosity and molecular transport are important to provide space and surface area for cell seeding before implantation. Controlled transport also plays an important role in delivery of nutrients and removal of waste, while maintaining a separation barrier similar to that of a basal lamina in native tissue. Finally, a bioengineered membrane with controlled transport properties will provide significant utility for the design of tissue scaffolds that deliver therapeutic proteins and/or growth factors. Consequently, there is a need to develop a device and a testing protocol that will facilitate systematic studies that characterize the relationships between the materials properties and the molecular transport properties of collagen membranes.

The goal of this project is to design, develop and validate a testing system to conduct biological transport analyses through collagen membranes. Students will use CAD design to construct the device, and biomaterials processing techniques to fabricate the scaffolds. Students will also use biochemical analyses to measure and transport through to the materials. This project has application to biomaterials, drug delivery, tissue engineering and regenerative medicine.

Research/Design Considerations:

- Rationale for a specific method of developing a testing system
  - Client needs
  - Manufacturing methods
  - Advantages/Limitations
- Biological transport calculations associated with methods for characterizing transport through the collagen membranes
- Biochemical analyses of membrane transport
  - What ECM molecules/peptides are important to study?
  - What experimental/practical parameters must be considered when selecting these materials?
  - What is the most efficient method for methods for measuring transport through the membrane?

What techniques can be developed to facilitate "high-throughput" screening of the system?
What statistical analyses should be considered to analyze this system?

Deliverables:

General

- Quarterly: MQP reports following BME MQP format
- Quarterly: Laboratory notebook for team (submit with report)
- Weekly progress reports and presentations
- Participation in BME Project Presentation Day (including abstracts and resumes)

Minimum Milestones for Quarterly Deliverables

1st term

- Introduction – background and significance
- Design – revised client statement, clear objectives, weighted
- Gantt Chart for remainder of project
    - Timeline, deadlines
    - Breakdown of responsibility (personal and group)
    - Types of modeling/testing/analysis

2nd term

- Reduce design to practice
- Experimental design
- Development of analysis methods
- Detailed report of proposed testing methods

3rd term/4th term

- Develop prototype device
- Develop method to measure transport through collagen membranes
- Biochemical analyses of molecular transport in response to precisely designed membranes
- Recommendations for future developments of the scaffolds

Expectation of work

- 17 hrs/week per student x 4 = 68 hrs/wk
- Work as team
- Individual responsibilities
- Grading policy

  A. exceed stated objectives and advisors expectations (take control of project)
  B. complete stated objectives and meet advisors expectations (follow advisors advice well)
  C. complete stated objectives below advisors expectations
  NR. do not complete stated objectives
  The advisors reserve the right to grade the group as a team or to grade each student individually as appropriate.

Budget

- $156-*/person reimbursed by BME Department at the end of the project (additional funds may be available)

Website

- Set up a website for the MQP team on myWPI

Academic Dishonesty and Plagiarism

In your *Campus Planner and Resource Guide* (p. 91-92 or www.wpi.edu/Pubs/Policies/Judicial/sect5.html) there is a statement regarding Academic Honesty. You should read and understand this policy and the implications for any violation of that policy. Understand that all of the work you present as your own, must be your own, and not the work of anyone else. Use of reference materials from research literature and Websites MUST be cited and referenced appropriately.

We trust that each of you will exhibit academic honesty and you will not tolerate dishonesty. If we discover obvious violations of the Academic Honesty policy, our response will be a grade of zero for that assignment. Additionally, **any occurrences of academic dishonesty will be reported to the Department Chair and the Dean of Student Life, as required by WPI.**

Department of Biomedical Engineering Major Qualifying Project Syllabus – 2006/2007

This document outlines the requirements for completing a Major Qualifying Project (MQP) in the Department of Biomedical Engineering (BME) at WPI. The full requirements can be found on the BME Department's website on the Student Resources page (http://www.wpi.edu/Academics/Depts/BME/Resources/project.html). There is also a tutorial for writing your MQP report, also on the Student Resources page, under "MQP Report Information Packet". Taken together these documents allow you to plan ahead for your project deadlines.

Briefly, the requirements for completing an MQP are:

1) Keep a notebook (one per project group) of all design work used in your project. This notebook is to be turned in to the BME office at the conclusion of your project.

2) Write a report that documents your MQP using the format outlined in the MQP report tutorial.

3) Present your project at Project Presentation Day, either at the BME event or in the department of your major (WPI) advisor. You are required to stay for the entire event unless your group is presenting in another department as well.

Important Dates for 2006/07 MQP's:

- February 5, 2007: MQP Titles submitted to the BME department
- March 20, 2007: Resumes (One page, no set format) submitted to the BME department
- March 27, 2007: Abstracts (Two page, IEEE format) submitted to the BME department
- March 27, 2007: **(optional)** Digital photographs of your project submitted to the BME department
- April 17, 2007: Project Presentation Day
- April 26, 2007, CDR deadline (**IMPORTANT NOTE**: I will NOT sign CDR forms without verification from Lisa Wall, Lab Manager that the Team's benchspace in the MQP Lab has been cleaned-up).

Appendix B – Meeting with Katie Bush

Date: September 18, 2006
Time: 2:00 pm All group members present
Scribe: Rachael Buteau

Client meeting with Katie Bush

Katie first described some of the key components of the device. She discussed the development of the collagen membranes and gave us a range of membrane thickness between 50 to 200 microns. These membranes were described as being a "snot like" material that could withstand various pressures, such as binder clips used during the drying process. The membranes were usually made around a mesh Nytex material as well to help in the handling process. The membranes sometimes have topographical features on them, using a negative replicate method; however, our studies would just be focusing on flat membranes. For testing, these membranes would first be placed in a phosphate buffer solution (PBS) that did not contain any type of cations to hydrate.

Katie also described the chamber system to us. She requested that the donor and receiver chamber remain the same size and should hold 3-5 mL. These chambers would need to be this size due to the fact that various assays required samples of approximately 100 µl to be removed during testing. These chambers would also need to be non-protein binding as well and allow pipette tips to be placed in and out of the chambers for sampling. Katie's current device, PermeGear Horizontal Diffusion Cell, would be demonstrated to the group at a later time.

The research that was currently going on in the lab was also discussed. Katie is working to fabricate collagen with topographical features that are similar to the natural skin. This topographical feature represents the basal lamina of the natural skin. Keratinoctyes would be seeded onto this to allow for proliferation. Below this topographical collagen would be a dermal sponge. This sponge would need to contain fibroblasts due to the fact that is hypothesized that fibroblasts send signals to keratinocytes in order for proliferation to occur. In order to understand and characterize this process, it is necessary to understand diffusion through collagen. Currently, EGF, KGF and interlukin-1 are thought to help in this process. With this information, Katie suggested that we look more into the assays in order to see what molecules could be used within our system along with the proper way to validate the system. Both the Bradford and BCA protein assays were suggested as a starting point. By looking at these assays, we could also decide a specific pore size necessary for the collagen membranes we would be testing.

A secondary meeting would be held September 20, 2006 to see the PermeGear demonstration.

Meeting adjourned 3 pm.

Appendix C – Glossary of Terms for the PCC

Tier 1

Effective – able to be developed to use minimal materials (small amounts of membranes and molecules) while being able to use a range of different materials as well (different types of membranes, different molecules); the device must effectively measure molecular transport rate of various molecules through a variety of membranes.

Durable – can withstand multiple uses, cleanings, and transport; can be maintained throughout the usual wear and tear of device usage

Safe- can in no way harm the user while using the device for testing; for example, no sharp edges

Easily used – is able to be assembled, tested and cleaned by the user in a simple, straight-forward manner without complicated steps that one would need training for

Practical to make – device needs to be realistic in terms of cost, material(s) and time to be designed, developed and validated

Tier 2

Versatile – device is able to use multiple types of molecules and membranes

Efficient – device uses minimal amount of materials to obtain quality results

Organic resistant – can withstand the use of organic materials (will not degrade when used with organic solvents)

Sterilizable – able to be sterilized, not just cleaned

No leakage – device will not leak when chambers are filled and membrane is placed in between and clamped; device will be sealed to prevent loss of solution which may alter testing; and also sealed from outside environment

Non-porous – material(s) of device will not contain any type of pores that may absorb solution used in diffusion

Long-term – can withstand use over an extended period of time (undefined quantitatively)

Acid/Base resistant – material is able to be used with acid and base solutions for testing or cleaning and not affect the material(s) or its performance

Easily to clean – can simply take pieces apart and clean thoroughly

Portable – can be moved within the lab and to other labs easily

User-independent – the device needs to be user-friendly enabling it to have any user who will understand how to operate tests and obtain samples/results from it

Easily assembled – pieces must be simple and put together by user without a struggle to utilize tested materials and membranes; the device will hold together sturdily

Bench-top fit – device must be able to be placed on lab bench and take up minimal space for convenience

Affordable – the device must remain within a price range that the client considers lower cost in comparison to other devices on the market

Simple design – design of device must be something that designers can actually develop and implement

Tier 3

Controls mixing – device must have ability for the user to define the mixing rate to provide homogeneous solutions in the donor and receiver chambers

Controls temperature – device have the ability for the user to control the temperature of testing conditions

High-throughput – device must be able to run multiple tests at the same time with multiple molecules

Compatible cell co-culture – device must be made of material in which cells can be cultured in as well

Conservative use of materials – amount of materials used, including membranes and molecules, is minimal but still enough to perform testing

Ethanol resistant – device material(s) not degraded by the use of ethanol for cleaning purposes

Gamma resistant – the quality of device material(s) are not affected by gamma radiation

Autoclavable – the device material(s) can withstand temperatures for sterilization in an autoclave

Sterilizable – the device material(s) can withstand sterilization techniques done over an extended amount of time (not yet quantitatively defined)

Acid-cleaned – the device material(s) can maintain quality with the use of acids to clean

Soap and hot water – the areas of the device can be accessed to clean thoroughly with soap and hot water

Tier 4

Spectrophotometry – the system will be large enough to allow samples (~1 ml) and the device won't compromise the liquid's composition that would alter a spectrophotometry test

ELISA – the system won't excrete, absorb, alter, or denature proteins that would interfere with an ELISA test; device must also allow for several samples

HPLC – the system won't alter, absorb, or excrete chemicals of any kind that might interfere with HPLC testing

Range of Membrane Geometry - device can hold membrane that vary in thickness and general shape

Various Molecules – the device can transport multiple molecules as well as some simultaneously

Accuracy – the device will produce results that are repeatable

Precision – the device will perform compatibly with other devices

Sterilizable – the device can be cleaned by sterilization techniques

Biocompatible – the device will not adversely affect biological components added to the system; the device may provide favorable conditions for the biological components added

Small area membrane – tested materials that will be conservatively used

Least amount of molecules – tested materials that will be conservatively used

Appendix D – Final Pair-wise Comparison Charts

Katie Bush – weighted 100%

Tier 1						
OBJECTIVES	Effective	Durable	Safe	Easily Used	Practical to Make	Total
Effective		0.5	1	0.5	1	3.0
Durable	0.5		0.5	0.5	1	2.5
Safe	0	0.5		0.5	0.5	1.5
Easily used	0.5	0.5	0.5		1	2.5
Practical to Make	0	0	0.5	0		0.5

Tier 2			
Objectives for Effective	Versatile	Efficient	Total
Versatile		1	1.0
Efficient	0		0

Tier 2							
Objectives for Durable	Chemical Resistant	Sterilizable	No leakage	Non-porous	Long term	Acid/Base Resistant	Total
Chemical Resistant		0.5	0	0	0	0	0.5
Sterilizable	0.5		0	0.5	0	0.5	1.5
No leakage	1	1		0.5	0.5	0.5	3.5
Non-porous	1	0.5	0.5		0.5	0.5	3.0
Long term	1	1	0.5	0.5		0.5	3.5
Acid/Base Resistant	1	0.5	0.5	0.5	0.5		3.0

Tier 2						
Objectives for Easily Used	Easy to clean	Portable	User-independent	Easily assembled	Benchtop fit	Total
Easy to clean		1	1	0.5	0.5	3.0
Portable	0		1	0.5	0.5	2.0
User-independent	0	0		0	0	0
Easily assembled	0.5	0.5	1		1	3.0
Benchtop fit	0.5	0.5	1	0		2.0

Tier 2			
Objectives for Practical to Make	Affordable	Simple Design	Total
Affordable		1	1.0
Simple Design	0		0

Tier 3			
Objectives for Versatile	Uses Various Membranes	Various Assays	Total
Uses Various Membranes		0	0
Various Assays	1		1.0

Tier 3						
Objectives for Efficient	Controls Mixing	Controls Temperature	High-throughput	Compatible cell coculture	Conservative Use of Materials	Total
Controls Mixing		0.5	0.5	1	0.5	2.5
Controls Temperature	0.5		0.5	1	0.5	2.5
High-throughput	0.5	0.5		1	0.5	2.5
Compatible cell coculture	0	0	0		0	0
Conservative Use of Materials	0.5	0.5	0.5	1		2.5

Tier 3				
Objectives for Sterilizable	Ethanol resistant	Gamma resistant	Autoclavable	Total
Ethanol resistant		1	1	2.0
Gamma resistant	0		0	0
Autoclavable	0	1		1.0

Tier 3				
Objectives for Cleaned	Sterilizable	Acid-cleaned	Soap and hot water	Total
Sterilizable		0.5	0.5	1.0
Acid-cleaned	0.5		0.5	1.0
Soap and hot water	0.5	0.5		1.0

Tier 4				
Objectives for Various Assays	Spectrophotometry	ELISA	HPLC	Total
Spectrophotometry		1	1	2.0
ELISA	0		0	0
HPLC	0	0		0

Tier 4			
Objectives for Uses Various Materials	Range of Membrane Geometry	Various Molecules	Total
Range of Membrane Geometry		0	0
Various Molecules	1		1.0

Tier 4			
Objectives for Various High throughput	Accuracy	Precision	Total
Accuracy		0.5	0.5
Precision	0.5		0.5

Tier 4			
Objectives for Compatible with Cell coculture	Sterilizable	Biocompatible	Total
Sterilizable		0.5	0.5
Biocompatible	0.5		0.5

Tier 4			
Objectives for Conservative Use of Materials	Small area membrane	Least amount of molecules	Total
Small area membrane		0.5	0.5
Least amount of molecules	0.5		0.5

Rachael Buteau – weighted 25% (75% MQP team)

Tier 1							
OBJECTIVES	Effective	Durable	Safe	Easily Used	Practical to Make	Total	Weighted Total
Effective		1	1	1	1	4.0	1.0
Durable	0		1	0	0	1.0.	0.25
Safe	0	0		1	0	1.0	0.25
Easily used	0	1	0		0.5	1.5	0.375
Practical to Make	0	1	1	0.5		2.5	0.675

Tier 2				
Objectives for Effective	Versatile	Efficient	Total	Weighted Total
Versatile		1	1.0	0.25
Efficient	0		0	0

Tier 2								
Objectives for Durable	Chemical Resistant	Sterilizable	No leakage	Non-porous	Long term	Acid/Base Resistant	Total	Weighted Total
Chemical Resistant		0.5	0	0	0	0	0.5	0.125
Sterilizable	0.5		0	0	0	0.5	1.0	0.25
No leakage	1	1		0	1	1	4.0	1.0
Non-porous	1	1	1		1	1	5.0	1.25
Long term	1	1	0	0		1	3.0	0.75
Acid/Base Resistant	1	0.5	0	0	0		1.5	0.375

Tier 2							
Objectives for Easily Used	Easy to clean	Portable	User-independent	Easily assembled	Benchtop fit	Total	Weighted Total
Easy to clean		1	0	0.5	0	1.5	0.375
Portable	0		0	0.5	0.5	1.0	0.25
User-independent	1	1		1	1	4.0	1.125
Easily assembled	0.5	0.5	0		0	1.0	0.25
Benchtop fit	1	0.5	0	1		2.5	0.675

Tier 2				
Objectives for Practical to Make	Affordable	Simple Design	Total	Weighted Total
Affordable		0.5	0.5	0.125
Simple Design	0.5		0.5	0.125

Tier 3				
Objectives for Versatile	Uses Various Membranes	Various Assays	Total	Weighted Total
Uses Various Membranes		0.5	0.5	0.125
Various Assays	0.5		0.5	0.125

Tier 3							
Objectives for Efficient	Controls Mixing	Controls Temperature	High-throughput	Compatible cell coculture	Conservative Use of Materials	Total	Weighted Total
Controls Mixing		0.5	1	1	0.5	3.0	0.75
Controls Temperature	0.5		1	1	0.5	3.0	0.75
High-throughput	0	0		1	0.5	1.5	0.375
Compatible cell coculture	0	0	0		1	1.0	0.25
Conservative Use of Materials	0.5	0.5	0.5	0		1.5	0.375

Tier 3					
Objectives for Sterilizable	Ethanol resistant	Gamma resistant	Autoclavable	Total	Weighted Total
Ethanol resistant		1	1	2.0	0.5
Gamma resistant	0		0.5	0.5	0.125
Autoclavable	0	0.5		0.5	0.125

Tier 3					
Objectives for Cleaned	Sterilizable	Acid-cleaned	Soap and hot water	Total	Weighted Total
Sterilizable		0.5	1	1.5	0.375
Acid-cleaned	0.5		0.5	0.5	0.125
Soap and hot water	0	0.5		0.5	0.125

Tier 4					
Objectives for Various Assays	Spectrophotometry	ELISA	HPLC	Total	Weighted Total
Spectrophotometry		1	1	2.0	0.5
ELISA	0		0.5	0.5	0.125
HPLC	0	0.5		0.5	0.125

Tier 4				
Objectives for Uses Various Materials	Range of Membrane Geometry	Various Molecules	Total	Weighted Total
Range of Membrane Geometry		0.5	0.5	0.125
Various Molecules	0.5		0.5	0.125

Tier 4				
Objectives for Various High throughput	Accuracy	Precision	Total	Weighted Total
Accuracy		0.5	0.5	0.125
Precision	0.5		0.5	0.125

Tier 4				
Objectives for Compatible with Cell coculture	Sterilizable	Biocompatible	Total	Weighted Total
Sterilizable		1	1	0.25
Biocompatible	0		0	0

Tier 4				
Objectives for Conservative Use of Materials	Small area membrane	Least amount of molecules	Total	Weighted Total
Small area membrane		0	0	0
Least amount of molecules	1		1	0.25

Thomas Jenket -- weighted 25% (75% MQP team)

Tier 1

OBJECTIVES	Effective	Durable	Safe	Easily Used	Practical to Make	Total	Weighted Total
Effective		0.5	1	0.5	0	2.0	0.5
Durable	0.5		1	0.5	0.5	2.5	0.675
Safe	0	0		0.5	0	0.5	0.125
Easily used	0.5	0.5	0.5		0.5	2.0	0.5
Practical to Make	1	0.5	1	0.5		3.0	0.75

Tier 2

Objectives for Effective	Versatile	Efficient	Total	Weighted Total
Versatile		0.5	0.5	0.125
Efficient	0.5		0.5	0.125

Tier 2

Objectives for Durable	Chemical Resistant	Sterilizable	No leakage	Non-porous	Long term	Acid/Base Resistant	Total	Weighted Total
Chemical Resistant		1	0	0	0.5	0.5	2.0	0.5
Sterilizable	0		0	0.5	0	0.5	1.0	0.25
No leakage	1	1		1	1	1	5.0	1.25
Non-porous	1	0.5	0		0.5	1	3.0	0.75
Long term	0.5	1	0	0.5		1	3.0	0.75
Acid/Base Resistant	0.5	0.5	0	0	0		1.0	0.25

Tier 2

Objectives for Easily Used	Easy to clean	Portable	User-independent	Easily assembled	Benchtop fit	Total	Weighted Total
Easy to clean		1	0.5	0.5	0.5	2.5	0.675
Portable	0		0	0.5	0.5	1.0	0.25
User-independent	0.5	1		0	0.5	2.0	0.5
Easily assembled	0.5	0.5	1		1	3.0	0.875
Benchtop fit	0.5	0.5	0.5	0		1.5	0.375

Tier 2

Objectives for Practical to Make	Affordable	Simple Design	Total	Weighted Total
Affordable		0.5	0.5	0.125
Simple Design	0.5		0.5	0.125

Tier 3				
Objectives for Versatile	Uses Various Membranes	Various Assays	Total	Weighted Total
Uses Various Membranes		0	0	0
Various Assays	1		1.0	0.25

Tier 3							
Objectives for Efficient	Controls Mixing	Controls Temperature	High-throughput	Compatible cell coculture	Conservative Use of Materials	Total	Weighted Total
Controls Mixing		0.5	0.5	1	0	2.0	0.5
Controls Temperature	0.5		0.5	1	1	3.0	0.375
High-throughput	0.5	0.5		1	0.5	2.5	0.675
Compatible cell coculture	0	0	0		0	0	0
Conservative Use of Materials	1	0	0.5	1		2.5	0.675

Tier 3					
Objectives for Sterilizable	Ethanol resistant	Gamma resistant	Autoclavable	Total	Weighted Total
Ethanol resistant		1	1	2.0	0.5
Gamma resistant	0		0	0	0
Autoclavable	0	1		1.0	0.25

Tier 3					
Objectives for Cleaned	Sterilizable	Acid-cleaned	Soap and hot water	Total	Weighted Total
Sterilizable		1	0	1.0	0.25
Acid-cleaned	0		0	0	0
Soap and hot water	1	1		2.0	0.5

Tier 4					
Objectives for Various Assays	Spectrophotometry	ELISA	HPLC	Total	Weighted Total
Spectrophotometry		1	1	2.0	0.5
ELISA	0		1	1.0	0.25
HPLC	0	0		0	0

Tier 4				
Objectives for Uses Various Materials	Range of Membrane Geometry	Various Molecules	Total	Weighted Total
Range of Membrane Geometry		0.5	0.5	0.125
Various Molecules	0.5		0.5	0.125

Tier 4				
Objectives for Various High throughput	Accuracy	Precision	Total	Weighted Total
Accuracy		0.5	0.5	0.125
Precision	0.5		0.5	0.125

Tier 4				
Objectives for Compatible with Cell coculture	Sterilizable	Biocompatible	Total	Weighted Total
Sterilizable		0.5	0.5	0.125
Biocompatible	0.5		0.5	0.125

Tier 4				
Objectives for Conservative Use of Materials	Small area membrane	Least amount of molecules	Total	Weighted Total
Small area membrane		0	0	0
Least amount of molecules	1		1.0	0.25

Christina Mezzone -- weighted 25% (75% MQP team)

Tier 1							
OBJECTIVES	Effective	Durable	Safe	Easily Used	Practical to Make	Total	Weighted Total
Effective		1	1	1	1	4.0	1.0
Durable	0		0	1	1	2.0	0.5
Safe	0	1		1	1	3.0	0.75
Easily used	0	0	0		1	1.0	0.25
Practical to Make	0	0	0	0		0	0

Tier 2				
Objectives for Effective	Versatile	Efficient	Total	Weighted Total
Versatile		0.5	0.5	0.125
Efficient	0.5		0.5	0.125

Tier 2								
Objectives for Durable	Chemical Resistant	Sterilizable	No leakage	Non-porous	Long term	Acid/Base Resistant	Total	Weighted Total
Chemical Resistant		1	0	0	0	1	2.0	0.5
Sterilizable	0		0	0	0	1	1.0	0.25
No leakage	1	1		0.5	1	1	4.5	1.125
Non-porous	1	1	0.5		1	1	4.5	1.125
Long term	1	1	0	0		1	3.0	0.75
Acid/Base Resistant	0	0	0	0	0		0	0

Tier 2							
Objectives for Easily Used	Easy to clean	Portable	User-independent	Easily assembled	Benchtop fit	Total	Weighted Total
Easy to clean		0	0.5	0	0	0.5	0.125
Portable	1		0	0	.5	1.5	0.375
User-independent	0.5	1		1	1	3.5	0.875
Easily assembled	1	1	0		.5	2.5	0.675
Benchtop fit	1	0.5	0	0.5		2.0	0.5

Tier 2				
Objectives for Practical to Make	Affordable	Simple Design	Total	Weighted Total
Affordable		0.5	0.5	0.125
Simple Design	0.5		0.5	0.125

Tier 3				
Objectives for Versatile	Uses Various Membranes	Various Assays	Total	Weighted Total
Uses Various Membranes		0 .5	0.5	0.125
Various Assays	0.5		0.5	0.125

Tier 3							
Objectives for Efficient	Controls Mixing	Controls Temp	High-throughput	Compatible cell coculture	Conservative Use of Materials	Total	Weighted Total
Controls Mixing		0.5	0.5	1	0.5	2.5	0.675
Controls Temperature	0.5		0.5	1	0.5	2.5	0.675
High-throughput	0.5	0.5		0	1	2.0	0.5
Compatible cell coculture	0	0	1		0	1.0	0.25
Conservative Use of Materials	0.5	0.5	0	1		2.0	0.5

Tier 3					
Objectives for Sterilizable	Ethanol resistant	Gamma resistant	Autoclavable	Total	Weighted Total
Ethanol resistant		1	1	2.0	0.5
Gamma resistant	0		0	0	0
Autoclavable	0	1		1.0	0.25

Tier 3					
Objectives for Cleaned	Sterilizable	Acid-cleaned	Soap and hot water	Total	Weighted Total
Sterilizable		0.5	0.5	1.0	0.25
Acid-cleaned	0.5		0	0.5	0.125
Soap and hot water	0.5	1		1.5	0.375

Tier 4					
Objectives for Various Assays	Spectrophotometry	ELISA	HPLC	Total	Weighted Total
Spectrophotometry		1	1	2.0	0.5
ELISA	0		0	0	0
HPLC	0	1		1.0	0.25

Tier 4				
Objectives for Uses Various Materials	Range of Membrane Geometry	Various Molecules	Total	Weighted Total
Range of Membrane Geometry		0	0	0
Various Molecules	1		1.0	0.125

Tier 4				
Objectives for Various High throughput	Accuracy	Precision	Total	Weighted Total
Accuracy		0.5	0.5	0.125
Precision	0.5		0.5	0.125

Tier 4				
Objectives for Compatible with Cell coculture	Sterilizable	Biocompatible	Total	Weighted Total
Sterilizable		0	0	0
Biocompatible	1		1.0	0.25

Tier 4				
Objectives for Conservative Use of Materials	Small area membrane	Least amount of molecules	Total	Weighted Total
Small area membrane		0.5	0.5	0.125
Least amount of molecules	0.5		0.5	0.125

George D. Pins, Ph.D. – weighted 50%

Tier 1							
OBJECTIVES	Effective	Durable	Safe	Easily Used	Practical to Make	Total	Weighted Total
Effective		1	1	1	1	4.0	2.0
Durable	0		1	1	0.5	2.5	1.25
Safe	0	0		0	0.5	0.5	0.25
Easily used	0	0	1		1	2.0	1.0
Practical to Make	0	0.5	0.5	0		1.0	0.5

Tier 2				
Objectives for Effective	Versatile	Efficient	Total	Weighted Total
Versatile		0	0	0
Efficient	1		1.0	0.5

Tier 2								
Objectives for Durable	Chemical Resistant	Sterilizable	No leakage	Non-porous	Long term	Acid/Base Resistant	Total	Weighted Total
Chemical Resistant		0	0	0	0	0	0	0
Sterilizable	1		0	0	0	0	1.0	0.5
No leakage	1	1		0.5	1	0.5	4.0	2.0
Non-porous	1	1	0.5		1	0.5	4.0	2.0
Long term	1	1	0	0		0	2.0	1.0
Acid/Base Resistant	1	1	0.5	0.5	1		4.0	2.0

Tier 2							
Objectives for Easily Used	Easy to clean	Portable	User-independent	Easily assembled	Benchtop fit	Total	Weighted Total
Easy to clean		1	1	0.5	1	3.5	1.75
Portable	0		0	0	0	0	0
User-independent	0	1		0	1	2.0	1.0
Easily assembled	0.5	1	1		1	3.5	1.75
Benchtop fit	0	1	0	0		1.0	0.5

Tier 2				
Objectives for Practical to Make	Affordable	Simple Design	Total	Weighted Total
Affordable		0.5	0.5	0.25
Simple Design	0.5		0.5	0.25

Tier 3				
Objectives for Versatile	Uses Various Membranes	Various Assays	Total	Weighted Total
Uses Various Membranes		0	0	0
Various Assays	1		1.0	0.5

Tier 3							
Objectives for Efficient	Controls Mixing	Controls Temp	High-throughput	Compatible cell coculture	Conservative Use of Materials	Total	Weighted Total
Controls Mixing		0.5	0.5	1	0.5	2.5	1.25
Controls Temperature	0.5		0.5	1	0.5	2.5	1.25
High-throughput	0.5	0.5		1	0.5	2.5	1.25
Compatible cell coculture	0	0	0		0	0	0
Conservative Use of Materials	0.5	0.5	0.5	1		2.5	1.25

Tier 3					
Objectives for Sterilizable	Ethanol resistant	Gamma resistant	Autoclavable	Total	Weighted Total
Ethanol resistant		1	0	1.0	0.5
Gamma resistant	0		0	0	0
Autoclavable	1	1		2.0	1.0

Tier 3					
Objectives for Cleaned	Sterilizable	Acid-cleaned	Soap and hot water	Total	Weighted Total
Sterilizable		0	0	0	0
Acid-cleaned	1		0.5	1.5	0.75
Soap and hot water	1	0.5		1.5	0.75

Tier 4					
Objectives for Various Assays	Spectrophotometry	ELISA	HPLC	Total	Weighted Total
Spectrophotometry		1	1	2.0	1.0
ELISA	0		1	1.0	0.5
HPLC	0	0		0	0

Tier 4				
Objectives for Uses Various Materials	Range of Membrane Geometry	Various Molecules	Total	Weighted Total
Range of Membrane Geometry		0	0	0
Various Molecules	1		1.0	0.5

Tier 4				
Objectives for Various High throughput	Accuracy	Precision	Total	Weighted Total
Accuracy		0.5	0.5	0.25
Precision	0.5		0.5	0.25

Tier 4				
Objectives for Compatible with Cell coculture	Sterilizable	Biocompatible	Total	Weighted Total
Sterilizable		0	0	0
Biocompatible	1		1.0	0.5

Tier 4				
Objectives for Conservative Use of Materials	Small area membrane	Least amount of molecules	Total	Weighted Total
Small area membrane		0.5	0.5	0.25
Least amount of molecules	0.5		0.5	0.25

Appendix E - Weighted Totals from PCCs for Weighted Objectives Tree

Tier 1	
OBJECTIVES	Total with weights
Effective	7.5
Durable	5.175
Safe	2.875
Easily used	4.625
Practical to Make	2.425
	22.6

Tier 2	
Objectives for Effective	Total with weights
Versatile	1.5
Efficient	0.75
	2.25

Tier 2	
Objectives for Durable	Total with weights
Chemical Resistant	1.625
Sterilizable	2.75
No leakage	8.875
Non-porous	8.125
Long term	6.75
Acid/Base Resistant	5.625
	33.75

Tier 2	
Objectives for Easily Used	Total with weights
Easy to clean	5.925
Portable	2.875
User-independent	3.5
Easily assembled	6.55
Benchtop fit	4.05
	22.9

Tier 2	
Objectives for Practical to Make	Total with weights
Affordable	1.625
Simple Design	0.625
	2.25

Tier 3	
Objectives for Versatile	Total with weights
Uses Various Materials	0.25
Various Assays	2.0
	2.25

Tier 3	
Objectives for Efficient	Total with weights
Controls Mixing	5.675
Controls Temperature	5.55
High-throughput	5.3
Compatible cell coculture	0.5
Conservative Use of Materials	5.3
	22.325

Tier 3	
Objectives for Sterilizable	Total with weights
Ethanol resistant	4.0
Gamma resistant	0.125
Autoclavable	2.625
	6.75

Tier 3	
Objectives for Cleaned	Total with weights
Sterilizable	1.875
Acid-cleaned	2.0
Soap and hot water	2.75
	6.625

Tier 4	
Objectives for Various Assays	Total with weights
Spectrophotometry	4.5
ELISA	0.875
HPLC	0.375
	5.75

Tier 4	
Objectives for Uses Various Materials	Total with weights
Range of Membrane Geometry	0.25
Various Molecules	1.875
	2.125

Tier 4	
Objectives for Various High throughput	Total with weights
Accuracy	1.125
Precision	1.125
	2.25

Tier 4	
Objectives for Compatible with Cell coculture	Total with weights
Sterilizable	0.875
Biocompatible	1.375
	2.25

Tier 4	
Objectives for Conservative Use of Materials	Total
Small area membrane	0.875
Least amount of molecules	1.375
	2.25

Appendix F – Molecules Chart

All information found on Sigma Aldrich and Pierce websites

Name	Mol Wt	Radius (nm)
Mannitol	182.2	0.40
Testosterone	288.43	
Sodium fluorescein	376 D	0.50
Glucose	180 D	0.37
Human albumin	67 KD	3.55
FITC-D 4KD	4400 D	1.30
FITC-D 20 KD	19600 D	3.20
FITC- D 40 KD	28,900	4.50
FITC-BSA	67,000	3.62
Rhodamine D, 70 KD	70,000	6.40
FITC-D 70 KD	71,200	6.40
FITC-IgG	150,000	5.23
FITC-D, 150 KD	150,000	8.25
acetylsalicylic acid	180.16	
benzoic acid	122.12	
caffeine	194.19	
chloramphenicol	323.13	
dinitrochlorobenzene	202.55	
hippuric acid	179.18	
nicotinic acid	123.11	
phenol	94.11	
salicyclic acid	138.12	
thiourea	76.12	
urea	60.06	
USP alcohol		
isopropyl myristate		
Hydrocortisone	362.47	
tamoxifen	371.51	
BHCl		
PHCl		
Fluorescein	300 Da	0.86
polypeptide	1200	2.40
lactalbumin	14500	3.80
ovalbumin	45000	5.60
FC fragement	50000	5.80
BSA	50000	6.00
f(ab)2 fragment	68000	7.20
IgA	100000	9.40
IgG*	150000	11.00
IgM	970000	15.00
S-IgA		28.00
Vitamin B 12	1300	
Cytochrome C	13400	

Appendix G– Metrics for Rankings

EFFECTIVE

Versatile

Objective: Compatible to various assays
Units: Ranking the versatility on a scale of 1 (meets none) to 3 (meets all)
Metric: Measure the versatility of the device by seeing how many types of assays can be carried out using the device out of the assays specified by our objectives. On a scale of 1 to 3, assign the following ratings: 1 – can be used for one, 2 – can be used for two, 3 – can be used for all three assays

Objective: Uses various materials
Units: Ranking the variety of materials able to be used on a scale of 1 (meets none) to 3 (meets all)
Metric: Measure the variety of materials able to be used by deciding if the device can hold various sized membranes and use a variety of molecules. On a scale of 1 to 3, assign the following ratings: 1 – Can not hold various membrane sizes or use a variety of molecules, 2 – may be able to hold various membrane sizes or use various molecules, 3 – can hold various membranes and various molecules.

Efficient

Objective: High throughput
Units: Ranking the ability to place the device in a high-throughput system on a scale of 1 to 4
Metric: Measure the ability to be a high-throughput device based on number of actual devices used in a system. One a scale of 1 to 4, assign the following ratings: 1 – 6 devices, 2 – 12 devices, 3 – 18 devices, 4 – 24 devices

Objective: Controls temperature
Units: Ranking the devices ability to control temperature on a scale of 1 (can not) to 3 (can)
Metric: On a scale of 1 to 3, assign the following ratings to temperature control: 1 – can not control temperature, 2 –variable temperature between 25-40 C, 3 – maintains constant temperature between 25-40 C

** For the specific function of controlling temperature, more variables within the objective needed to be taken into account. A binary system (0,1) was used to assign weather the device was able to meet specifics within the objective. See Below:

> **Sub-Objective**: Even distribution of heat from all sides
> **Metrics**: Assign the following ratings: 0 – means can not evenly distribute heat from all sides, 1 – means can evenly distribute heat from all sides
>
> **Sub-Objective**: No infiltration of water into system
> **Metrics**: Assign the following ratings: 0 – infiltration into the device could occur which could effect testing, 1 – no infiltration will occur into the device

Sub-Objective: Maintains temperature within 5 degrees of setting within 5 minutes
Metrics: Assign the following ratings: 0 – means of temperature control can not maintain the temperature within 5 degrees of setting within 5 minutes, 1 – means of temperature control can maintain the temperature within 5 degrees of setting within 5 minutes

Sub- Objective: Range of temperature can be set between 25-40 C
Metrics: Assign the following ratings: 0 – variable temperatures within the 25-40 C range can not be set, 1 – variable temperatures within the 25-40 C range can be set by the means

** Due to the fact that not all means of mixing were compatible with some means of temperature control, a yes/no system was used to determine which means of each function were compatible to each other. This would be used to determine the ranking of temperature control for the function-means of mixing.

Objective: Controls temperature (specific to mixing)
Units: Ranking the means ability to control temperature on a scale of 1 (Mixing compatible to one type of temperature control) to 4 (mixing compatible to all types of temperature control)
Metrics: On a scale of 1 to 4, assign the following rankings: 1 –mixing compatible to one type of temperature control, 2 – compatible to two types of temperature control, 3 – compatible to three types of temperature control, 4 – compatible to all types of temperature control

Objective: Compatible cell co-culture
Units: Ranking the devices ability to allow cell culture on a scale of 1 (meets none) to 3 (meets all)
Metric: Measure the devices ability to allow for cell culture based on whether it is sterilizable and biocompatibile. On scale of 1 to 3, assign the following ratings: 1 – is neither sterilizable or biocompatible, 2 - can be sterilizable or biocompatible, 3 – is both sterilizable and biocompatible

Objective: Homogeneously mixes
Units: Ranking the ability of the device to mix the chambers of 1 (can not mix) to 3 (homogenously mixes)
Metric: Measure the devices ability to homogenously mix based on amount of time it takes to mix chambers. On a scale of 1 to 3, assign the following ratings: 1 – can not homogenously mix, 2 – takes more than 5 minutes to mix, 3 – takes less than 5 minutes to mix

** For the specific function of homogeneously mixing, different means of mixing could not be easily weighted against the general objective of homogeneously mixes. This objective needed to be broken down for this specific function-means. See below:

Sub-Objective: Even mixing
Units: Ranking the ability of the means to mix the chambers on a scale of 0 (can not) to 1(can always)
Metrics: Assign the following rankings: 0 – means can never evenly mix, 0.5 – means may be able to evenly mix, 1 – means will definitely/always evenly mix

Sub-Objective: Control rate of mixing
Units: Ranking the ability of the means to control the rate of mixing in the chambers on a scale of 0 (can not) to 1(can always)
Metrics: Assign the following rankings: 0 – means can never control mixing, 0.5 – means may be able to control rate of mixing, 1 – means can control rate of mixing

Sub-Objective: Compatibility of mixing device to water
Units: Ranking the ability of the means to be compatible with water on a scale of 0 (can not) to 1(can always)
Metrics: The compatibility of the type of mixing was ranked based on what types of temperature control the mixing was compatible with from previously comparing each temperature control means to each mixing means (ex. the motor was ranked for compatibility using water based on its use with a water jacket, plate and snake system only). Assign the following rankings: 0 – means is not compatible, 0.5 – means may be compatible with water, 1 – means is always compatible with water

** Due to the fact that not all means of temperature control were compatible with some means of mixing, a yes/no system was used to determine which means of each function were compatible to each other. This would be used to determine the ranking of homogeneous mixing for the function of temperature control.

Objective: Homogeneously mixes (specific to temperature control)
Units: Ranking the means ability to homogeneously mixed on a scale of 1 (temperature control compatible to one type of mixing) to 4 (temperature control compatible to all type of mixing)
Metrics: On a scale of 1 to 4, assign the following rankings: 1 - compatible to one type of mixing, 2 – compatible to two types of mixing, 3 – compatible to three types of mixing, 4 – compatible to all types of mixing

Objective: Conservative use of materials
Units: Ranking the conservativeness of the device on a scale of 1 (meets none) to 3 (meets all)
Metric: Measure the ability for the device to conserve materials based on its minimal use of membranes and molecules. Minimal use of membranes is determined by complete membrane placed in system will be in contact with diffusion fluid when used. Minimal use of molecules is determined by amount of fluid in chambers actually necessary to drive diffusion. On a scale of 1 to 3, assign the following rankings: 1 – uses large amount

of molecules and membranes, 2 – uses a minimal amount of molecules or membranes, 3 – uses both minimal amounts of membranes and molecules.

SAFE
Objective: Safe
Units: Ranking the safety on a scale of 1 (worst) to 3 (best)
Metric: Measure the safety of the device by determining if the device contains any sharp edges. Sharp edges are defined as any portion of the device that can cut the user. On a scale of 1 to 3, assign the following ratings: 1 – more than 5 places with sharp edges , 2 – between/= 3 to 5 places with sharp edges, 3 – less than 3 places with sharp edges

DURABLE
Objective: Chemical resistant
Units: Ranking the resistance on a scale of 1 (worst) to 3 (best)
Metric: Measure the resistance of the device by determining if the device materials will be affected by chemicals. On a scale of 1 to 3, assign the following ratings: 1 – device deteriorates/degrades and is no longer usable, 2 – evidence of deterioration/degradation after exposure to chemicals but still usable, 3 – devices functions and materials remain unchanged if chemicals used

Objective: Long term
Units: Ranking the duration of the device on a scale of 1 (worst) to 3 (best)
Metric: Measure the duration of the device based on the number of times it can be used. On a scale of 1 to 3, assign the following ratings: 1 – can be used less than 20 times, 2 – can be used between 20 times to 40 times years, 3 – over 40 times

Objective: No leakage
Units: Ranking the leakage on a scale of 0 (leaks) to 1 (no leaks)
Metric: Assign the following ratings: 0 – device will leak, 1 – device will not leak.

Objective: Non-Porous
Units: Ranking the porosity on a scale of 0 (no) to 1 (yes)
Metric: On a scale of 0 to 1, assign the following: 0 – the device will allow the infiltration of molecules, 1 – it will not allow the infiltration of molecules

Objective: Sterilizable
Units: Ranking the ability to sterilize the device on a scale of 1 (meets none) to 3 (meets all)
Metric: Measure the ability to sterilize the device based on its ability to be sterilized with ethanol, gamma, or autoclaving method. On a scale of 1 to 3, assign the following ratings: 1 – can be sterilized by </= one method, 2 – can be sterilized by more than one method, 3 – can be sterilized by all methods

Objective: Acid-Base Resistant
Units: Ranking the resistance of the device on a scale of 1 (worst) to 3 (best)

Metric: Measure the resistance of the device based on how the device will be effected by the chemicals. On a scale of 1 to 3, assign the following ratings: 1 – device deteriorates/degrades and is no longer usable, 2 – evidence of deterioration/degredation after exposure to chemicals but still usable, 3 – devices functions and materials remain in tact if chemicals used.

PRACTICAL TO MAKE

Objective: Affordable
Units: Ranking the affordability the device on a scale of 0 (expensive) to 1 (inexpensive)
Metric: Measure affordability based on the price of the component of the device in question. Each component will be looked at separately and will be ranked on the specific price range that component may fall in (ex. – mixing – price and categorize each means of mixing On a scale of 0 to 1 assign the following: \

0 – component cost > $100
0.1 – component cost between $90-100
0.2 – component cost between $80-90
0.3 – component cost between $70-80
0.4 – component cost between $60-70
0.5 – component cost between $50-60
0.6 – component cost between $40-50
0.7 – component cost between $30-40
0.8 – component cost between $20-30
0.9 – component cost between $10-20
1 – component cost < $10

Objective: Simple design
Units: Ranking the simplicity the device on a scale of 1 (complex) to 3 (simple)
Metric: Measure simplicity of the device by the number of parts/pieces that are major components of the device. This includes stirrers, chambers, membrane holder and ports. On a scale of 1 to 3 assign the following: 1 – more than 10 pieces, 2 – between 5 and 10 pieces, 3 – less than 5 pieces.

EASILY USED

Objective: User-Independent
Units: Ranking how easy it would be for any user to use the devices on a scale of 1 (multiple interactions) to 3 (few interactions)
Metric: Measure dependency on based on the number of user/device interactions in protocol to reduce human error. On a scale of 1 to 3, assign the following values: 1 – more than 16 interactions, 2 – between 8-16 interactions, 3 – less than 8 interactions

Objective: Portable
Units: Ranking how portable the device is on a scale of 1 (fixed) to 3 (portable)

Metric: Measure portability based on the size of the device. On a scale of 1 to 3 assign the following: 1 – bigger than 4'x4', 2 – size between 2'x2' and 4'x4', 3 – size smaller than 2'x2'.

Objective: Easy to clean
Units: Ranking how easy it is to clean the device on a scale of 1 (hard) to 3 (easy)
Metric: Measure how easy it is to clean based on time to clean the device. On a scale of 1 to 3, assign the following ranking: 1 – more than 30 minutes, 2 –between 15-30 minutes, 3 – less than 15 minutes.

Objective: Easily assembled
Units: Ranking the ease to assemble the device 1 (hard) to 3 (easy)
Metric: Measure ease to assemble the device based on the time needed to assemble device. On a scale of 1 to 3, assign the following rating: 1 – more than 1 hour, 2 – 30 minutes to an hour, 3 – less than 30 minutes.

Appendix H – Justifications of Metrics

User Independent:

User independence is defined as having the testing protocol and the device work as intended regardless of who uses it. This does assume that the user has basic laboratory common sense (i.e. how to use a pipette, etc.). User independence is measured based on the amount of interactions a user has with the device. Lowering the number of interactions decreases the opportunities for error on the user's part, thereby increasing the device's independence.

Compatible to Various Assays:

There are three assays that should be able to be done with this device: ELISA, spectrophotometry, and HPLC. The metric is set up to measure the number of assays the device will be able to accommodate. The more assays that it can accommodate, the more versatile it is. Certain assays need a certain volume of solution in order to be used, this can dictate the size of the chambers needed as well as the sampling ports necessary.

Compatible Cell Co-cultures:

The device would potentially have the ability to host live cells in it. This would mean that the device would have to be sterilizible. This could be done through a chemical means or putting it through an autoclave. The device must also be biocompatible, which means that it should not be made out of toxic material.

Homogenously Mixing:

The device needs to be able to mix the chambers well enough so that no boundary layers at the membrane interface. Homogenously mixing takes care of the boundary layer by making sure that there are no concentration gradients within one single chamber, whether it is a donor or receptor chamber. This is meant to try to mimic the body's environment. The receptor chamber is supposed to impersonate the body, which would redistribute the molecules evenly. This device measures just passive diffusion, so no conductive or cell mediated diffusive forces are taken into account.

Another feature that is desired is to be able to control the rate of the mixing. This is important to ensure that the mixing doesn't compromise the membrane's integrity.

There was also a question of which mixing methods can be combined with which heating mechanisms. For example, a water bath cannot be used when a large stir rod is used for the stirring mechanism because the motor would short circuit when exposed directly to water.

Chemical Resistant:

The chambers must be chemically resistance to different solutions. The chambers cannot show signs of degradation or deterioration due to exposure to solvents or cleaning agents. If the chamber degrades too much then there's a risk of cross contamination from the heating source or leaking from the chamber. This can be determined by its ability to maintain a smooth surface on the face of the device and also by the seal between the connectors and the actual chambers.

Sterilizable:

The device must be able to be sterilized in order to ensure that there are no other factors contributing to the diffusion. The sterilization methods are by ethanol, gamma, or autoclaving.

Simple Design:

This is a common concept for engineers. Engineers strive to keep things in their simplest forms. This metric measure the number of pieces the device actually has. Fewer pieces are desired.

Appendix I – Weighted Function Means

Temperature Control Parameters					
	Even distribution of heat from all sides	No Infiltration	Maintains temp within 5 degrees of setting in 5 minutes	Range of temp 25-40	Total
Water Bath	1	0	1	1	3
Incubator	1	1	0	1	3
Jacket	1	0	1	1	3
Snake	0	1	1	1	3**
Heating Plate	0	1	1	0	2
Ratings: 0-never, .5-maybe, 1-always					
**Due to the fact that many of the various mean of temperature control had the same score, all temp control options had to be compared to mixing options					

Temperature Control vs. Mixing Options					
	Shaker	Motor Mixer	Stir Plate/bars	Bladder	Total
Water Bath	N	N	N	Y	1
Incubator	Y	N	Y	N	2
Jacket	Y	Y	Y	N	3
Plate	Y	Y	N	N	2
Snake	Y	Y	Y	Y	4
TOTAL (# of Y)	4	3	3	2	
The temp control with the most variability was ranked the highest					

Mixing Options vs. Mixing Capabilities				
	Even mixing	Control rate of mixing	Compatible w/ H2O	Total
Shaker	1	1	1	3
Motor	1	0.5	0	1.5
Bladder	0.5	0	1	1.5
Stir plate	0.5	0.5	1	2
Ratings: 0-never, .5-maybe, 1-always				
Multiple components of mixing needed to be taken into the consideration in ranking				

Temperature Control

Design Constraint	Plate	Water Bath	Water Jacket	Incubator	Snake
< $468	N/A	N/A	Y	N/A	Y
Time	N/A	N/A	Y	N/A	Y
Non-protein binding	N/A	N/A	N/A	N/A	N/A

Design Objectives	Weight %	Plate Score	Plate Weighted Score	Water Bath Score	Water Bath Weighted Score	Water Jacket Score	Water Jacket Weighted Score	Incubator Score	Incubator Weighted Score	Snake Score	Snake Weighted Score
High throughput	7.9	1	7.9	3	23.7	4	31.6	4	31.6	4	31.6
Controls Temp *	8.3	2	16.6	3	24.9	3	24.9	3	24.9	3	24.9
Homogenously mixing**	8.5	1	8.5	2	17	3	25.5	2	17	4	34
Safe	12.7	3	38.1	3	38.1	3	38.1	3	38.1	3	38.1
Chemical resistant	1.1	3	3.3	3	3.3	2	2.2	3	3.3	2	2.2
Long term	4.5	3	13.5	2	9	2	9	3	13.5	2	9
No leakage	6	1	6	0	0	0	0	1	6	0	0
Sterilizable	1.9	1	1.9	2	3.8	2	3.8	2	3.8	2	3.8
Acid-base resistant	3.8	3	11.4	3	11.4	2	7.6	3	11.4	2	7.6
Portable	2.6	3	7.8	2	5.2	3	7.8	1	2.6	3	7.8
TOTALS	167.8	21	115	23	136.4	24	150.5	25	152.2	25	159

* Based off of components of the objective rating

** Based off of how many types of mixing each means of temperature control was compatible with

Donor and Receiver Engagement

Design Constraint	Latch	Screw-lock	Draw Latch	Screw/ Clamp	Slide
< $468	Y	Y	Y	Y	Y
Time	Y	Y	Y	Y	Y
Non-protein binding	N/A	N/A	N/A	N/A	N/A

Design Objectives	Weight %	Latch Score	Latch Weighted Score	Screw-lock Score	Screw-lock Weighted Score	Draw Latch Score	Draw Latch Weighted Score	Screw Score	Screw Weighted Score	Slide Score	Slide Weighted Score
Chemical resistant	1.1	2	2.2	3	3.3	2	2.2	2	2.2	2	2.2
Long term	4.5	3	13.5	2	9	3	13.5	2	9	3	13.5
Sterilizable	1.9	3	5.7	2	3.8	3	5.7	3	5.7	3	5.7
Acid-base resistant	3.8	2	7.6	3	11.4	2	7.6	2	7.6	2	7.6
Affordable	7.7	1	7.7	0.9	6.93	1	7.7	0.9	6.93	0.8	6.16
Simple design	3	3	9	2	6	2	6	3	9	2	6
Easy to clean	5.3	2	10.6	2	10.6	1	5.3	3	15.9	1	5.3
Easily assembled	5.9	2	11.8	1	5.9	3	17.7	3	17.7	2	11.8
TOTALS	84.2	18	68.1	15.9	56.93	17	65.7	18.9	74.03	15.8	58.26

Holding Membrane

Design Constraint	O-ring	Cartridge	Press-fit	Multi-shape Slide	Teeth Cartridge
< $468	Y	Y	Y	Y	Y
Time	Y	Y	Y	Y	Y
Non-protein binding	N/A	N/A	N/A	N/A	N/A

Design Objectives	Weight %	O-ring Score	O-ring Weighted Score	Cartridge Score	Cartridge Weighted Score	Press-fit Score	Press-fit Weighted Score	Multi-shape Slide Score	Multi-shape Slide Weighted Score	Teeth Cartridge Score	Teeth Cartridge Weighted Score
Uses various materials	7.4	2	14.8	2	14.8	2	14.8	3	22.2	2	14.8
Conservative use of tested materials	7.9	3	23.7	3	23.7	3	23.7	2	15.8	2	15.8
Chemical resistant	1.1	3	3.3	3	3.3	3	3.3	3	3.3	3	3.3
Long term	4.5	2	9	3	13.5	3	13.5	3	13.5	3	13.5
No leakage	6	3	18	3	18	2	12	3	18	3	18
Sterilizable	1.9	2	3.8	3	5.7	3	5.7	3	5.7	3	5.7
Acid-base resistant	3.8	3	11.4	3	11.4	3	11.4	3	11.4	3	11.4
Affordable	7.7	1	7.7	0.9	6.93	0.9	6.93	0.7	5.39	0.8	6.16
Simple design	3	3	9	2	6	2	6	1	3	1	3
User-independent	3.1	3	9.3	3	9.3	1	3.1	2	6.2	2	6.2
Easy to clean	5.3	3	15.9	3	15.9	3	15.9	3	15.9	2	10.6
Easily assembled	5.9	3	17.7	2	11.8	1	5.9	1	5.9	2	11.8
Non-Porous	5.5	0	0	1	5.5	1	5.5	1	5.5	1	5.5
TOTALS	173.9	31	143.6	31.9	145.83	27.9	127.73	28.7	131.79	27.8	125.76

Mixing

Design Constraint	Shaker plate	Stir bars	Mechanical stirrers	Large stirrers	Large stirrers with wings	Flapper/ Rocking system	Inflatable Bladder
< $468	Y	Y	Y	Y	Y	Y	Y
Time	Y	Y	Y	Y	Y	Y	Y

Design Objectives	Weight %	Shaker plate score	Shaker plate Weighted Score	Stir bars score	Stir bars Weighted Score	Mechanical stirrers Score	Mechanical stirrers Weighted Score	Large stirrers Score	Large stirrers Weighted Score	Large stirrers with wings Score	Large stirrers with wings Weighted Score	Flapper/ Rocking system Score	Flapper/ Rocking system Weighted Score	Inflatable Bladder Score	Inflatable Bladder Weighted Score
High throughput	7.9	4	31.6	4	31.6	4	31.6	4	31.6	4	31.6	4	31.6	3	23.7
Controls temperature *	8.3	3	24.9	2	16.6	2	16.6	2	16.6	2	16.6	3	24.9	1	8.3
Compatible cell co-culture	0.7	3	2.1	2	1.4	2	1.4	2	1.4	2	1.4	2	1.4	3	2.1
Homogenously mixing**	8.5	3	25.5	2	17	2	17	2	17	2	17	2	17	2	17
Chemical resistant	1.1	3	3.3	3	3.3	2	2.2	2	2.2	2	2.2	2	2.2	2	2.2
Long term	4.5	3	13.5	3	13.5	2	9	2	9	2	9	2	9	3	13.5
No leakage	6	3	18	3	18	2	12	2	12	2	12	2	12	2	12
Sterilizable	1.9	2	3.8	3	5.7	3	5.7	3	5.7	3	5.7	3	5.7	2	3.8
Acid-base resistant	3.8	3	11.4	3	11.4	2	7.6	2	7.6	2	7.6	2	7.6	2	7.6
Affordable	7.7	1	7.7	1	7.7	0.8	6.16	0.7	5.39	0.6	4.62	0.9	6.93	0.4	3.08
Simple design	3	3	9	3	9	3	9	2	6	1	3	3	9	3	9
User-independent	3.1	3	9.3	3	9.3	2	6.2	2	6.2	2	6.2	3	9.3	3	9.3
Portable	2.6	3	7.8	3	7.8	3	7.8	3	7.8	3	7.8	3	7.8	2	5.2
Easy to clean	5.3	3	15.9	3	15.9	2	10.6	2	10.6	2	10.6	3	15.9	2	10.6
Easily assembled	5.9	3	17.7	3	17.7	3	17.7	2	11.8	2	11.8	3	17.7	3	17.7
TOTALS	203.4	43	201.5	41	185.9	34.8	160.56	32.7	150.89	31.6	147.12	37.9	178.03	33.4	145.08

* based off # of compatible means of temp control for each type of mixing
** based off sub-objective ranking totals

Multi-Chamber System

Design Constraint	Parallel Vertical	Parallel Horizontal	Series	Series and parallel
< $468	Y	Y	Y	Y
Time	Y	Y	Y	Y

Design Objectives	Weight %	Parallel Vertical Score	Parallel Vertical %	Parallel Horizontal Score	Parallel Horizontal %	Series Score	Series %	Series and parallel Score	Series and parallel %
High throughput	7.9	4	31.6	4	31.6	1	7.9	4	31.6
Compatible cell co-culture	0.7	2	1.4	3	2.1	1	0.7	3	2.1
Conservative use of tested materials	7.9	3	23.7	3	23.7	2	15.8	3	23.7
No leakage	6	3	18	3	18	2	12	3	18
Simple design	3	3	9	3	9	2	6	3	9
User-independent	3.1	2	6.2	2	6.2	2	6.2	2	6.2
Portable	2.6	3	7.8	3	7.8	2	5.2	2	5.2
Easy to clean	5.3	3	15.9	3	15.9	3	15.9	2	10.6
Easily assembled	5.9	3	17.7	3	17.7	2	11.8	2	11.8
TOTALS	135.1	26	131.3	27	132	17	81.5	24	118.2

Materials

Design Constraint	Acryllic	Polycarbonate
< $468	Y	Y
Time	Y	Y

Design Objectives	Weight %	Acryllic Score	Acryllic %	Polycarbonate Score	Polycarbonate %
Compatible to various assays	59.3	3	177.9	3	177.9
Uses various materials	7.4	3	22.2	3	22.2
High throughput	7.9	3	23.7	3	23.7
Controls Temp	8.3	2	16.6	3	24.9
Compatible cell co-culture	0.7	3	2.1	3	2.1
Chemical resistant	1.1	1	1.1	2	2.2
Long term	4.5	2	9	3	13.5
Sterilizable **	1.9	1	1.9	2	3.8
Acid-base resistant	3.8	2	7.6	2	7.6
Affordable	7.7	2	15.4	3	23.1
Easy to clean	5.3	1	5.3	2	10.6
TOTALS	312.3	23	282.8	29	311.6

Sampling port

Design Constraint	Open	Rubber			
< $468	Y	Y			
Time	Y	Y			
Design Objectives	Weight %	Open Score	Open %	Rubber Score	Rubber %
Compatible to various assays	59.3	3	177.9	3	177.9
Simple design	3	3	9	3	9
User-independent	3.1	3	9.3	3	9.3
Portable	2.6	3	7.8	3	7.8
Easy to clean	5.3	3	15.9	2	10.6
Easily assembled	5.9	3	17.7	3	17.7
Non-Porous	5.5	3	16.5	2	11
TOTALS	254.1	21	254.1	19	243.3

Appendix J – Price Information Used in Affordability Ranking

Product	Distributor	Dimensions	Price
O-ring	Allorings.com	ID: 7/8'' OD: 1'' W: 1/16''	50 @ $9.90
3 Blade Propeller	Cole-Parmer	1'' dia x 5/16'' bore diam	$24.00 each
Paddle Propeller	Cole-Parmer	¼'' x 2-5/8'' x 15''	$24.00 each
4 Blade Propeller	Sciencelab.com	50 diam x 350 mm long	$91.00 each
12V Air Pump	Aquatic Eco-systems, inc.	12V 25 W	$87.78 each
2'' Thick Polycarb	http://www.mcmaster.com/	1' x 1'	$188.46
	Plastics Unlimited	1' x 1'	$219.00
	GE Polymershapes	1' x 1'	$235.00
	Precision Punch and Plastics	12'' x 24''	$421.00
	Total Plastics	10'' x 32 ¾''	$379.88
Bessey Adjustable Clamp	McFeelys.com	2.5'' x 12''	$10.43
Adjustable Knuckle Catch	HomeDecorHardward.com		$6.40
Adjustable Catch	Neilsen Sessions	~5'' x 3''	$9.80
Draw Catch	Nielsen Sessions	5.07'' x 2.36''	$3.99
Nylon Snap Joiners	Woodworker's Supply	3/8'' diameter 3/8'' deep	10 @ $6.99
Polypropylene Non-threaded leurs	Home Depot	¼'' OD x 1/8'' ID	$2.47
		3/8'' OD x ¼'' ID	$3.23
Polypropylene Threaded leurs	Home Depot	¼'' OD x 1/8'' ID	$2.59
		3/8'' OD x ¼'' ID	$2.55
		½'' OD x 3/8'' ID	$2.99
		3/8'' OD x 3/8'' ID	$2.80
Clear Vinyl Tubing	Home Depot	OD x ID (inch) x Length	Price
		5/8'' x ½'' x 10'	$3.49
		5/8'' x ½'' x 20'	$5.79
		¾'' x ½'' x 10'	$5.99
		¾'' x 5/8'' x 10'	$4.98
		¾'' x 5/8'' x 20'	$8.99
		3/8'' x ¼'' x 10'	$2.39
		3/8'' x ¼'' x 20'	$3.99
Polyethylene tubing (non clear)	Home Depot	OD x ID (inch) x Length	Price
		½'' x 3/8'' x 25'	$6.49
		3/8'' x ¼'' x 25'	$4.99

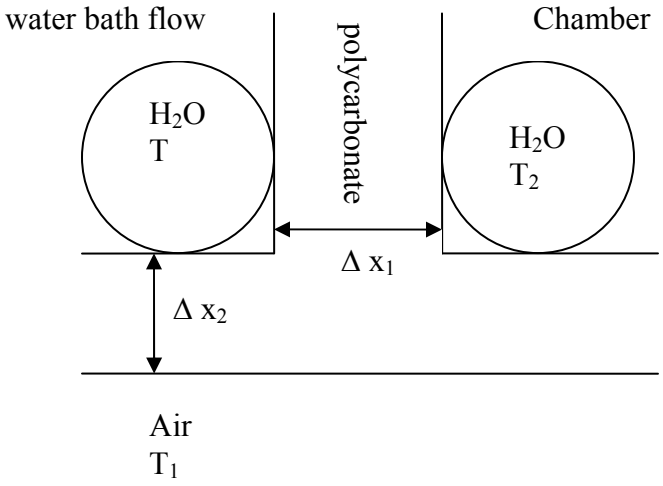
Appendix K – Temperature Calculations for Polycarbonate and Acrylic

November 13, 2006, 11:30am- 12:20pm
Meeting with Brian James Savilonis, Mechanical Engineering Professor at Worcester Polytechnic Institute

Team member present : Rachael Buteau

Objective: To understand the fundamentals of thermal resistance to determine whether machine-grade polycarbonate would be efficient to provide controlled temperature with a flowing water jacket.

System simplified to a one dimensional conduction problem to analyze.



$T_1 = 20$ °C

$T_2 = 30$ °C

$\Delta x_1 = .005$m

$\Delta x_2 = .015$m

k (machine grade polycarbonate) = 0.2 W/ (m*°C)
k (acrylic) = 1.4 (m*°C)

Qdot = ΔT/ R and R = Δx/ k → k= Thermal Conductivity - ASTM C177 Standard

$(T_2 - T_1)/ (R_2) = (T-T_2)/ (R_1)$

Solve for T. With k (machine grade polycarbonate)
$R_1 = .005/ 0.2 = .025$ m^2* °C/ W
$R_2 = .015/ 0.2 = .075$ m^2* °C/ W

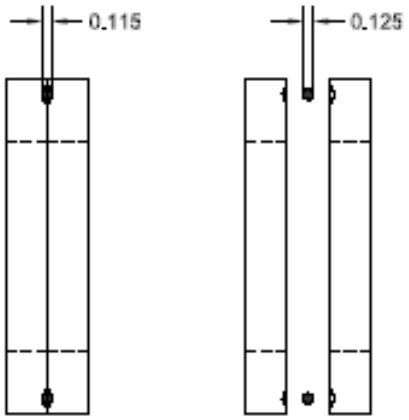
$T= T_2 + [(T_2-T_1)/R_2]*R_1 =$ 33.33 °C

Therefore, for polycarbonate if the desired temperature of the chamber is 30°C, then the water bath should be set at about 33.33°C; this is only about a 10% difference, and acrylic is about the same.

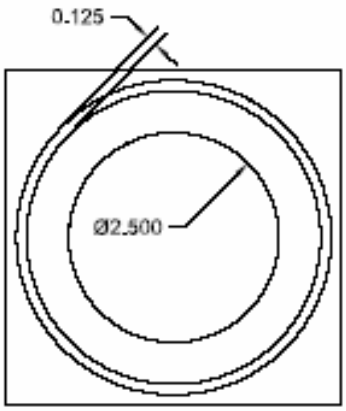
Appendix L – O-ring Seal

In the example below, the designer wants the 1/8" (.125") diameter O-Ring to compress .010" when the 2 blocks are sandwiched together, creating a seal. When this is done, the O-Ring will deform into a "football shape", as shown in the lefthand view. To compensate for the deformation of rubber, the designer must make an allowance for the material movement by machining the groove in the blocks as shown.

Example: 2 blocks being sealed by a 1/8" (.125") diameter O-Ring

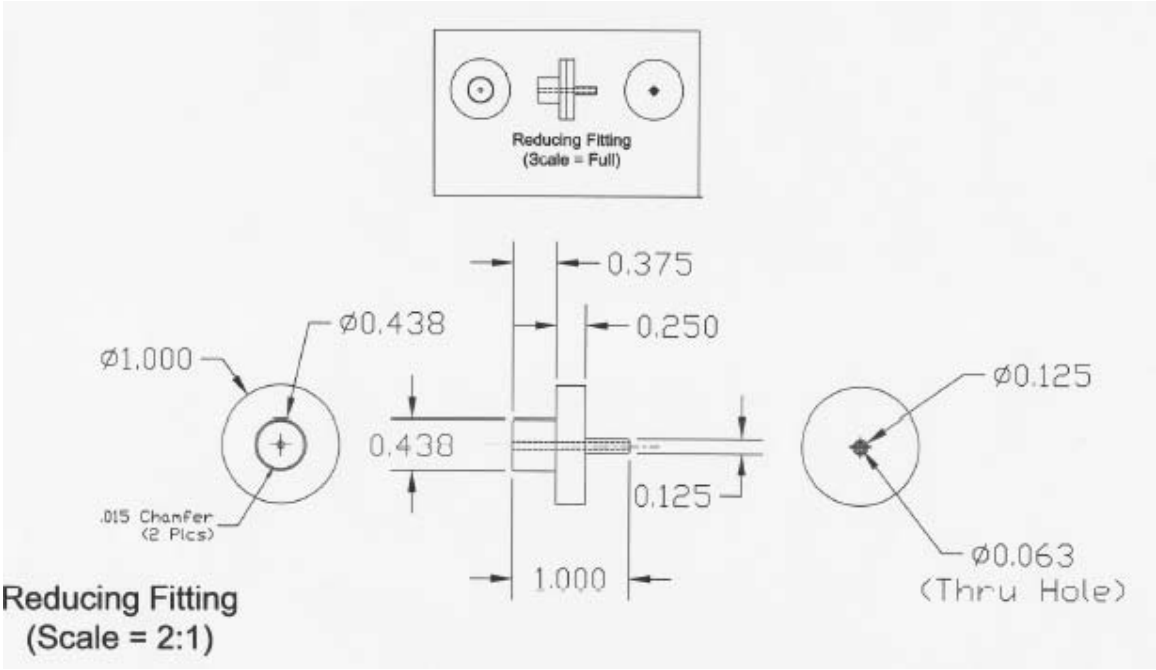


Side view showing the 2-1/2" thru hole (the dotted lines) and the machined groove in each mating block for sealing with a 1/8" (.125") diameter O-Ring



Front view showing a 2-1/2" diameter hole machined completely thru the block and a machined groove for a 1/8" (.125") diameter O-Ring for sealing

Appendix M – Tubing connector



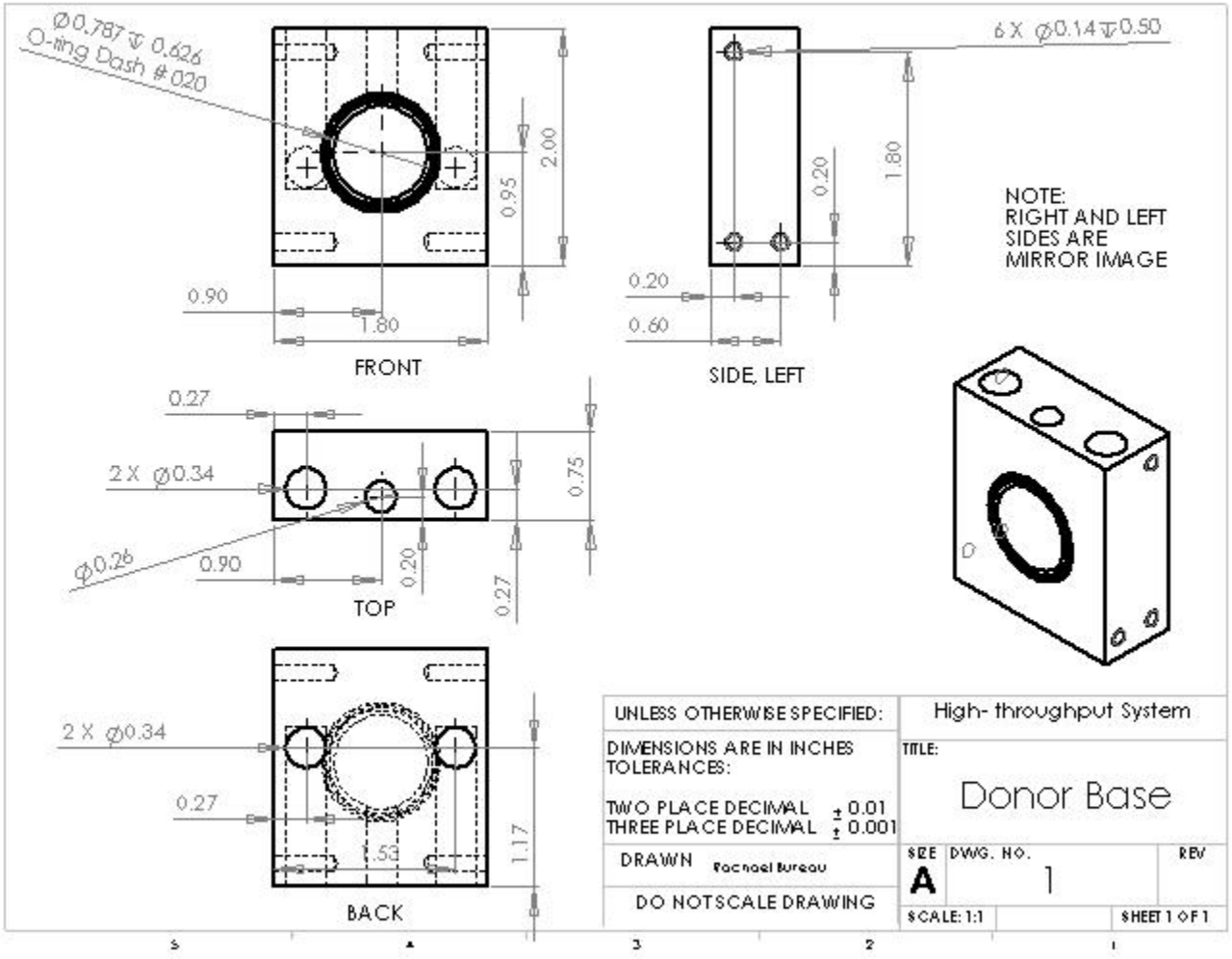
Appendix N - Budget

Product	Qty	Description	Retailer/ Distributor	Purchased by	Price
Tube Fitting 90 Deg Elbow	100	1/8" NPT polyethylene; PN 2808K49	McMaster Carr	Mezzone	$61.60
PVC Tubing	100ft	Tubing 1/8"; PN 5233K52	McMaster Carr	Mezzone	$10.00
O- ring Dash # 020	50	Silicone; PN 9396K104	McMaster Carr	Mezzone	$7.42
shipping on above three items					$8.50
Corner Clamp	1	Pony 3"	Home Depot	Mezzone	$8.49
Polycarbonate	2	2"thick; 4.875" X 7" and 4" X 9"	Plastics Unlimited	Jenket	$100.00
Gamma Globulin	1	Bovine blood; PN G5009-1G	Sigma-Aldrich	Account	$41.68
Polycarbonate	1	Clear 1/4" thick; 12" X 24"	MSC Direct	Account	$16.52
Corner Clamp	10	Pony 3"	Home Depot	Rachael	$93.87
Corner Braces	4	1"; packs of 4	Home Depot	Rachael	$7.10
Silicone	1	GE Silicone II; Clear	Home Depot	Rachael	$5.66
Caulk Gun	1		Home Depot	Rachael	$2.18
Polycarbonate	1	.75" thick; 7" X 8"	Plastics Unlimited	Rachael	$33.34
Rubber Stoppers	25	PN L-MS-1	Astro- Tex Company	Rachael	$39.25
Screws for donor chamber	100	8-32 1/2" stainless socket head cap	MSC Direct	Account	$6.27
Tube Fitting 90 Deg Elbow	10	1/8" NPT polyethylene; PN 2808K49	McMaster Carr	Jenket	$10.68
Silicone Tubing	25ft	1/8" ID , PN 51135K16	McMaster Carr	Mezzone	$15.25
Glucose HK Assay	2	G 3293 - 50 mL assay Reagent	Sigma-Aldrich	Account	$101.00
				TOTAL	**$568.81**

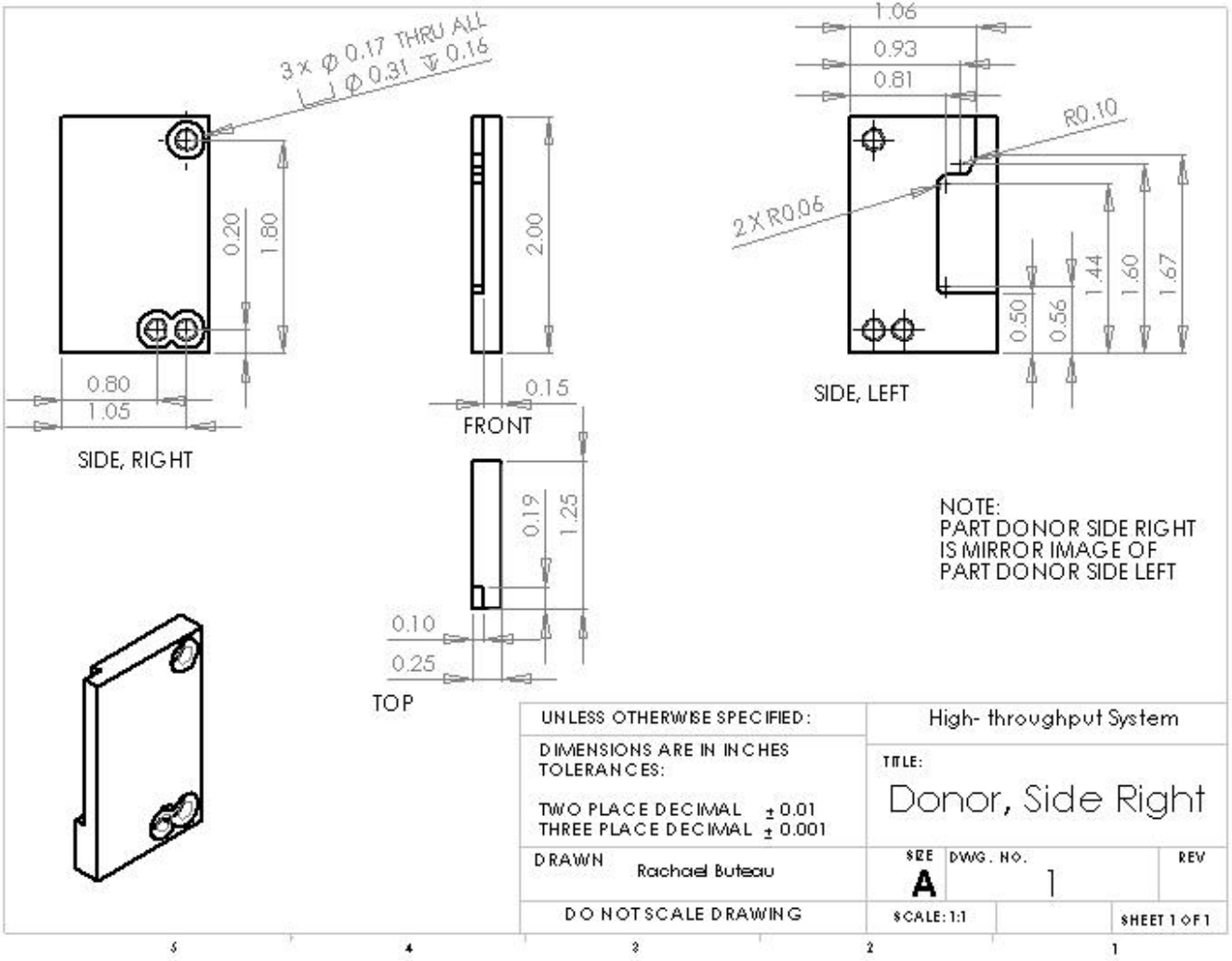
Appendix O – Final Budget

Product	Qty	Description	Retailer/ Distributor	Price
Tube Fitting 90 Deg Elbow	100	1/8" NPT polyethylene; PN 2808K49	McMaster Carr	$61.60
PVC Tubing	100ft	Tubing 1/8"; PN 5233K52	McMaster Carr	$10.00
O- ring Dash # 020	50	Silicone; PN 9396K104	McMaster Carr	$7.42
Shipping	$8.50			
Corner Clamp	1	Pony 3"	Home Depot	$8.49
Polycarbonate	2	2"thick; 4.875" X 7" and 4" X 9"	Plastics Unlimited	$100.00
Gamma Globulin	1	Bovine blood; PN G5009-1G	Sigma- Aldrich	$41.68
Polycarbonte	1	Clear 1/4" thick; 12" X 24"	MSC Industrial Direct Co., Inc.	$16.52
Corner Clamp	1	Pony 3"	Home Depot	$9.38
Corner Braces	1	1"; packs of 4	Home Depot	$1.78
Silicone	1	GE Silicone II; Clear	Home Depot	$5.66
Caulk Gun	1		Home Depot	$2.18
Polycarbonate	1	.75" thick; 7" X 8"	Plastics Unlimited	$33.34
Rubber Stoppers	25	PN L-MS-1	Astro- Tex Company	$39.25
Screws for donor chamber	100	8-32 1/4" stainless socket head cap screw; PN 5667050	MSC Industrial Direct Co., Inc.	$6.27
Glucose HK Assay	2	G 3293 - 50 mL assay Reagent	Sigma- Aldrich	$101.00
Head Screws	1	18−8 STAINLESS STEEL SOCKET HEAD CAP SCREW, 3/8"−16 THREAD, 1−1/2" LENGTH	McMaster Carr	$18.63
Plastic Thumb Heads	1	PLASTIC PRESS−FIT THUMB SCREW HEAD, TEE, BLACK, FITS 3/8" SCREW, 1−3/4" A, 15/32" B	McMaster Carr	$6.39
Silicone Tubing	25ft	1/8" ID , PN 51135K16	McMaster Carr	$15.25
Round Spindle cap tip	10	neoprene; 3/8-16; PN 97434617	MSC Industrial Direct Co., Inc.	$5.50
Anti-seize	1		AutoZone	$3.14
Fasteners			I. B. Barrows CO.	$7.61
Tube Fitting 90 Deg Elbow	10	1/8" NPT polyethylene; PN 2808K49	McMaster Carr	$10.68
				$520.27

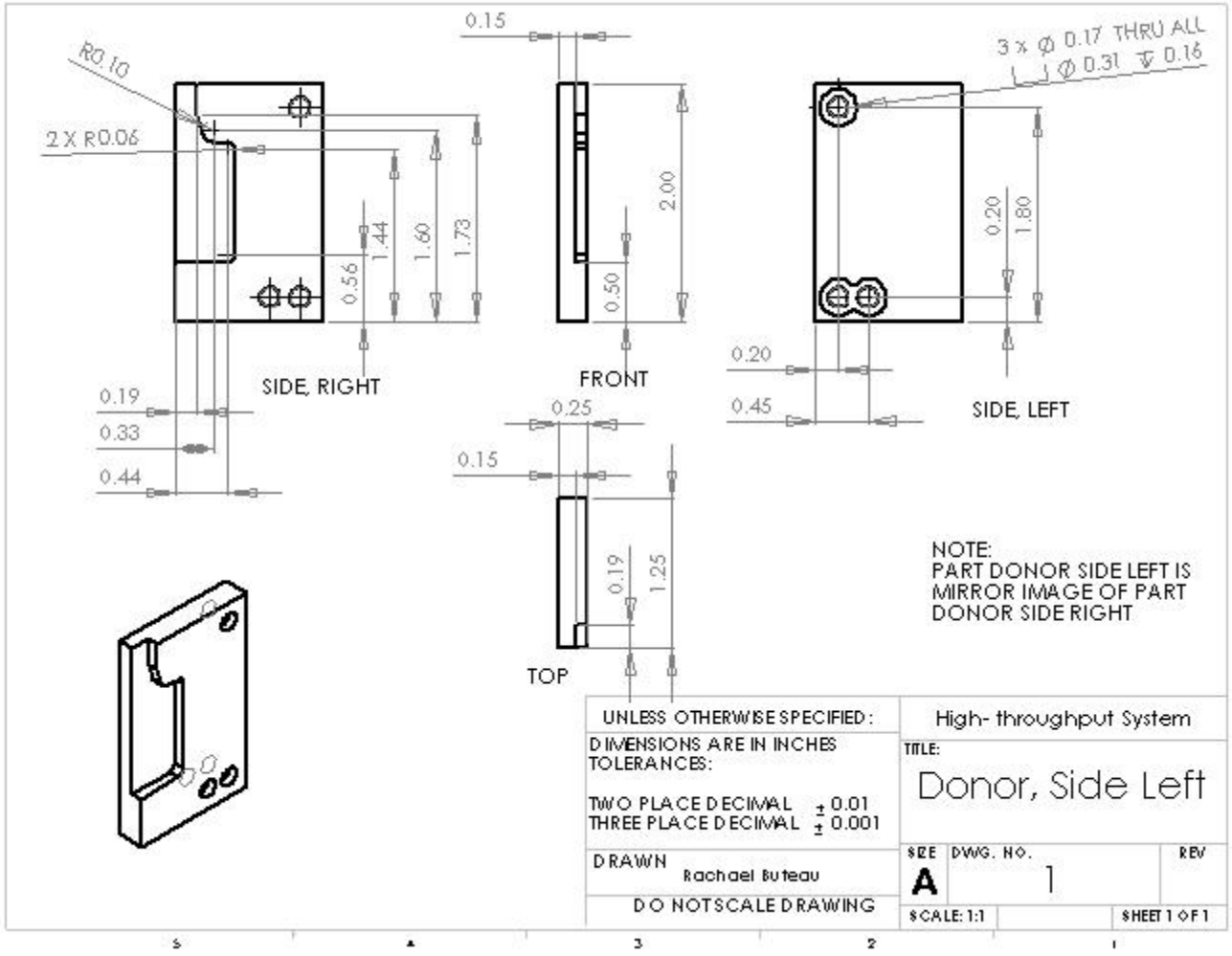
Appendix P – Donor Base



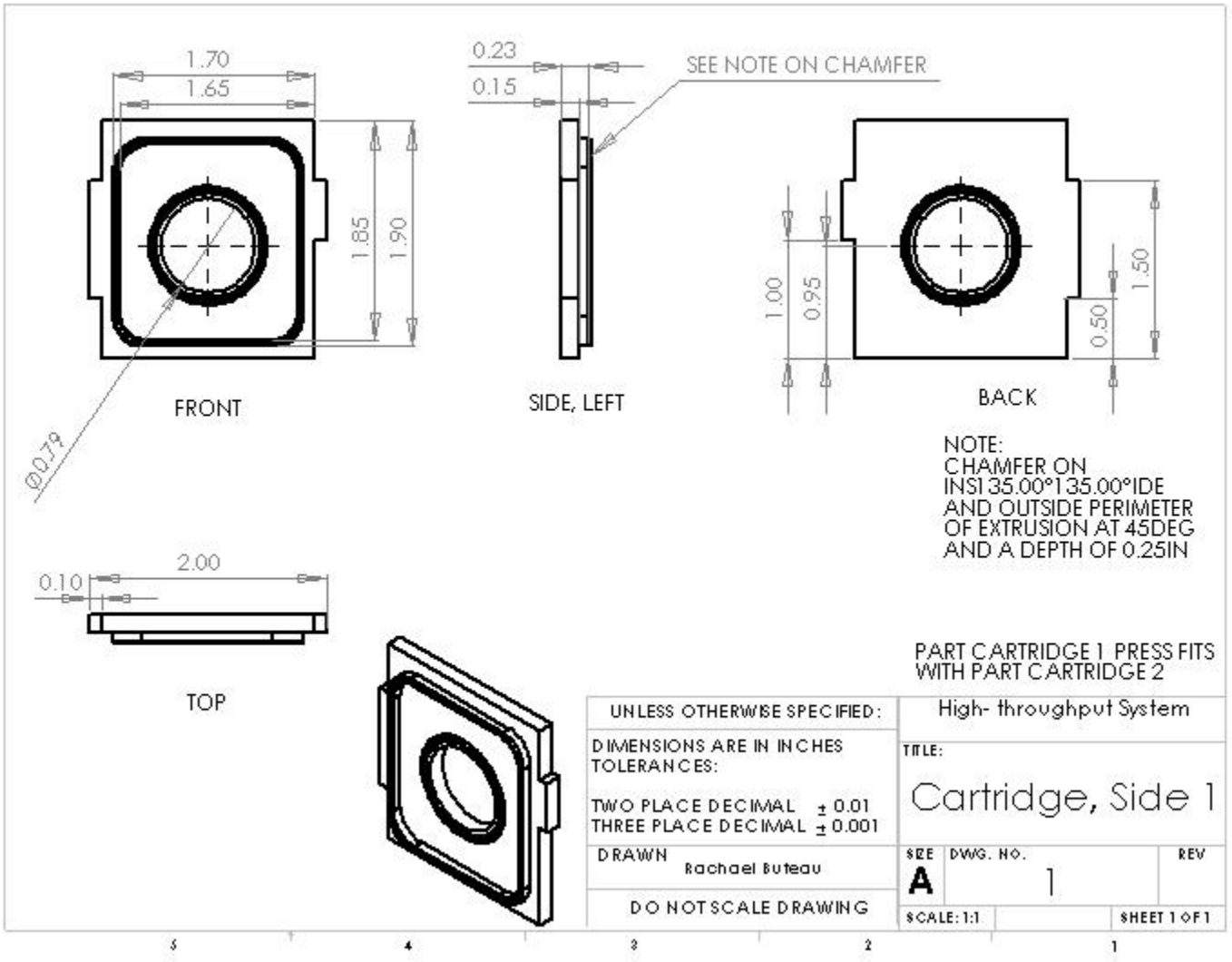
Appendix Q – Donor, Side Right



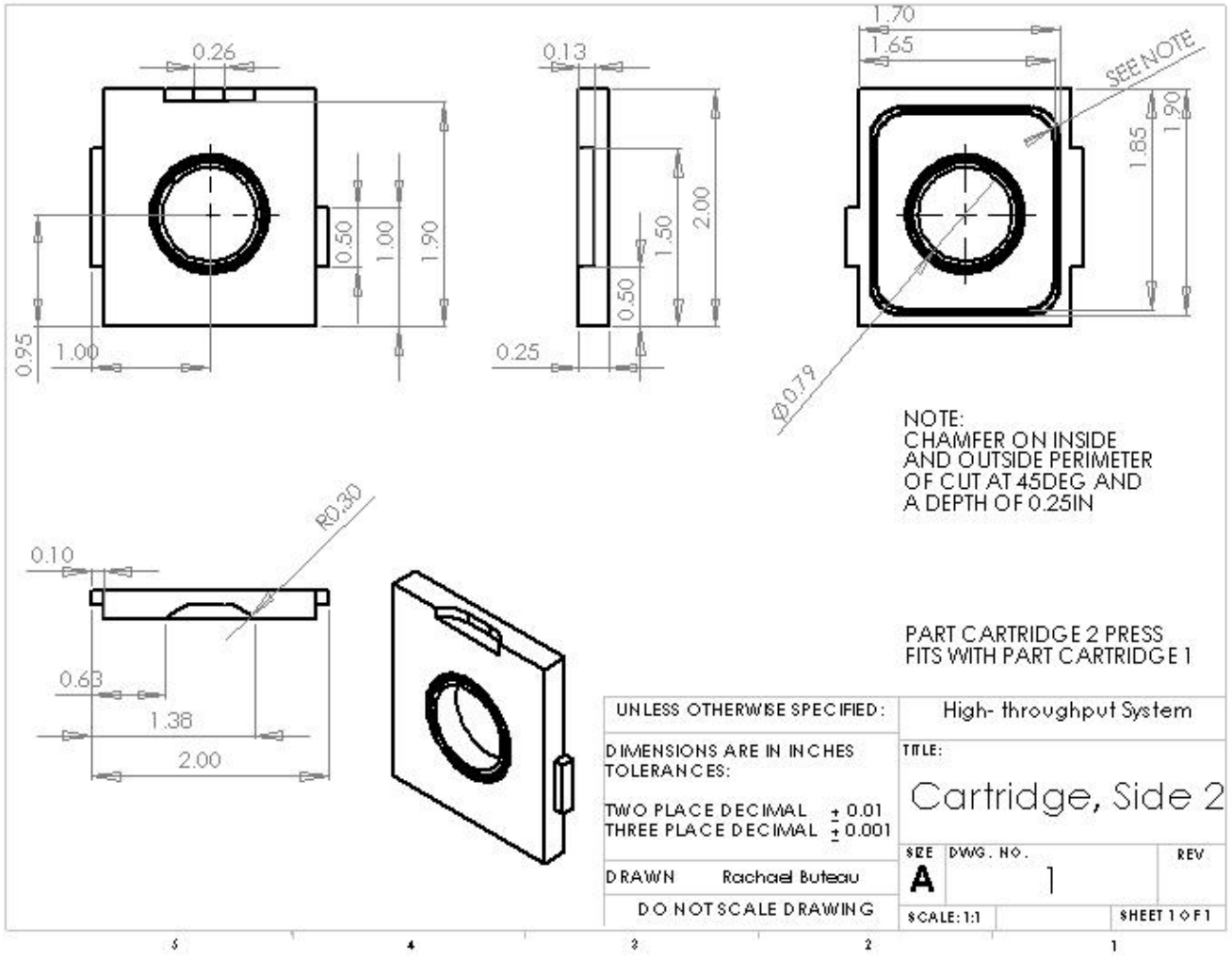
Appendix R – Donor, Side Left



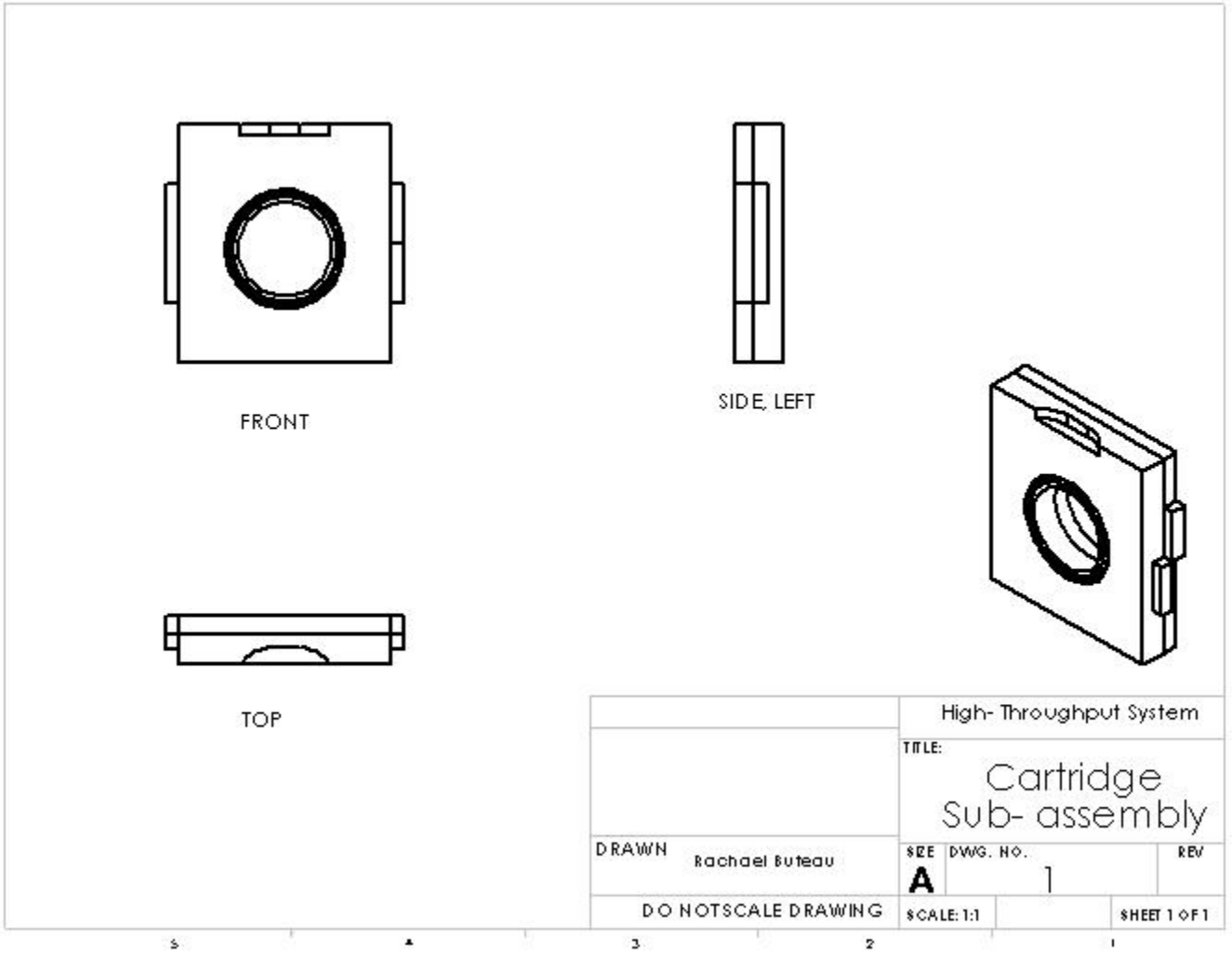
Appendix S – Cartridge Side 1



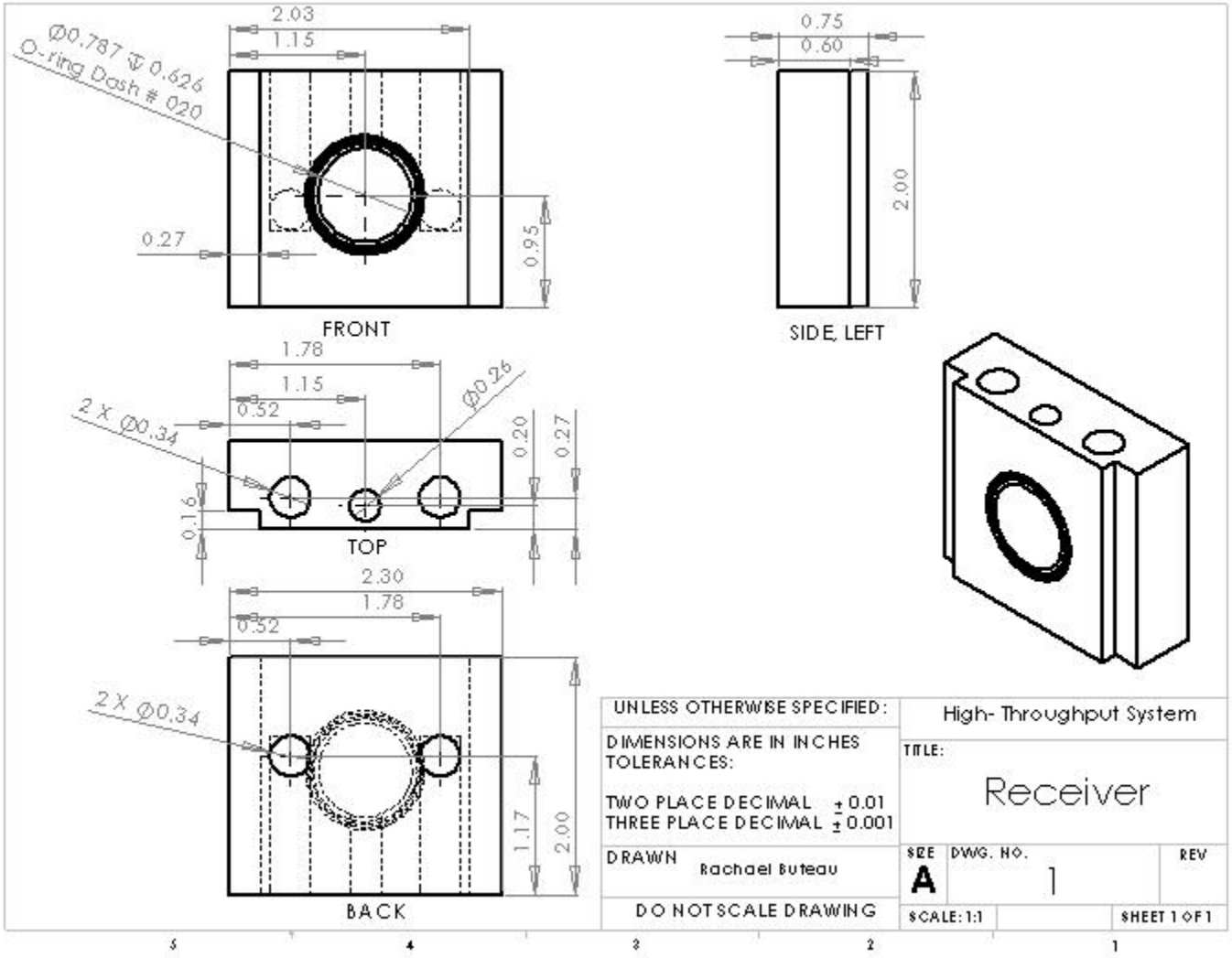
Appendix T – Cartridge, Side 2



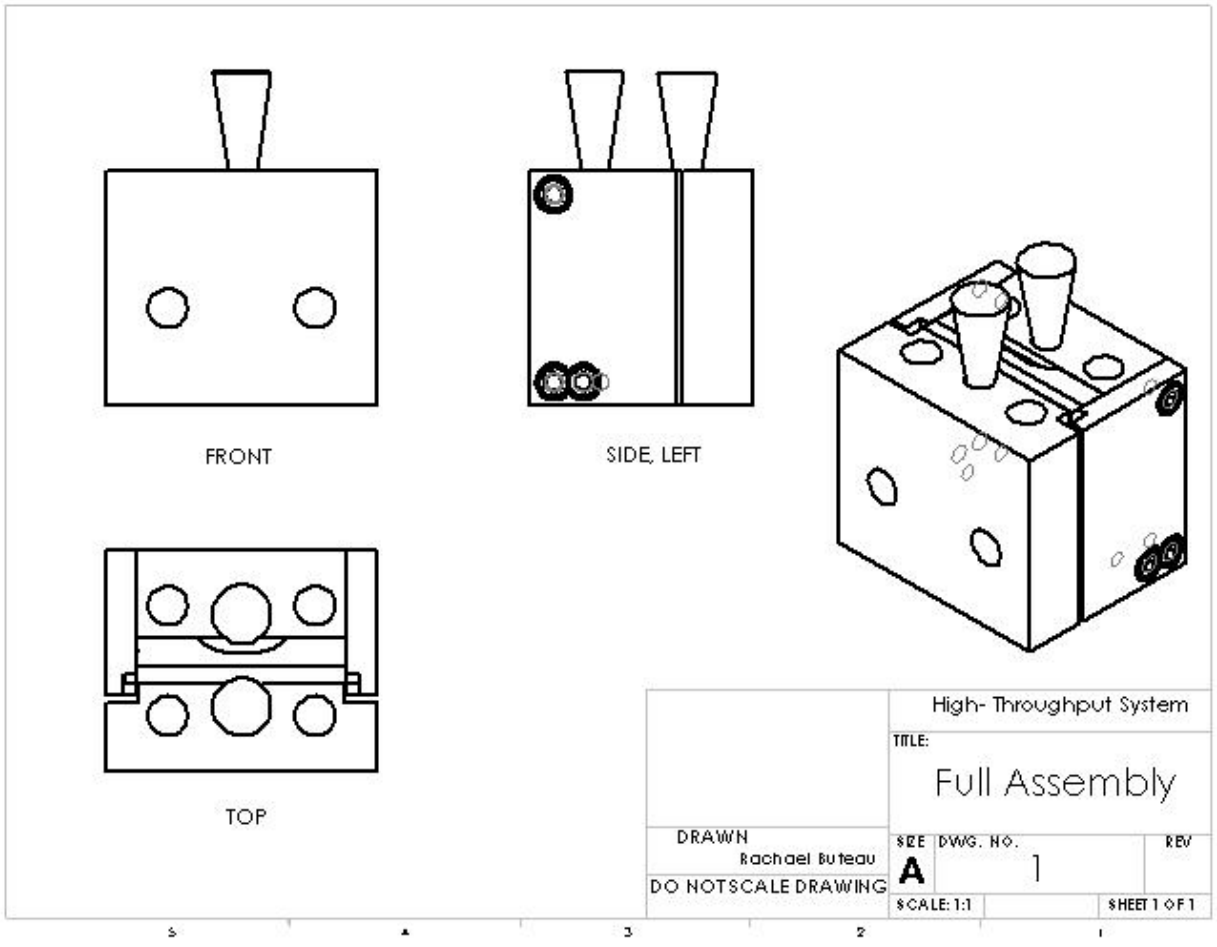
Appendix U – Cartridge Sub- assembly



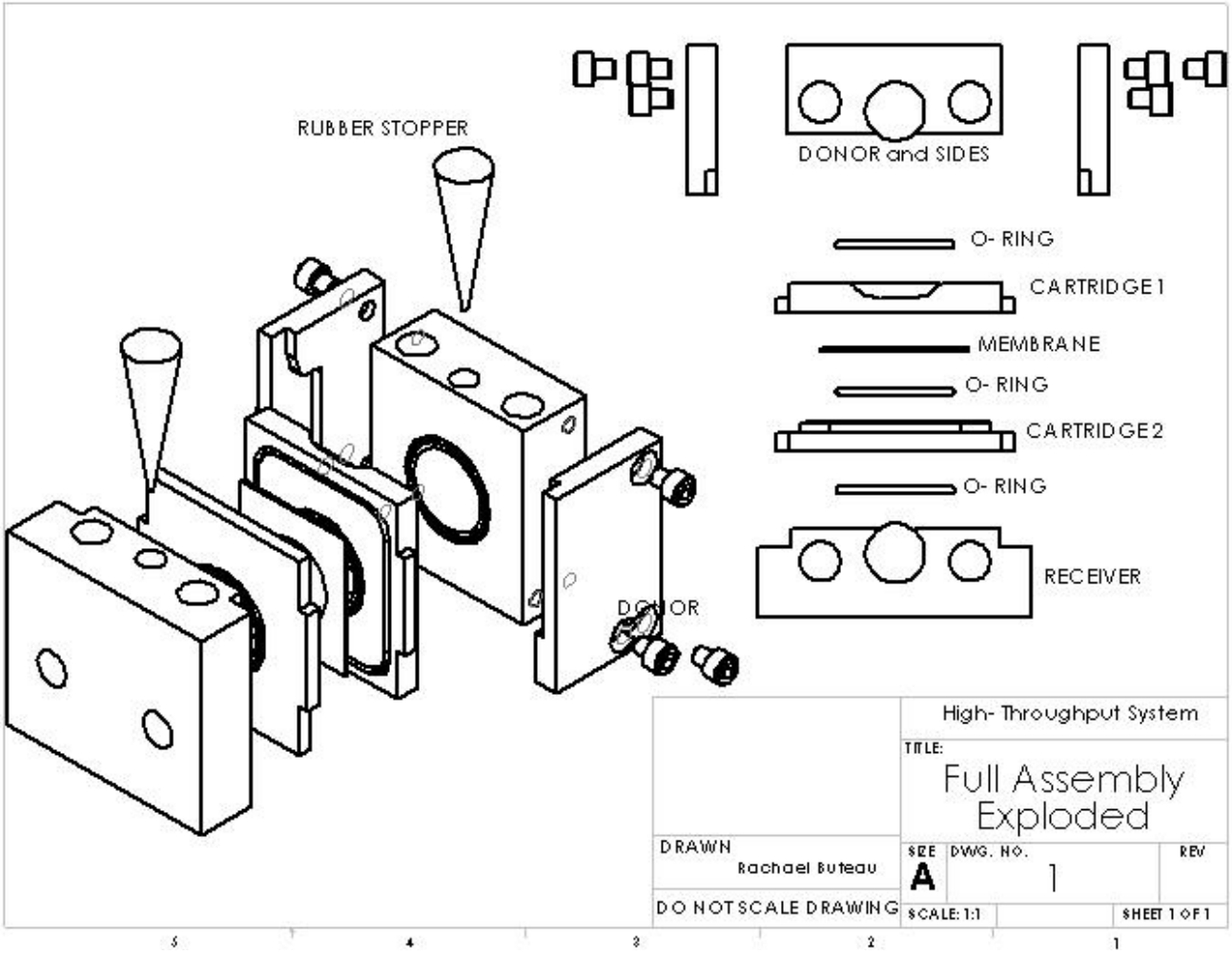
Appendix V – Receiver



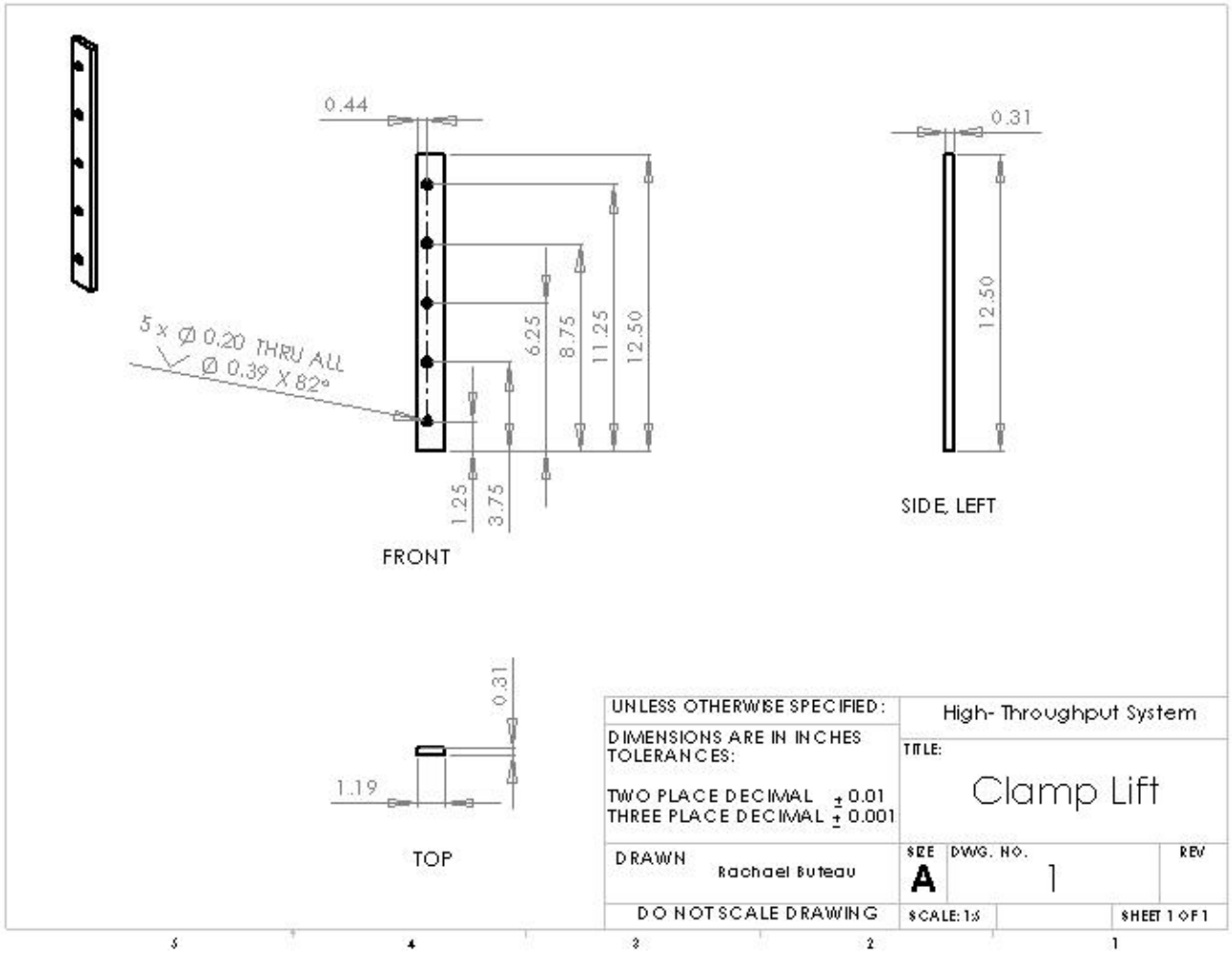
Appendix W – Full Assembly



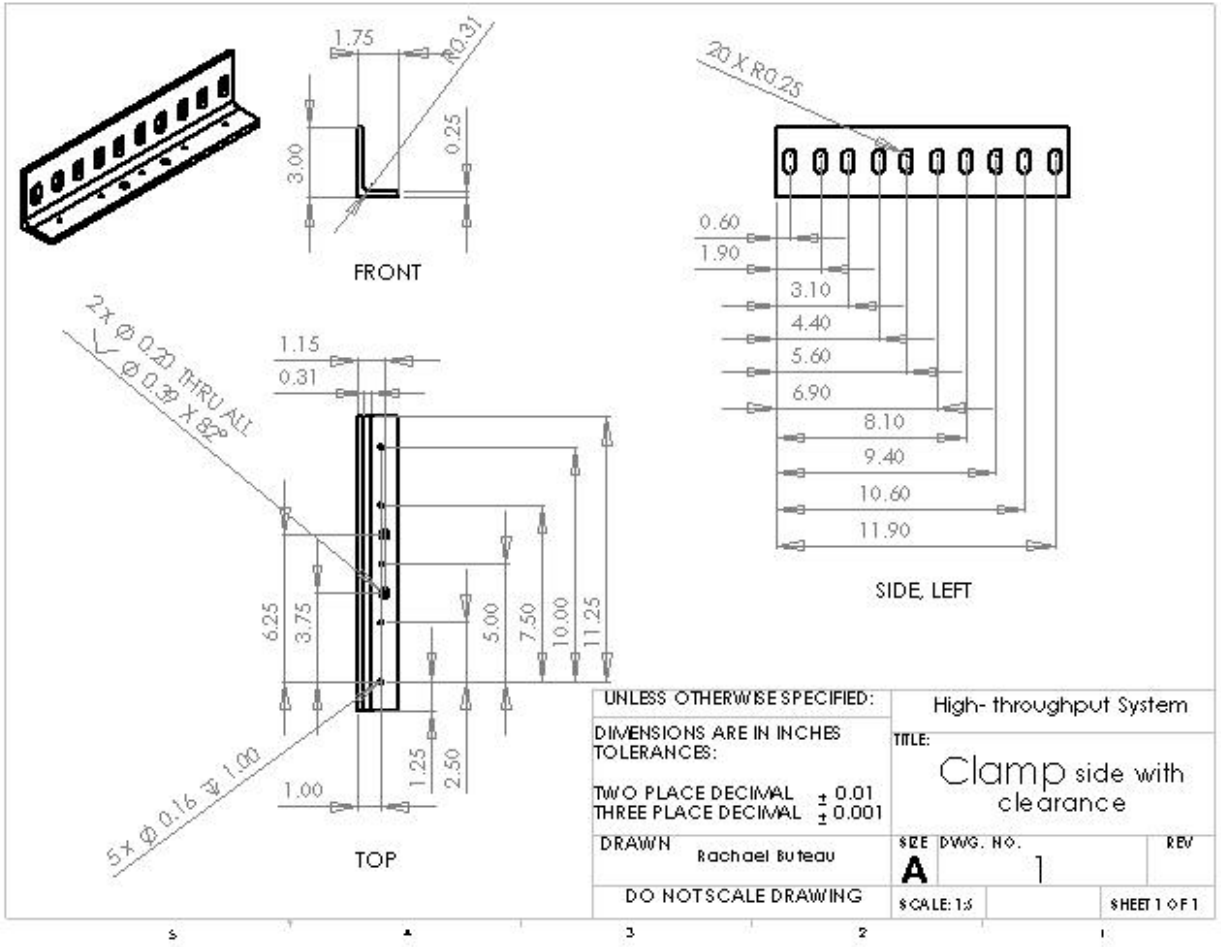
Appendix X – Full Assembly Exploded



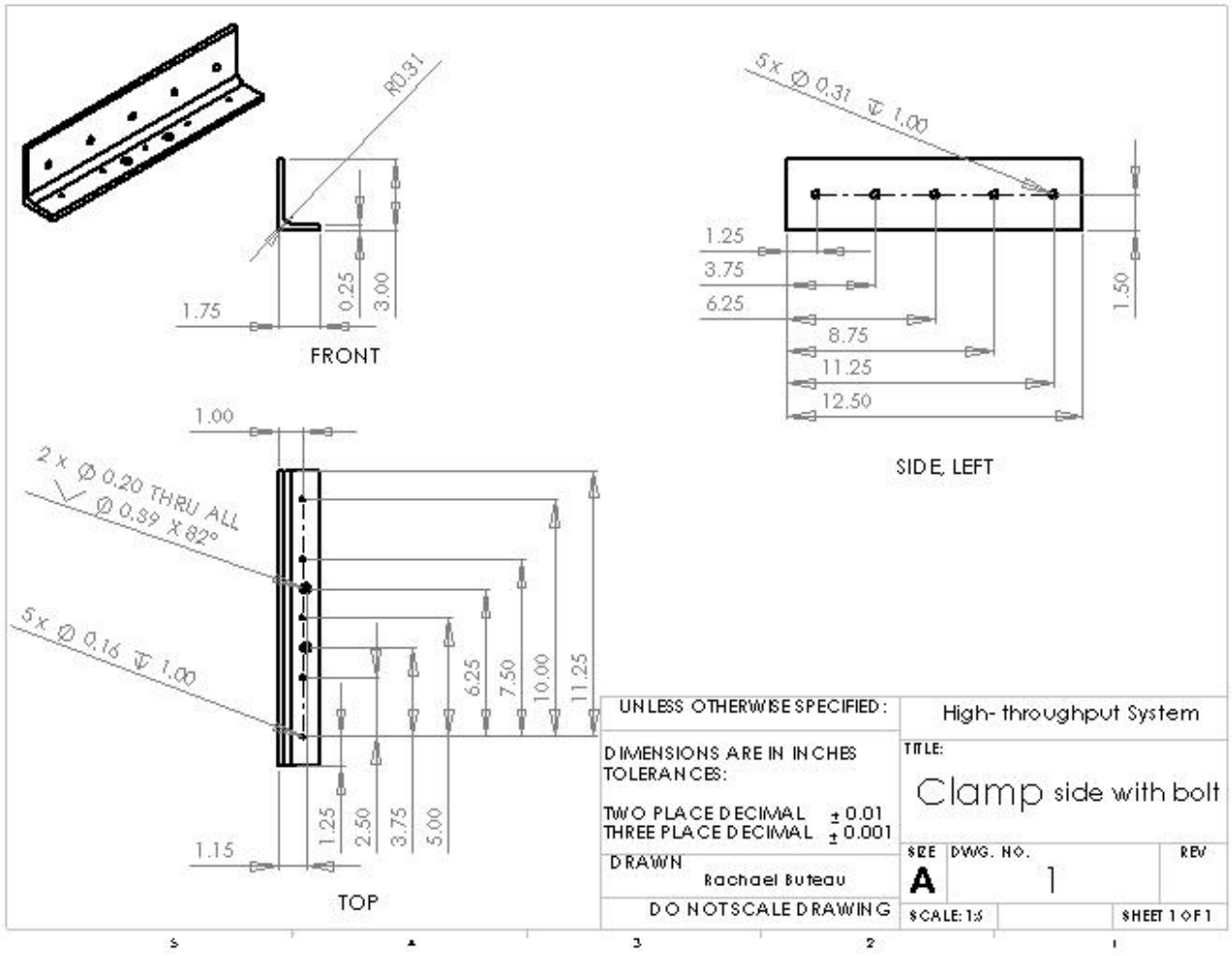
Appendix Y – Clamp Lift



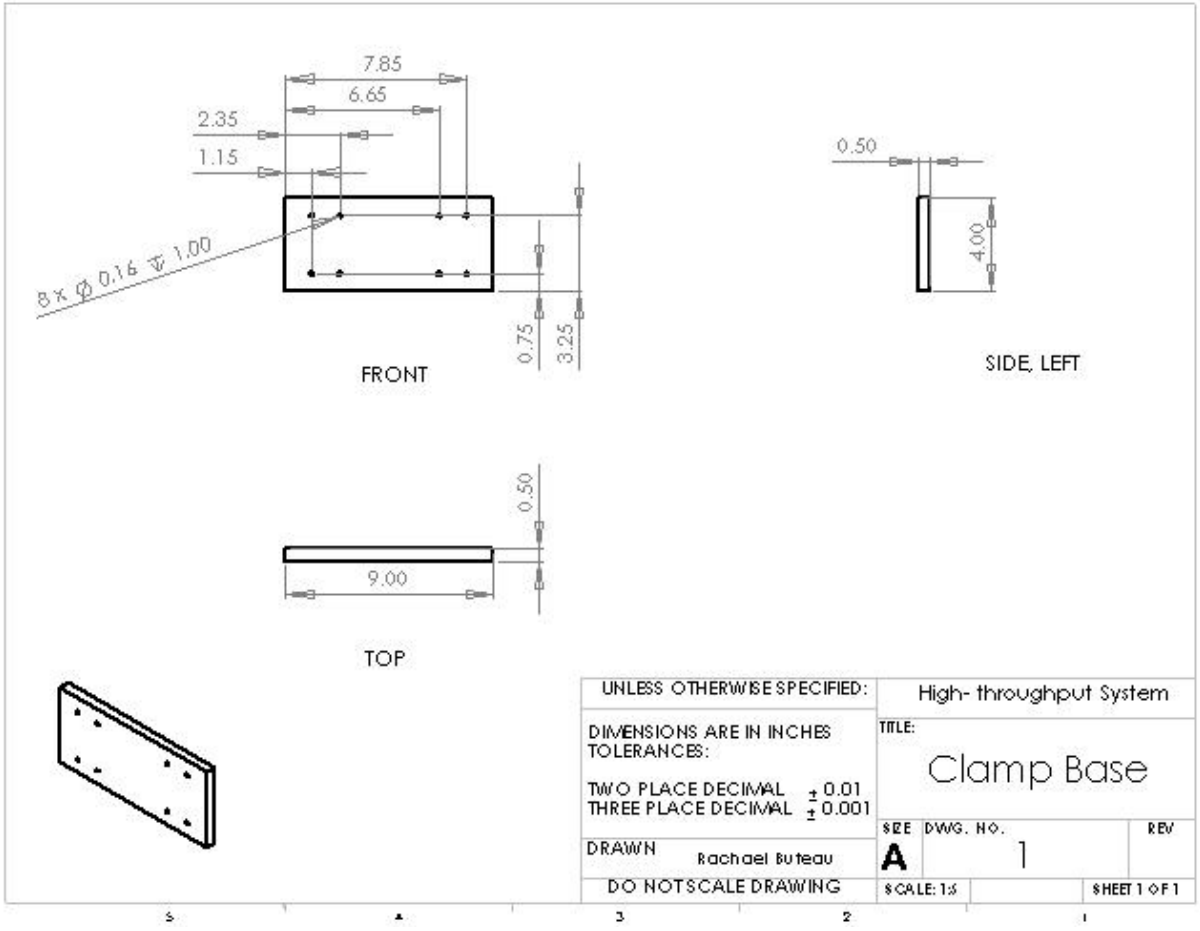
Appendix Z – Clamp side with clearance



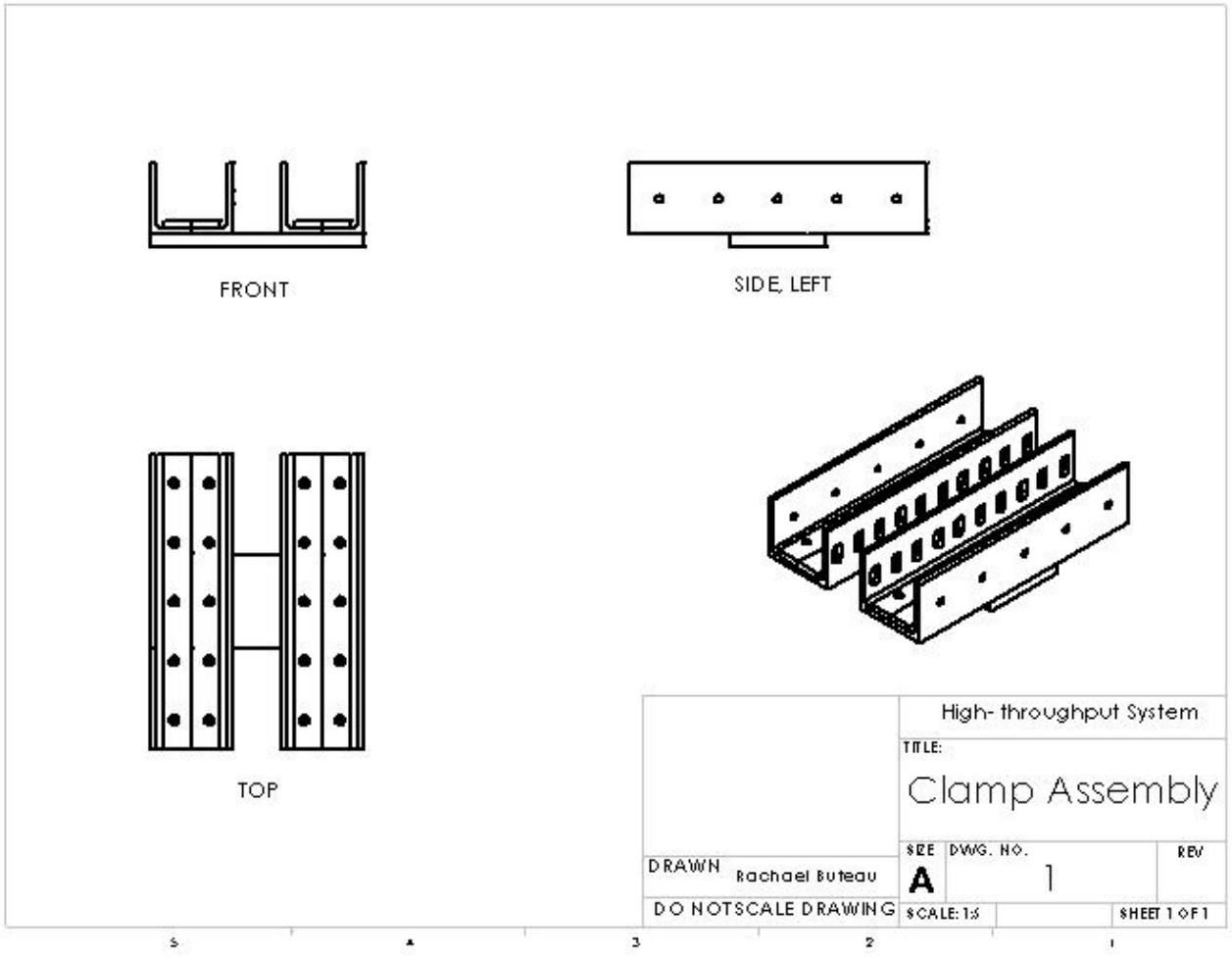
Appendix AA – Clamp side with bolts



Appendix AB – Clamp Base



Appendix AC – Clamp Assembly



Appendix AD – Rat Tail Tendon Collagen Membrane Protocol

Protocol: To create RTT Collagen Membranes
Protocol obtained from: Katie Bush

Materials:

- 13 Rat Tails
- Phosphate Buffer Solution (1x)
- Hemostat
- 3% acetic acid (48 ml acetic acid +1552 ml H_2O)
- Cheese cloth and funnel
- 2 L Beaker
- 4 rotor bottles
- Stir plate
- Dripping flask
- 30 % NaCl solution (96g/320 ml)
- 0.6% acetic acid (2.4 ml acetic acid in 397.6 ml ddH_2O)
- Dialysis bags and clips
- 1mN HCl (4ml/4L)
- Metal tray
- Freeze dryer
- 5mM HCl

RTT Collagen

1) Thaw 13 rat tails in 1x PBS (non-sterile). Squeeze out as much blood as possible
2) Pull tendons using hemostat. Avoid getting bloody tendons
3) Place tendon strands in 1xPBS
4) Put rinsed tendons into 1000ml of 3% acetic acid and stir overnight at 4 C.
5) Filter solution through 4 layers of cheese cloth using a funnel into a 2L beaker.
6) Pour filtrate into rotor bottle and centrifuge for 2 hours at 4 C at 8590 RPM
7) Decant supernatant into 2L beaker discarding pellet
8) At 4 C, drip 320 ml (~350 ml/hr) of 30 % NaCl solution into supernatant and allow to sit for at least 1 hour (can go overnight).
9) Pour entire solution and precipitate into rotor bottles and spin at 4960 RPM at 4 C for 30 minutes.
10) Decant supernatant and discard. Save any gelatinous material and any pellet.
11) In 2L beaker, re-suspend pellets in 400 mL of 0.6% acetic acid and spin overnight at 4 C or as long as necessary to dissolve pellet.
12) Place collagen solution into dialysis bags (slightly longer that 1 ft.) and double clip bags. In 4L flask, dialyze collagen into 1mN HCl five times with a minimum of 4 hours changing the dialysant.
13) Pour collagen solution onto metal tray in freeze dryer and run program 2.
14) Store collagen at 4 C and measure out and dissolve in 5 mM HCl to obtain desired concentration.

.Appendix AE – PermeGear® Assembly Protocol

Purpose: Instructions to assemble PermeGear Diffusion Chamber

Materials:

- Water Bath
- PeremeGear Device – Side-by-Side Horizontal ®
    - Clamping fixture
    - Glass donor chamber
    - Glass receiver chamber
    - Two stir bars
    - Two port pluds
- ¼" ID silicone tubing
- 2 x ¼" to 7/16" tube reduction connectors
- Small stir plate

Protocol:

1. Place chamber fixture on small stir plate
2. Put two chambers next to each other and connect the two water ports closer together using silicone tubing
3. Attach 4" of silicone tubing to the smaller end of each tubing connector
4. Attach the 4" tubing and connector to each of the remaining water ports.
5. Attach water bath tube to the larger end of the tubing connector to develop a closed system.
6. Place mechanical stir bars individually within the donor and receiver chamber
7. Place membrane in between donor and receiver chamber once it has been fully hydrated (refer to Diffusion Assay for particular molecules)
8. Push two chambers together and hold as you place chambers within their fixture
9. Secure chambers within fixture and seal chambers by tightening the screw on the fixture
10. Place the appropriate amount of solution with each of the chambers based on the molecule being studied.

    Donor: Glucose solution; Receiver: PBS*:* 3.5 mL per chamber

    Donor: BSA solution; Receiver: PBS*:* 3.5 mL per chamber

    Donor: Gamma Globulin solution; Receiver: PBS*:* 3.5 mL per chamber

Appendix AF – High-Throughput System Assembly Protocol

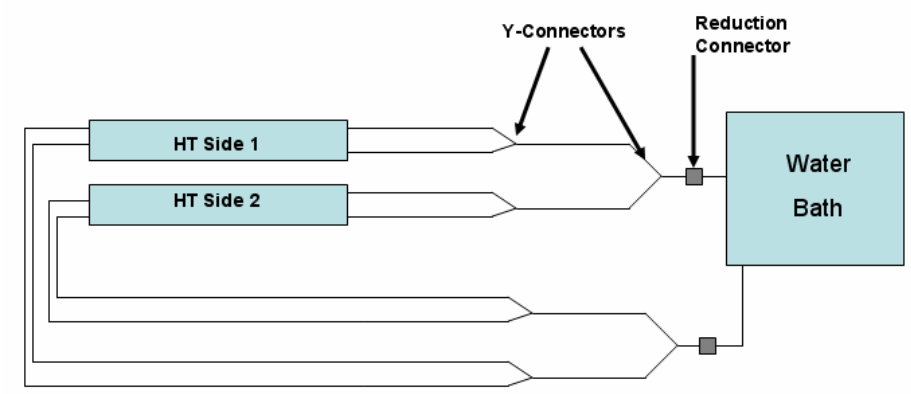
Purpose: Instructions to assemble High-Throughput System

Materials:

- Water Bath
- High-throughput system
  - Clamping fixture
  - 10 polycarbonate donor chambers
  - 10 polycarbonate receiver chambers
  - 10 cartridges
  - 20 sampling port plugs
- 1/8" ID silicone tubing
- 2 x 7/16" to 1/8" tube reduction connectors
- 6 Y-connectors
- Shaker Plate(VWR Orbital Shaker DS-500)

Protocol:

1) Place 5 donor and receiver chamber pairs within each row of the clamping fixture
2) Slide each individual donor chamber back through the spaces in the side plate of each row of the clamping fixture
3) Allow each receiver chamber to abut the claming device
4) Attach snake system tubing using following method:
   a. Donor-to-donor, leaving each end connector unattached
   b. Receiver-to receiver, leaving each end connector unattached
5) Attach end connectors based on the following diagram:



6) Allow water to flow through system for 40 minutes for heating while hydrating 10 collagen membranes for 30 minutes
7) As system is heating, place entire system on top of VWR Orbital Shaker Plate
8) Once membranes fully hydrated, place one collagen membrane in one cartridge by pressing the two sides of the cartridge together
9) Slide cartridge down and back into its appropriate donor chamber (labeled on top)

10) Press-fit cartridge in by pushing receiver chamber into it and clamping donor and receiver chamber together
11) Place the following amounts of pre-heated solution individually within each chamber:
    a. Donor chamber: 5 mg/ml molecular solution – 5 mL
    b. Receiver chamber: Phosphate Buffer Solution (PBS) – 5 mL
12) Place rubber stoppers in each sampling port
13) Being mixing the entire system at shaker plate setting of 150
14) Sample each receiver based on appropriate molecular diffusion study

Appendix AG – Protocol for Making Solutions of 5 mg/ml

Purpose: To make solutions with a definitive concentration fore testing purposes

Materials:

- Dry protein product
    - Bovine Serum Albumin – Sigma Aldrich
    - D +/-Glucose – Sigma Aldrich
    - Gamma Globulin - Pierce
- Phosphate Buffer Solution as diluents (non-sterile)
- Scale (preferably digital)
- Stir Plate and Stir Bar
- Graduated Cylinder
- Refrigerator

Protocol:

- Weigh 1 gram of dry protein product on the scale.
- Measure 200 ml of PBS using the graduated cylinder.
- Dissolve the protein in the PBS solution in the graduated cylinder until there is no solid matter using the stir plate.
- Place in labeled, glass container and refrigerate until ready for use.

Appendix AH - Diffusion Assay for Glucose

Purpose: To carry out an experiment to study the diffusion of a particular molecule through collagen membranes

Materials:

- Set-up diffusion device/ high-throughput system
- Microcentrifugation tubes (VWR)
- Pipette tips (small – yellow, large – blue)
- Glucose solution 5mg/ml – refer to solutions protocol
- 1x Phosphate buffer solution (PBS) non-sterile

Protocol:

1) Set-up diffusion device/system with appropriate protocol
2) Heat appropriate amount of glucose solution and PBS depending on device being used for 30 minutes before testing:

   PermeGear:
   Glucose solution– 5 mL
   PBS – 8 mL

   High-throughput device:
   Glucose Solution – 6 mL/donor chamber per chamber being used
   PBS – 10 mL/receiver chamber per chamber being used

3) Take a 300 µL sample from receiver chamber at time 0, every minute over the course of 10 minutes and at the 30 minute mark, placing each sample taken into a labeled microcentrifuge tube with proper pipette
4) Refill receiver chamber with 300 µL of PBS after taking each sample to restore the original amount of solution within the receiver chamber.
5) Once all samples have been taken, remove all liquid from both the chambers.
6) Disconnect the water bath system
7) Dismantle and clean high-throughput system as stated in the cleaning protocol.
8) Freeze microcentrifuge tubes at 4ºC until ready to carry out assay.

Appendix AI - Diffusion Assay for Bovine Serum Albumin (BSA)

Purpose: To carry out an experiment to study the diffusion of BSA through collagen membranes

Materials:

- Set-up diffusion device/ high-throughput system
- Pipette tips
- BSA solution (5 mg/ml) – refer to solutions protocol
- 1x Phosphate buffer solution (PBS) non-sterile

Protocol:

1) Set-up diffusion device/system using appropriate protocol
2) Heat appropriate amount of BSA solution and PBS depending on device being used for 30 minutes before testing:

   PermeGear:
   BSA solution– 5 mL
   PBS – 8 mL

   High-throughput device:
   BSA Solution – 6 mL/donor chamber per chamber being used
   PBS – 10 mL/receiver chamber per chamber being used

3) Take a 150 µL sample from receiver chamber at time 0 and every half hour over the course of 4 hours, placing each sample taken into a labeled microcentrifuge tube using a pipette
4) Refill receiver chamber with 150 µL of phosphate buffer solution after taking each sample to restore the original amount of solution within the receiver chamber.
5) Once all samples have been taken, remove all liquid from both the donor and receiver chamber.
6) Disconnect the water bath system
7) Dismantle and clean high-throughput system as stated in the cleaning protocol.
8) Freeze microcentrifuge tubes at 4ºC until ready to carry out assay.

Appendix AJ - Diffusion Assay for Gamma Globulin

Purpose: To carry out an experiment to study the diffusion of a particular molecule through collage membranes

Materials:

- Set-up diffusion device/ high-throughput system
- Pipette tips
- BSA solution (5 mg/ml) – refer to solutions protocol
- 1x Phosphate buffer solution (PBS) non-sterile

Protocol:

1) Set-up diffusion device/ high-throughput system with appropriate protocol
2) Heat appropriate amount of Gamma Globulin solution and PBS depending on device being used for 30 minutes before testing:

   PermeGear:
   Gamma Globulin solution– 5 mL
   PBS – 8 mL

   High-throughput device:
   Gamma Globulin Solution – 6 mL/donor chamber per chamber being used
   PBS – 10 mL/receiver chamber per chamber being used

3) Take a 150 µL sample from receiver chamber at time 0 and every two hours over the course of 8 hours and at 24 hours, placing each sample taken into a labeled microcentrifuge tube using a pipette.
4) Refill receiver chamber with 150 µL of phosphate buffer solution after taking each sample to restore the original amount of solution within the receiver chamber.
5) Once all samples have been taken, remove all liquid from both the donor and receiver chamber.
6) Disconnect the water bath system
7) Dismantle and clean high-throughput system as stated in the cleaning protocol.
8) Freeze microcentrifuge tubes at 4ºC until ready to carry out assay.

Appendix AK – BCA Protocol for BSA

Purpose: To measure the concentration of BSA in a solution.
Reference: Pierce- BCA Protein Kit http://www.piercenet.com/files/1745dh5.pdf

Materials:

- 1x Phosphate buffer solution (PBS) as diluents
- BSA ampules (1 ml @ 2 mg/ml)
- Pipette tips
- 96 Well Plate and covers
- BCA Reagent A and B (50:1 ratio of A:B)
- Spectrophotometer (96 well plate reader) and Softmax Pro software

Protocol:

Standard Dilutions:

Vial	Volume of Diluents (µl)	Volume and Source of Protein	Final Protein Concentration (µg/ml)	Total Final Volume (µl)
A	0	150 µl of Stock	2,000	150
B	50	150 µl of Stock	1,500	100
C	200	200 µl of Stock	1,000	200
D	100	100 µl of vial B dilution	750	200
E	200	200 µl of vial C dilution	500	200
F	200	200 µl of vial E dilution	250	200
G	200	200 µl of vial F dilution	125	350
H	200	50 µl of vial G dilution	25	250
I	200	0	0	200

1. Mix Reagent A and B to a 50:1 ratio with the following formula:
   (# standards + # unknowns) x (# of replicates) x (200 µl of Reagent per sample) = Total volume of Reagent needed
2. Pipette 25 µl of the protein sample into one well of the 96 well plate and add 200 µl of the Reagent A and B mix and mix for 30 seconds on a shaker plate.
3. Cover and incubate for 30 minutes at 37º C.
4. Cool plate to room temperature
5. Read 96 well plate in spectrophotometer at 562 nm.

Samples:

1. Pipette 200 µl of PBS into a well to act as a control for this reading.
2. Pipette 25 µl of solution with unknown concentration into one well of the 96 well plate.
3. Add 200 µl of the Reagent A and B mix and shake on a shaker plate for 30 seconds.
4. Cover and incubate for 30 minutes at 37º C.
5. Cool plate to room temperature.
6. Place 96 well plate in the plate reader and read at 562 nm.

Appendix AL – BCA Protocol for Gamma Globulin

Purpose: To measure the concentration of gamma globulin in a solution.
Reference: Pierce- BCA Protein Kit http://www.piercenet.com/files/1745dh5.pdf

Materials:

- 1x Phosphate buffer solution (PBS) as diluents
- Gamma Globulin - Bovine blood; Sigma-Aldrich PN G5009-1G
- Pipette tips
- 96 Well Plate and covers
- BCA Reagent A and B (50:1 ratio of A:B)
- Spectrophotometer (96 well plate reader) and Softmax Pro software

Protocol:

Standard Dilutions:

Vial	Volume of Diluents (µl)	Volume and Source of Protein	Final Protein Concentration (µg/ml)	Total Final Volume (µl)
A	0	150 µl of Stock	2,000	150
B	50	150 µl of Stock	1,500	100
C	200	200 µl of Stock	1,000	200
D	100	100 µl of vial B dilution	750	200
E	200	200 µl of vial C dilution	500	200
F	200	200 µl of vial E dilution	250	200
G	200	200 µl of vial F dilution	125	350
H	200	50 µl of vial G dilution	25	250
I	200	0	0	200

1. Mix Reagent A and B to a 50:1 ratio with the following formula:
   (# standards + # unknowns) x (# of replicates) x (200 µl of Reagent per sample) = Total volume of Reagent needed
2. Pipette 25 µl of the protein sample into one well of the 96 well plate and add 200 µl of the Reagent A and B mix and mix for 30 seconds on a shaker plate.
3. Cover and incubate for 30 minutes at 37º C.
4. Cool plate to room temperature.
5. Read plate using spectrophotometer at 562 nm.

Testing an unknown protein concentration:

1. Pipette 200 µl of PBS into a well to act as a control for this reading.
2. Pipette 25 µl of solution with unknown concentration into one well of the 96 well plate.
3. Add 200 µl of the Reagent A and B mix and shake on a shaker plate for 30 seconds.
4. Cover and incubate for 30 minutes at 37º C.
5. Cool plate to room temperature.
6. Place 96 well plate in the plate reader and read at 562 nm.

Appendix AM- Glucose (HK) Assay

Protocol: To measure the concentration of glucose in a solution
Reference: Sigma Aldrich (HK) Assay Kit Bulletin – Product Code: GAHK-20
http://www.sigmaaldrich.com/sigma/bulletin/gahk20bul.pdf
Materials:

- Glucose (HK) Assay Kit
  - Glucose (HK) Assay Reagent (Produce Code G3293)
  - Glucose Standard Solution (Product Code G 3285)
- 96-well plate
- Epindorf tubes
- Plastic cuvets
- Pipette tips

Protocol:
Standard

1) Pipette the following amount into labeled glass test tubes

Tube	Water (µl)	Glucose Standard (µl)	Obtained Concentration
A	250	0	0.00 g/L
B	245	5	0.02 g/L
C	240	10	0.04 g/L
D	235	15	0.06 g/L
E	230	20	0.08 g/L

2) Add 500 µl of assay reagent into first tube, cap tube and vortex
3) Repeat for each test tube
   a. Allow 30-60s between additions to each test tube
4) Incubate tubes for 30 minutes at 37 ºC
5) Starting with tube A, add 500 µl of 12 N H2SO4 into each tube
   a. Allow 30-60s between additions to each test tube
6) Vortex each tube carefully
7) Remove 100 µl sample from each tube and place in well
   a. Vortex each tube before sampling
8) Read plate at 540 nm.

Sample

1) Pipette water into one glass test tube as a control
2) Take 100 µl of each sample collected and place in epidorf tube.
3) Add 1 ml of assay reagent into epindorf tube with sample.
4) Repeat for each sample taking during test
   a. Allow 30-60 s between additions to each test tube
5) Incubate tubes for 15 minutes at room temperature (between 18-35ºC)
6) Vortex each tube carefully.
7) Remove 1 ml sample from each tube and place cuvet.
8) Read cuvet with proper UV reader at 340 nm.

Appendix AN - Plate Reader and Softmax Pro Software Protocol

Purpose: Directions for using spectrophotometer for all standards and experiments

Protocol:

1) Turn on spectrophotometer by flipping switch located in rear of machine
2) Log-in to the computer
3) Open program
   Click Programs
   Find spf312(softmax pro) and click to open
4) Once program is opened, highlight plate 1
5) Click Setup on the menu bar and adjust the wavelength to the proper wavelength needed to test specific molecule in use:
    a. Glucose = 540 nm
    b. BCA Assay molecules = 562 nm
6) Under automixing and blanking:
    a. Check automixing box (make sure time = 5 seconds)
    b. Turn blanking off
    c. Click O.K.

Standards

7) Click Template on the menu bar
    a. Choose group →**standards**
    b. Type in specific concentrations of each well of 96 well plate
        i. High-light well
        ii. Type in concentration (from protocol)
        iii. Click assign
    c. High-light well with no assigned concentration
        i. Click group → blank
    d. Click O.K. to complete

Samples

8) Click Experiment on the menu bar
    a. Click new plate and add another plate to the file
9) High-light newly added plate
10) Click Template on the menu bar
    a. Choose group → **samples**

Reading Plate

11) Place standard plate into plate reader
12) Click on standard plate within document
13) Click Read on the menu bar
14) Repeat 11 through 13 for sample plate as well
15) Results will be recorded, *Results will graph when linear fit chosen*

Appendix AO – Pore Size Analysis

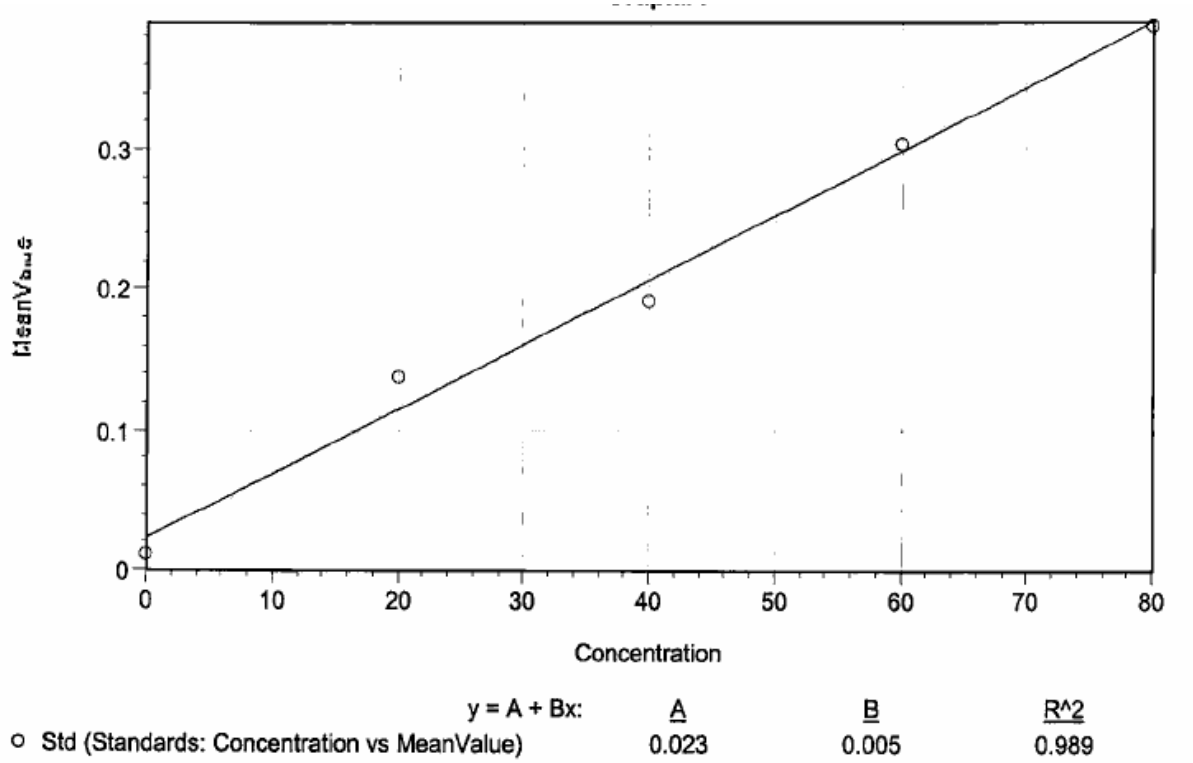
Area in Pixels^2	Area (unitless)	Micrometer^2	Radius in Micrometers	Radius in Nanometers	Diameter in Nanometers
76	0.0048	0.1197	0.20	195.23	390.46
89	0.0056	0.1401	0.21	211.27	422.53
57	0.0036	0.0898	0.17	169.07	338.14
94	0.0059	0.1480	0.22	217.12	434.24
58	0.0037	0.0913	0.17	170.55	341.10
37	0.0023	0.0583	0.14	136.22	272.44
55	0.0035	0.0866	0.17	166.08	332.16
49	0.0031	0.0772	0.16	156.76	313.52
33	0.0021	0.0520	0.13	128.64	257.29
52	0.0033	0.0819	0.16	161.49	322.97
56	0.0035	0.0882	0.17	167.58	335.16
39	0.0025	0.0614	0.14	139.85	279.70
72	0.0045	0.1134	0.19	190.02	380.04
61	0.0038	0.0961	0.17	174.90	349.81
62	0.0039	0.0976	0.18	176.33	352.66
60	0.0038	0.0945	0.17	173.46	346.93
66	0.0042	0.1039	0.18	181.93	363.86
46	0.0029	0.0724	0.15	151.88	303.77
66	0.0042	0.1039	0.18	181.93	363.86
49	0.0031	0.0772	0.16	156.76	313.52
88	0.0055	0.1386	0.21	210.08	420.15
81	0.0051	0.1276	0.20	201.55	403.09
78	0.0049	0.1228	0.20	197.78	395.56
75	0.0047	0.1181	0.19	193.94	387.88
55	0.0035	0.0866	0.17	166.08	332.16
71	0.0045	0.1118	0.19	188.70	377.39
53	0.0033	0.0835	0.16	163.03	326.06
61	0.0038	0.0961	0.17	174.90	349.81
66	0.0042	0.1039	0.18	181.93	363.86
45	0.0028	0.0709	0.15	150.22	300.45
33	0.0021	0.0520	0.13	128.64	257.29
92	0.0058	0.1449	0.21	214.80	429.59
77	0.0049	0.1213	0.20	196.51	393.02
52	0.0033	0.0819	0.16	161.49	322.97
52	0.0033	0.0819	0.16	161.49	322.97
63	0.0040	0.0992	0.18	177.75	355.50
73	0.0046	0.1150	0.19	191.34	382.67
69	0.0043	0.1087	0.19	186.02	372.04
50	0.0031	0.0787	0.16	158.35	316.70
57	0.0036	0.0898	0.17	169.07	338.14
44	0.0028	0.0693	0.15	148.55	297.09
45	0.0028	0.0709	0.15	150.22	300.45
63	0.0040	0.0992	0.18	177.75	355.50
42	0.0026	0.0661	0.15	145.13	290.26
57	0.0036	0.0898	0.17	169.07	338.14
62	0.0039	0.0976	0.18	176.33	352.66
47	0.0030	0.0740	0.15	153.53	307.05
41	0.0026	0.0646	0.14	143.39	286.79
68	0.0043	0.1071	0.18	184.67	369.33
56	0.0035	0.0882	0.17	167.58	335.16
Conversion Factor				**Average =**	**343.88**
126 pixels in 5 micrometers				**Standard Deviation**	**42.74**

Appendix AP – Standard Results for Glucose, BSA, and Gamma Globulin

Glucose Standard Readings

Sample	Concentration	Wells	BackConcCalc	Values	MeanValue	Std.Dev.	CV%
St01	0.000	A1	-2.776	0.010	0.011	0.002	15.7
		B1	-2.122	0.013			
		C1	-2.776	0.010			
St02	20.000	A2	24.477	0.135	0.138	0.003	2.2
		B2	25.131	0.138			
		C2	25.785	0.141			
St03	40.000	A3	35.596	0.186	0.192	0.005	2.6
		B3	37.340	0.194			
		C3	37.558	0.195			
St04	60.000	A4	59.142	0.294	0.303	0.008	2.7
		B4	62.631	0.310			
		C4	61.759	0.306			
St05	80.000	A5	76.148	0.372	0.387	0.013	3.4
		B5	81.599	0.397			
		C5	80.509	0.392			

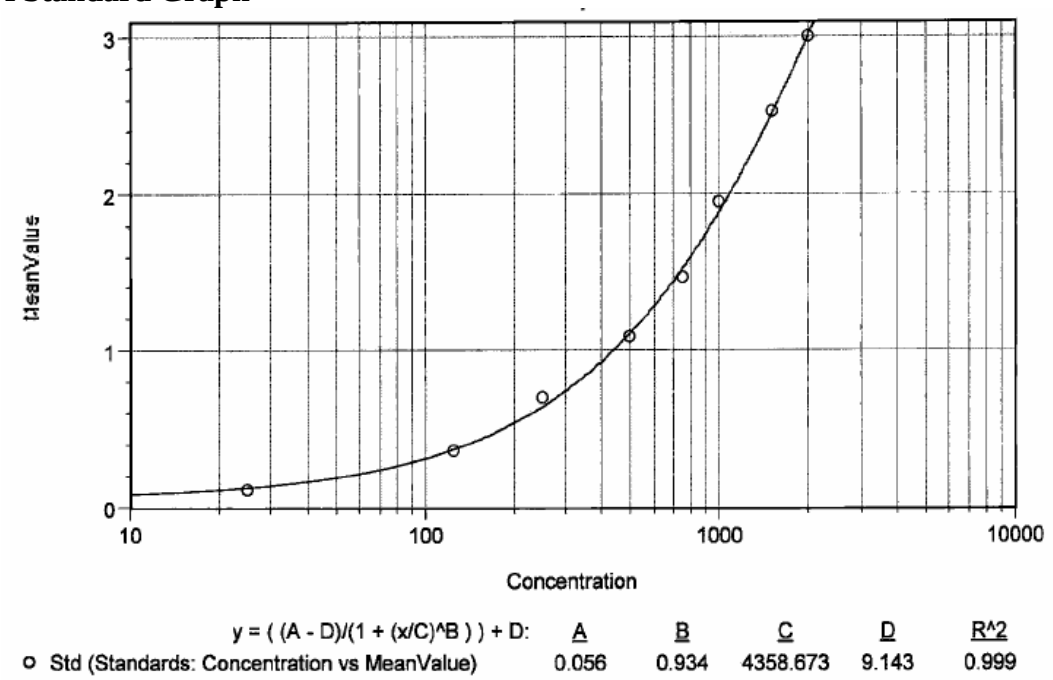
Glucose Standard Curve



BSA Standard Readings

Sample	Concentration	Wells	BackConcCalc	Values	MeanValue	Std.Dev.	CV%
St01	2000.000	A1	1907.366	2.929	3.003	0.069	2.3
		A2	1998.195	3.015			
		A3	2053.408	3.066			
St02	1500.000	B1	1400.081	2.394	2.522	0.124	4.9
		B2	1520.290	2.530			
		B3	1623.566	2.642			
St03	1000.000	C1	993.458	1.882	1.943	0.056	2.9
		C2	1050.327	1.959			
		C3	1073.628	1.990			
St04	750.000	D1	694.244	1.442	1.468	0.055	3.7
		D2	751.406	1.531			
		D3	687.292	1.431			
St05	500.000	E1	465.515	1.058	1.089	0.047	4.3
		E2	513.729	1.143			
		E3	470.557	1.067			
St06	250.000	F1	240.079	0.625	0.699	0.107	15.4
		F2	338.520	0.822			
		F3	252.192	0.650			
St07	125.000	G1	116.510	0.355	0.367	0.013	3.4
		G2	122.141	0.368			
		G3	127.370	0.380			
St08	25.000	H1	20.201	0.116	0.116	0.002	1.7
		H2	19.473	0.114			
		H3	20.931	0.118			
St09	0.000	A7	0.750	0.059	0.059	0.001	1.0
		A8	0.750	0.059			
		A9	1.042	0.060			

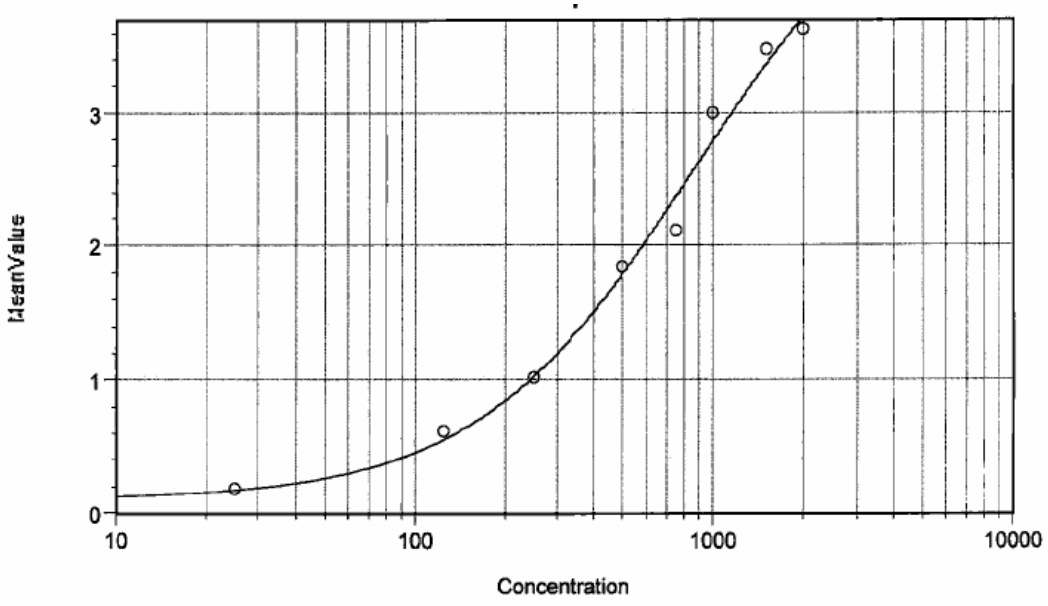
BSA Standard Graph



Gamma Globulin Standard Readings

Sample	Concentration	Wells	BackConcCalc	Values	MeanValue	Std.Dev.	CV%
St01	2000.000	A4	1845.589	3.631	3.628	0.025	0.7
		A5	1876.543	3.651			
		A6	1802.053	3.602			
St02	1500.000	B4	1577.231	3.434	3.470	0.035	1.0
		B5	1664.357	3.503			
		B6	1626.921	3.474			
St03	1000.000	C4	1073.666	2.902	2.994	0.080	2.7
		C5	1172.834	3.029			
		C6	1191.111	3.051			
St04	750.000	D4	599.509	2.053	2.116	0.055	2.6
		D5	636.546	2.138			
		D6	644.613	2.156			
St05	500.000	E4	485.423	1.765	1.845	0.076	4.1
		E5	518.582	1.853			
		E6	543.199	1.916			
St06	250.000	F4	246.373	1.013	1.014	0.049	4.8
		F5	260.663	1.064			
		F6	233.383	0.966			
St07	125.000	G4	137.054	0.601	0.611	0.010	1.6
		G5	141.925	0.620			
		G6	140.129	0.613			
St08	25.000	H4	28.431	0.185	0.187	0.002	1.1
		H5	28.720	0.186			
		H6	29.584	0.189			
St09	0.000	A7	Range?	0.061	0.061	0.001	0.9
		A8	Range?	0.061			
		A9	Range?	0.062			

Gamma Globulin Standard Curve



	y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
o	Std (Standards: Concentration vs MeanValue)	0.101	1.19	860.637	5.055	0.991

Appendix AQ – Results for PermeGear® Trials: Glucose

Abs = Absorbancy

Trial 1					
Time (mins)	**Abs 1**	**Abs 2**	**Abs 3**	**Avg Abs**	**Concentration (mg/ml)**
2	0.2498	0.2494	0.2494	0.250	0.072
4	0.6154	0.6154	0.6149	0.615	0.178
6	0.7336	0.7336	0.7349	0.734	0.213
8	0.8604	0.8614	0.8613	0.861	0.249
10	1.3159	1.3171	1.3159	1.316	0.381
Trial 2					
Time (mins)	**Abs 1**	**Abs 2**	**Abs 3**	**Avg Abs**	**Concentration (mg/ml)**
2	0.3048	0.3053	0.3053	0.305	0.088
4	0.4265	0.4262	0.4265	0.426	0.124
6	0.8407	0.841	0.841	0.841	0.244
8	1.2468	1.2472	1.2468	1.247	0.361
10	1.5299	1.5305	1.5299	1.530	0.443
Trial 3					
Time (mins)	**Abs 1**	**Abs 2**	**Abs 3**	**Avg Abs**	**Concentration (mg/ml)**
2	0.4917	0.4921	0.4932	0.492	0.143
4	0.7764	0.7758	0.7778	0.777	0.225
6	1.0878	1.0878	1.0878	1.088	0.315
8	1.4074	1.4074	1.4074	1.407	0.408
10	1.7545	1.7545	1.761	1.757	0.509

Appendix AR – Results for PermeGear® Trials: BSA

** Only hour increments used to calculate diffusion coefficients (rows B, D, F and H)**

Trial 1 Absorbance Values

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.134	0.134	0.134									
	0.134	0.134	0.134									
B	0.173	0.173	0.177									
	0.173	0.173	0.177									
C	0.212	0.215	0.220									
	0.212	0.215	0.220									
D	0.251	0.665	0.258									
	0.251	0.665	0.258									
E	0.286	0.295	0.296									
	0.286	0.295	0.296									
F	0.319	0.327	0.331									
	0.319	0.327	0.331									
G	0.339	0.346	0.361									
	0.339	0.346	0.361									
H	0.361	0.364	0.375							0.001	-0.001	-0.001
	0.361	0.364	0.375							0.001	-0.001	-0.001

Trial 2 Absorbance Values

A	0.189	0.186	0.194									
	0.189	0.186	0.194									
B	0.208	0.211	0.147									
	0.208	0.211	0.147									
C	0.236	0.242	0.246									
	0.236	0.242	0.246									
D	0.264	0.263	0.262									
	0.264	0.263	0.262									
E	0.275	0.288	0.286									
	0.275	0.288	0.286									
F	0.284	0.288	0.289									
	0.284	0.288	0.289									
G	0.303	0.314	0.324									
	0.303	0.314	0.324									
H	0.317	0.323	0.327							0.001	-0.000	-0.000
	0.317	0.323	0.327							0.001	-0.000	-0.000

Trial 3 Absorbance Values

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.095	0.085	0.093									
	0.095	0.085	0.093									
B	0.131	0.134	0.136									
	0.131	0.134	0.136									
C	0.165	0.161	0.162									
	0.165	0.161	0.162									
D	0.193	0.198	0.198									
	0.193	0.198	0.198									
E	0.221	0.221	0.230									
	0.221	0.221	0.230									
F	0.251	0.255	0.255									
	0.251	0.255	0.255									
G	0.282	0.278	0.278									
	0.282	0.278	0.278									
H	0.292	0.293	0.301							0.002	-0.001	-0.000
	0.292	0.293	0.301							0.002	-0.001	-0.000

Appendix AS – Results for PermeGear® Trials: Gamma Globulin

Trial 1

0.186	0.188	0.189
0.186	0.188	0.189
0.284	0.292	0.294
0.284	0.292	0.294
0.357	0.367	0.363
0.357	0.367	0.363
0.427	0.446	0.432
0.427	0.446	0.432

Trial 2

0.331	0.300	0.241
0.331	0.300	0.241
0.385	0.408	0.512
0.385	0.408	0.512
0.588	0.909	0.562
0.588	0.909	0.562
0.713	1.005	0.677
0.713	1.005	0.677

Appendix AT – Results for High-throughput System: Glucose Using Dialysis Membrane

Abs = Absorbancy

Trial 1					
Time (mins)	**Abs 1**	**Abs 2**	**Abs 3**	**Avg Abs**	**Concentration (mg/ml)**
2	0.0970	0.0971	0.0970	0.097	0.028
4	0.4620	0.4617	0.4617	0.462	0.134
6	0.8948	0.8957	0.8939	0.895	0.259
8	1.1879	1.1879	1.1879	1.188	0.344
10	1.3476	1.3501	1.3451	1.348	0.391
30	2.4000	2.4000	2.4000	2.400	0.696
Trial 2					
Time (mins)	**Abs 1**	**Abs 2**	**Abs 3**	**Avg Abs**	**Concentration (mg/ml)**
2	0.0950	0.0949	0.0947	0.095	0.028
4	0.4010	0.4007	0.4016	0.401	0.116
6	0.6421	0.6421	0.6426	0.642	0.186
8	1.0428	1.0415	1.0415	1.042	0.302
10	1.2077	1.2058	1.2095	1.208	0.350
30	2.1402	2.1402	2.1402	2.140	0.621
Trial 3					
Time (mins)	**Abs 1**	**Abs 2**	**Abs 3**	**Avg Abs**	**Concentration (mg/ml)**
2	0.1032	0.1032	0.1036	0.103	0.030
4	0.4467	0.4470	0.4474	0.447	0.130
6	0.7854	0.7847	0.7833	0.784	0.227
8	0.9985	0.9974	0.9985	0.998	0.289
10	1.1775	1.1792	1.1792	1.179	0.342
30	2.3765	2.3765	2.4045	2.386	0.692

Appendix AU – Results for High-throughput System: Glucose Using SAC Membranes

Abs = Absorbency

Trial 1					
Time (mins)	**Abs 1**	**Abs 2**	**Abs 3**	**Avg Abs**	**Concentration (mg/ml)**
2	0.3671	0.3673	0.3679	0.367	0.107
4	0.7417	0.743	0.7417	0.742	0.215
6	1.3847	1.3903	1.3903	1.388	0.403
8	1.7744	1.7812	1.7812	1.779	0.516
10	2.2382	2.2798	2.3018	2.273	0.659
Trial 2					
Time (mins)	**Abs 1**	**Abs 2**	**Abs 3**	**Avg Abs**	**Concentration (mg/ml)**
2	0.424	0.424	0.4237	0.424	0.123
4	1.217	1.217	1.217	1.217	0.353
6	2.1656	2.1656	2.21826	2.183	0.633
8	2.6263	2.7355	2.5806	2.647	0.768
10	2.6775	2.6775	2.6775	2.678	0.776
Trial 3					
Time (mins)	**Abs 1**	**Abs 2**	**Abs 3**	**Avg Abs**	**Concentration (mg/ml)**
2	0.9984	0.9884	0.9906	0.992	0.288
4	2.1656	2.1492	2.1826	2.166	0.628
6	3.2796	3.2796	3.2796	3.280	0.951
8	3.4345	3.4345	3.5014	3.457	1.002
10	3.6775	3.8024	3.8024	3.761	1.091

Appendix AV – Results for High-throughput System: BSA Using SAC Membranes

Trial 1 Absorbance Values

0.212	0.210	0.232
0.212	0.210	0.232
0.349	0.362	0.353
0.349	0.362	0.353
0.484	0.525	0.520
0.484	0.525	0.520
0.624	0.629	0.654
0.624	0.629	0.654

Trial 2 Absorbance Value

0.250	0.250	0.245
0.250	0.250	0.245
0.485	0.488	0.486
0.485	0.488	0.486
0.628	0.642	0.633
0.628	0.642	0.633
0.766	0.780	0.782
0.766	0.780	0.782

Trial 3 Absorbance Values

0.324	0.329	0.312
0.324	0.329	0.312
0.377	0.393	0.379
0.377	0.393	0.379
0.504	0.505	0.515
0.504	0.505	0.515
0.607	0.668	0.592
0.607	0.668	0.592

Appendix AW – Results for High-throughput System: Gamma Globulin Using SAC Membranes

Trial 1 Absorbance Values

0.812	0.808	0.831
0.812	0.808	0.831
1.428	1.430	1.447
1.428	1.430	1.447
1.862	1.971	1.914
1.862	1.971	1.914
2.206	2.255	2.189
2.206	2.255	2.189

Trial 2 Absorbance Value

0.669	0.649	0.652
0.669	0.649	0.652
1.217	1.260	1.201
1.217	1.260	1.201
1.647	1.715	1.698
1.647	1.715	1.698
2.024	2.083	2.126
2.024	2.083	2.126

Trial 3 Absorbance Values

0.671	0.704	0.686
0.671	0.704	0.686
1.172	1.218	1.169
1.172	1.218	1.169
1.572	1.554	1.573
1.572	1.554	1.573
1.893	1.914	1.822
1.893	1.914	1.822

Appendix AX – Rearranged Fick's Law Variable Substitutions

$$\frac{\left(-L \times V \times \ln\left(\frac{(C_a - 2C_b)}{C_a}\right)\right)}{2A_{eff}} = D_{eff} \times t$$

- L = Membrane thickness= ~ 100 um
- V = Chamber volume
  - PermeGear = 3 mL
  - High-throughput System = 5 mL
- C = Concentration, C_a = 5 mg/ml, C_b = unknown
- A = Area of the membrane effected by diffusion = opening of chamber in contact with membrane
  - PermeGear = 0.6364 cm^2
  - High-throughput system = 3.29867 cm^2
- t = time
- D = Diffusion Coefficient

Appendix AY – Calculations for Rearranged Fick's Law: Glucose

PermeGear ®

Concentrations in mg/ml

Time	Trial 1	Trial 2	Trial 3
2	0.072292	0.0884	0.142634
4	0.17824	0.123533	0.225009
6	0.212657	0.243618	0.315147
8	0.24945	0.36125	0.407739
10	0.381346	0.443286	0.508925

Rearranged Ficks Law (cm^2)

Trial 1	Trial 2	Trial 3
6.92E-04	8.49E-04	1.38E-03
1.74E-03	1.19E-03	2.22E-03
2.10E-03	2.42E-03	3.18E-03
2.48E-03	3.68E-03	4.20E-03
3.90E-03	4.60E-03	5.36E-03

High-Throughput

Concentrations in mg/ml

Time	Trial 1	Trial 2	Trial 3
2	0.106556	0.123	0.287815
4	0.215219	0.353	0.628082
6	0.402646	0.633	0.951084
8	0.515891	0.768	1.002472
10	0.659247	0.776	1.090622

Rearranged Ficks Law (cm^2)

Trial 1	Trial 2	Trial 3
3.30E-04	3.82E-04	9.27E-04
6.82E-04	1.15E-03	2.19E-03
1.33E-03	2.21E-03	3.63E-03
1.75E-03	2.78E-03	3.88E-03
2.32E-03	2.82E-03	4.34E-03

Dialysis Membrane

Concentrations in mg/ml

Time	Trial 1	Trial 2	Trial 3
2	0.02814	0.027511	0.030
4	0.133922	0.116319	0.130
6	0.259492	0.186257	0.227
8	0.344491	0.302161	0.289
10	0.390804	0.350223	0.342
30	0.696	0.620658	0.692

Rearranged Ficks Law (cm^2)

Trial 1	Trial 2	Trial 3
8.58E-05	8.39E-05	9.14E-05
4.17E-04	3.61E-04	4.04E-04
8.31E-04	5.87E-04	7.23E-04
1.12E-03	9.76E-04	9.33E-04
1.29E-03	1.14E-03	1.11E-03
2.47E-03	2.16E-03	2.46E-03

Appendix AZ – Calculations for Rearranged Fick's Law: BSA

PermeGear®

Concentrations in mg/ml

Time	Trial 1	Trial 2	Trial 3
1	0.0268	0.04244	0.04791
2	0.05079	0.074463	0.077679
3	0.073847	0.104216	0.087629
4	0.091116	0.121855	0.102514

Rearranged Ficks Law (cm^2)

Trial 1	Trial 2	Trial 3
2.54E-04	4.04E-04	4.56E-04
4.84E-04	7.13E-04	7.44E-04
7.07E-04	1.00E-03	8.41E-04
8.75E-04	1.18E-03	9.87E-04

High-Throughput

Concentrations in mg/ml

Time	Trial 1	Trial 2	Trial 3
1	2.26E-04	3.87E-04	2.72E-04
2	4.42E-04	4.90E-04	6.74E-04
3	7.15E-04	7.18E-04	9.63E-04
4	9.39E-04	9.66E-04	1.27E-03

Rearranged Ficks Law (cm^2)

Trial 1	Trial 2	Trial 3
6.86E-07	1.17E-06	8.23E-07
1.34E-06	1.48E-06	2.04E-06
2.17E-06	2.18E-06	2.92E-06
2.85E-06	2.93E-06	3.84E-06

Appendix BA – Calculations for Rearranged Fick's Law: Gamma Globulin

PermeGear

Concentrations in mg/ml

Time	Trial 1	Trial 2
1	0.057198	0.0292
2	0.09459	0.057199
3	0.159464	0.07061
4	0.188805	0.0947

Rearranged Ficks Law (cm^2)

Trial 1	Trial 2
5.46E-04	2.77E-04
9.09E-04	5.46E-04
1.55E-03	6.75E-04
1.85E-03	9.10E-04

High-Throughput

Concentrations in mg/ml

Time	Trial 1	Trial 2	Trial 3
1	0.440473	0.408999	0.400985
2	0.566038	0.518257	0.525953
3	0.65898	0.591142	0.614102
4	0.692376	0.712945	0.651235

Rearranged Ficks Law (cm^2)

Trial 1	Trial 2	Trial 3
1.47E-03	1.35E-03	1.32E-03
1.95E-03	1.76E-03	1.79E-03
2.32E-03	2.04E-03	2.14E-03
2.46E-03	2.54E-03	2.29E-03

Appendix BB –Analysis of PermeGear® vs. High-throughput System: Glucose

Data source: Deff Comparison in Glucose Statistical Analysis

Normality Test: Passed (P = 0.520)

Equal Variance Test: Passed (P = 0.834)

Group Name	N	Missing	Mean	Std Dev	SEM
P- Avg KD	3	0	0.000451	0.0000806	0.0000465
HT - Avg KD	3	0	0.000335	0.0000873	0.0000504

Difference 0.000116

t = 1.698 with 4 degrees of freedom. (P = 0.165)

95 percent confidence interval for difference of means: -0.0000740 to 0.000307

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.165).

Power of performed test with alpha = 0.050: 0.183

The power of the performed test (0.183) is below the desired power of 0.800.
Less than desired power indicates you are more likely to not detect a difference when one actually exists. Be cautious in over-interpreting the lack of difference found here.

Appendix BC –Analysis of PermeGear® vs. High-throughput System: BSA

Data source: Deff Comparison in BSA Statistical Analysis

Normality Test: Passed (P = 0.559)

Equal Variance Test: Passed (P = 0.469)

Group Name	N	Missing	Mean	Std Dev	SEM
KD - P	3	0	0.000213	0.0000464	0.0000268
KD - HT	3	0	0.000255	0.0000667	0.0000385

Difference -0.0000421

t = -0.897 with 4 degrees of freedom. (P = 0.420)

95 percent confidence interval for difference of means: -0.000172 to 0.0000882

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.420).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.
Less than desired power indicates you are more likely to not detect a difference when one actually exists. Be cautious in over-interpreting the lack of difference found here.

Appendix BD –Analysis of PermeGear® vs. High-throughput System: Gamma Globulin

Data source: Deff Comparison in GG Stat Analysis

Normality Test: Passed (P = 0.501)

Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
P- Avg KD	2	0	0.000165	0.0000898	0.0000635
HT - Avg KD	2	0	0.000164	0.00000354	0.00000250

Source of Variation	DF	SS	MS	F	P
Between Groups	1	1.323E-023	1.323E-023	3.277E-015	1.000
Residual	2	0.00000000808	0.00000000404		
Total	3	0.00000000808			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 1.000).

Power of performed test with alpha = 0.050: 0.058

The power of the performed test (0.058) is below the desired power of 0.800.
Less than desired power indicates you are more likely to not detect a difference when one actually exists. Be cautious in over-interpreting the lack of difference found here.