**Nanoscience Technology Assay Housing**

**Final Report**

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**Client: Dr. James Hickman**

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**Definitions**

**Important Acronyms:**

**µCCA:** Micro Cell Culture Analog

**MEA:** Micro Electrode Array

**MECA:** Micro Electrode Cantilever Array

**bioMEMS:** Biomedical Microelectromechanical Systems

**PDMS:** Polydimethylsiloxane, a silicon-based organic polymer

**Important Terms/Variables:**

**Chamber:** The area on which cells grow (includes, but is not limited to, area of MEA/MECA)

**Housing/array:** The combination of the entire design, including all chambers, channels, and other materials

**Channel:** Delivers fluid through the housing

**Residence Time, tr:** The average time that a given liquid particle will spend inside the chamber

**Hydraulic Resistance, RH:** Resistance to fluid flow generated by friction with chamber and channel walls

**Shear stress, τw:** The stress experienced on the cell surface parallel to the direction of flow

**Viscosity, η:** Measure of a fluid’s tendency to flow – for water (and cell growth medium), equal to 0.1$^{g}/\_{cm\*s}$

**L, W, and h:** The dimensions of the chambers (length, width, and height, respectively)

# Need/Scope

           For this project, the client is Dr. James Hickman at the University of Central Florida’s NanoScience Technology Center. Dr. Hickman’s group designs and constructs assays based on functional units of organ systems for drug screening. These assays can determine the effect of drugs on the functionality of the organ systems simulated through the use of various novel techniques created by Hickman’s group. Several of these assays have already been created, including models of human liver, motor neurons, skeletal muscle and cardiac muscle [1]. Because the human body is complex, a more sophisticated system of drug screening is desired. Drugs do not just affect the targeted systems, but can have a variety of effects on the other tissues in the body. An integrated, whole-body system of individual assays could predict these effects with more accuracy, and, in doing so, assist in the validation of drugs. Effective drugs would progress in the design process with more confidence, and ineffective drugs filtered out before production with minimal economic loss. Additionally, testing drug candidates with human cells cultured on chips will decrease the number of animals used in live trials.

The goal of this project is to combine different individual units (micro cell culture analogs, or µCCAs), each consisting of a different organ tissue, so that they can interact and be studied as a single functional model. In order to combine the individual units into one whole-body system, there are two main areas of consideration that must be addressed: the microfluidic packaging and the electric/optical packaging of the system. Microfluidic considerations include the physical layout of the individual units and the fluid dynamics in the system. This system must be as physiologically relevant as possible so that the cells behave as they do *in vivo*. The electrical/optical considerations have to do with the measurement of the functionality of organ tissues by bioMEMS devices. Each individual unit contains a microelectrode array (MEA) or micro electrode cantilever array (MECA). Multiple electrical inputs and outputs are required for this device, as well as microscope access. Furthermore, the skeletal and cardiac muscle subunits must be accessible by laser for force analysis. These two areas of consideration are not mutually exclusive, as the subunits must be organized in a layout that can be easily observed by microscope and laser, and the inputs and outputs are both accessible and organized.

# Design Requirements

For this project, the functional organ models have already been created and tested.

Additionally, some groups have created multi-organ systems using a variety of methods [2]. However, in order to create a multi-organ system that can be observed in great detail, certain design specifications must be met. This project focuses on four cell types: cardiomyocytes (heart muscle cells), hepatocytes (liver cells), motoneurons, and skeletal muscle cells. Each organ model presents unique challenges, and other factors involve the system as a whole and the interaction between the subunits.

All of the µCCA’s use a bioMEMS system to analyze and observe certain cell properties. Both the hepatocytes and the motoneurons use MEAs for this purpose. These arrays are mounted on a square that is 15mmx15mmx500µm, and contain 16 electrodes and one reference. Both the skeletal muscle and heart muscle systems employ MECAs that allow the researcher to determine contractile strength of the muscle cells. The cantilevers are the same size as the MEAs, but require laser access from the bottom of the chip in order to measure the force developed in the muscle. Furthermore, all four chambers will need to be accessible by optical microscopein reflective mode from the top of the chip for monitoring cell viability during the cell lifetimes.

 When designing the individual units and chambers, there are certain constraints that must be met. First, each chamber must be at least as large as the MEAs/MECAs (and therefore greater than 15mmx15mmx500µm). In addition, the chamber should be constructed so as to be as physiologically realistic as possible – this means that the liquid residence times, shear stress, and liquid-to-cell ratios should all be within physiological ranges, as defined in Table 1.

Additionally, it is better if the organ subunits interact in a physiologically meaningful way. The ratios of cells in this device should mimic those in the body. By using the average cell numbers of different organs [3, 4], we can estimate that the cell ratios for this array would be approximately 1 motoneuron: 4167 skeletal muscle cells: 3.33 \* 104 cardiomyocytes: 4.02 \* 106 hepatocytes. Additionally, not all organ systems receive equivalent blood flow in the human body [5, 6]. Therefore, the array must have the capability of modulating the flow rate to individual units such that the flow ratios are 1(motoneurons): 1(cardiomyocytes): 4.44(skeletal muscle): 7.33(hepatocyte). Unfortunately, it is not feasible to create a system that matches the above cell ratios – doing so would require chambers far too large for this application (dimensions on the order of meters). It is, however, possible to match the fluid flow ratios listed.

Table - Specific design requirements with metrics

|  |  |
| --- | --- |
| Specification | Metric |
| Physiological Shear Stress | 2 dynes/cm2 (within 5%) |
| Physiological residence times | Within 10% of physiological values |
| Reasonably small Dimensions | Maximum 3x3cm chambers, minimum 1.5x1.5 cm |
| Physiological liquid:cell ratio | 1:2 is ideal, no greater than 5:1 |
| Physiological relative flow rates | Within 5% of physiological values |
| Laminar flow | Re < 2300 |
| Optical (microscope) access for all chambers | Reflective mode, 0.8x-35x |
| Laser access for MECAs | 633 nm wavelength through glass |

In order to quantify the design specifications, the metrics shown in Table 1 were created. These metrics were chosen in such a way that they reflect the relative importance of different system parameters – shear stress being the most important (as cells behave differently under non-physiological shear stresses), and liquid-to-cell ratio being relatively unimportant.

# Design Analysis

**Design details and dimensions**

In order to obtain relatively uniform flow across the each chamber, the chambers are rectangular, with sub-branched channels as inlets and outlets. This design, along with some of the important dimensions, is shown in Figure 1.



**L1**

**L2**

**L**

**W**

Figure - Design of the chamber and sub-branching channels

 The sub-branching channels are created such that the form a 45˚ angle with the main channel – thus, $L\_{1}=\frac{W}{\sqrt{2}}$. Given that the four sub-branches are evenly spaced through the width of the chamber, it can be shown that the length of the short sub-branches is $L\_{2}=\frac{\sqrt{10}}{6}W$. Note that since W is different for each chamber, each cell type will have different lengths of sub-branches.

**Analysis of Chamber Dimensions**

 Many design alternatives were considered to meet the design requirements listed in Table 1. These alternatives were discussed in greater detail in the progress report, but detailed analysis of the final solution is shown here. The final housing and array features a single molded PDMS layer, chambers in parallel with varying lengths of channels leading to them, a single inlet/outlet, and rectangular chambers.

 The chamber dimensions were calculated in order to ensure physiologically relevant values for shear stress, liquid:cell ratio, and residence time. These values were calculated using a system of equations and inequalities derived from the metrics displayed in Table 1. Each chamber type has its own unique system of equations made up of Equations 1-5.

Equation - Derived from the equation for shear stress, relates the width, height, and shear stress to the flow rate

$$Q=\frac{Wh\_{liq}^{2}τ\_{w}}{6η}$$

Equation - Relates the height, length, and shear stress to the liquid residence time

$$t\_{r}=\frac{6ηL}{h\_{liq}τ\_{w}}$$

Equation - Limitations on the width of the chamber, set by the design metrics

$$1.5\leq W\leq 3$$

Equation - Limitations on the length of the chamber, set by the design metrics

$$1.5\leq L\leq 3$$

Equation - Limitations on the height of the chamber, determined by the metric for liquid:cell ratio

$$h\_{liq}\leq 5\*h\_{cell}$$

 It is important to note that the system of equations and inequalities described by Equations 1-5 does not, in most cases, have a unique solution. Given the nature of the design metrics and the presence of inequalities, certain parameters can be varied for each cell type (e.g. increasing width to decrease the height, or vice-versa). From the metrics certain values are known: $τ\_{w}=2^{dynes}/\_{cm^{2}}$ for each chamber, and both $t\_{r}$ and $h\_{cell}$ are found for each cell type in the literature [7, 8]. Finally, $Q\_{heart}$, the smallest flow rate, is found by setting $W\_{heart}$ and $L\_{heart}$ to 1.5cm, the smallest possible dimensions. Since the fluid flow ratios between the cell types are known, the flow rates for each branch are defined and only the chamber dimensions remain unknown. Values for these dimensions are determined to ensure the design metrics are satisfied. Table 2 displays the calculated chamber dimensions, as well as the residence times and liquid:cell ratios for each cell type. The chamber heights displayed in Table 2 represent the total height of the chamber, which includes the height of the liquid and the height of the cells.

Table - Calculated Chamber Dimensions, residence times, and liquid:cell ratios

|  |  |  |  |
| --- | --- | --- | --- |
| **Cell Type** | **Residence time,** $t\_{r}$ (s) | **Liquid:cell** | **Chamber Dimension (**$W x L x h$**, in mm)** |
| **Heart** | 77.5 | 1.162 | 15 x 15 x 0.1081 |
| **Neuron** | 124.3 | 1.000 | 20.256 x 20.715 x 0.100 |
| **Muscle** | 80 | 2.120 | 20 x 28.28 x 0.156 |
| **Liver** | 69.9 | 4.448 | 30 x 25.92 x 0.1262 |

**Analysis of Channel Lengths**

The lengths of the channels leading to each chamber are varied to obtain physiological relative flow rates – changing the lengths of the channels alters the hydraulic resistance of each branch, therefore controlling the relative flow rate. The hydraulic resistance of each branch is a sum of the resistance of the chamber and of the channels. The channels are of square cross section, 40µm x 40µm in size. As shown in Figure 1, the channels split four ways going into every chamber, and merge back into a single channel going out of the chamber. Thus, the total hydraulic resistance is represented by Equation 6.

Equation - The total hydraulic resistance of a given branch

$$R\_{branch}=R\_{chamber}+R\_{channel}+R\_{sub-branches}$$

Given that the chambers and channels both have rectangular cross sections, the hydraulic resistances can be simplified: $R\_{chamber}$ and $R\_{channel}$ are simply rectangular channels, while $R\_{sub-branches}$ is calculated by adding the four inlet sub-branches in parallel, and adding those in series to the four parallel outlet sub-branches. It can be shown that this is equivalent to the parallel-summed resistances of just two sub-branches, of lengths $L\_{1}$ and $L\_{2}$, as shown in Figure 1. Thus, by substituting in the equation for the resistance of a rectangular channel, the branch resistance can be rewritten as Equation 7. $R\_{L\_{1}}$ and $R\_{L\_{2}}$ also represent channels with rectangular cross sections.

Equation - Total hydraulic resistance of a given branch

$$R\_{branch}=\frac{12ηL\_{chamber}}{W\_{chamber}h\_{chamber}^{3}}+\frac{12ηL\_{channel}}{W\_{channel}h\_{channel}^{3}}+\left(\frac{1}{R\_{L\_{1}}}+\frac{1}{R\_{L\_{2}}}\right)^{-1}$$

The ratios of the flow rates are known, and therefore the ratios of the branch resistances can be found by the equation for a flow divider and the equation for the total resistance shown in Equations 8 and 9, respectively.

Equation – The equation for a flow divider for parallel branches

$$\frac{Q\_{Branch}}{Q\_{Total}}=\frac{R\_{Total}}{R\_{Branch}}$$

Equation 9 – The total resistance of a system in parallel

$$R\_{Total}=\left(\frac{1}{R\_{branch1}}+\frac{1}{R\_{branch2}}+\frac{1}{R\_{branch3}}+\frac{1}{R\_{branch4}}\right)^{-1}$$

Together, these reduce to a series of equations, shown as Equations 10-12, that relate the channel lengths for each branch.

Equation 10 - Relates the length of the channels in the neuron branch to the length of the channels in the cardiac branch

$$L\_{neuron}=L\_{heart}-0.3247$$

Equation 11 - Relates the length of the channels in the skeletal muscle branch to the length of the channels in the cardiac branch

$$L\_{muscle}=\frac{L\_{heart}}{4.44}-1.002$$

Equation 12 - Relates the length of the channels in the hepatocyte branch to the length of the channels in the cardiac branch

$$L\_{liver}=\frac{L\_{heart}}{7.33}-1.685$$

Next, the shortest branch (the hepatocyte branch) is set to be 97.3 mm in length. This value represents the length of channels needed in that branch (excluding sub-branches and chamber dimensions), and is determined by the dimension of the entire housing. This value is then plugged into Equations 10-12 to determine the total channel lengths for each branch. Table 3 shows the lengths of these channels, as well as the resulting flow rates in each chamber/branch.

Table - Relative lengths and flow rates for each branch

|  |  |  |
| --- | --- | --- |
| **Cell Type** | **Channel Length (mm)** | **Flow Rate (uL/min)** |
| **Heart** |  725.3 | 10.128 |
| **Neuron** |  724.9 | 10.128 |
| **Muscle** |  162.3 | 44.97 |
| **Liver** |  97.3 | 74.22 |

The summation of the flow rates in each branch gives a total flow rate through the housing of 139.5 µL/min – this is the flow rate that the pump used for the array will be set to.

**Safety Analysis**

 In this design, there are several primary vectors to be concerned about when analyzing for safety. Through the utilization of DesignSafe® software, these risks were analyzed and evaluated in order to prepare for safe usage of the system and to minimize risk in its implementation.

 Unintentional contact with cells while plating the chambers is a very serious concern. This process is done manually, and has the potential for biohazard contamination if care is not taken in this aspect of assembly. Steps which can be taken to minimize risk are primarily the proper usage of personal protective equipment, such as gloves, goggles, and a lab coat. Additionally, caution should be exercised whenever biohazard materials are being handled, but particularly when the cells are being plated, with the applicant taking care not to splash or spill any of the cells. If these safety protocols are followed, the risk of contact with the cells is significantly decreased, and once the system is sealed the risk should be negligible.

 During experiments, the pump provides continuous flow throughout the system. This raises concern over the possibility of a leak, spill, or rupture of the system, which would lead to the uncontrolled ejection of potentially dangerous liquid. The fluid contained in the system should not be handled directly, as it may contain harmful substances or chemicals. Testing system integrity with non-toxic fluids before experimentation begins and ensuring that the system operates well within the flow rate parameters can minimize the risk of fluid expulsion from the system. Additionally, care should be taken at all times during experimentation to wear proper safety equipment, in case leaks arise. Through the preliminary testing of system integrity and the utilization of safety equipment, the risk of dangerous fluid exposure can be lessened to acceptable levels.

 Lasers can pose a risk to users, as they focus a large amount of energy over an extremely small area. Carelessness while operating the lasers during testing could lead to blindness, or skin burns. However, this risk is not large and can be primarily avoided by remaining cautious while activating the laser, and through wearing safety goggles to protect one’s eyes.

# Specific Details of Chosen Design

**Summary of Chosen Design**

 The device will have a single layer design, with channels and chambers machined into the same housing piece; branch points for the parallel organ chambers machined into the housing after the inlet and before the outlet; rectangular chambers with multiple inlets and outlets; laser access by way of glass mounted to the entirety of the underside of the µCCA housing; chambers with no microstructures; and clear, PDMS housing for optical access to the chambers. The housing will consist of two pieces of PDMS: a cap and a body. The body will have the channels and chambers machined into it, while the cap will contain the inlets and outlets. Between the cap and body of the PDMS housing will be a layer of clear silicone, acting as a gasket to seal the system. The cap will attach to the body of the cell array via four screws on the corners to apply compression equally over the surface of the chip. Sealed to the bottom of the body via oxygen plasma bonding will be a single layer of glass. In each chamber will be a silicon chip, either a MECA or MEA, for functional analysis of the cells. These silicon chips will also be attached to the PDMS housing through oxygen plasma bonding.

 The layout of the device can be seen, in a general form, in Figure 2. The chambers will be in parallel, with channels connecting them. While Figure 2 is not drawn to scale, it does illustrate the outline of the system. Each chamber varies in size, but contains a single bioMEMS chip embedded in it, on which the cells will grow. The lengths of the microchannels for each branch are varied by snaking the channels. This controls the hydraulic resistances of each branch to create physiological flow ratios.

Figure - Top view of the housing and assembly. This figure shows the important design elements but is not drawn to scale.

## Layers

In this device, the biggest design consideration is easy access to the cells to assess viability. Ideally, the cell viability can be determined by optical viewing of the chambers while the device is fully assembled. The channels will be small indentations cut out from the top of the PDMS housing – in Figure 3, these channels are not pictures as they would be going into/out of the page. These channels will branch out from the inflow, and feed directly into the 4 chambers. These chambers are all different dimensions, and have varying cell heights as well. The MEAs and MECAs lie directly below the cells, and held up by PDMS “ledges”. There is a small amount of empty space below the MEAs and MECAs where lasers have access to their underside for cell analysis, and though this is only necessary for the MECAs it is included for the MEAs as well. The depth of this space varies based on the heights of the individual chambers. Figure 3 shows a side-view cross section of the four adjacent chambers of varying heights and dimensions. Once again, the figure is not drawn to scale but rather illustrates the important design elements.

Housing

Glass

MEA or MECA

Cells

Fluid

Cap

Silicone Gasket

PDMS

Figure - Side section view of a chamber showing laser access

The PDMS housing assembly is bonded to a thin piece of glass which is attached beneath the housing. This glass has the correct optical properties to allow the lasers to shine through to interrogate the MECAs. The BioMEMS devices (MEAs and MECAs) are set into grooves designed into the bottom of the cell chambers, such that the top of the devices is flush with the floor of the chamber. They are oxygen plasma bonded there so that no fluid can pass through and interfere with the electronics, which are located on the bottom of each chip. The BioMEMS devices have electronic leads which will be drawn through small holes drilled into the side of the PDMS assembly prior to bonding to the glass underside. These holes are drilled into the side of the housing such that they do not interfere with the fluidic system, and only intersect with the empty space below the MEMS devices. Above the PDMS assembly is a cap also created out of PDMS. A molded silicone gasket is placed between the cap and the bottom assembly, and the pieces are joined via titanium screws, creating a seal. The clear optical properties of the PDMS allow for the viewing of cells through the top of the housing using microscopes, and having a lid on top sealing the system together allows for the creation of a closed fluidic system. The selected design allows for the plating of cells before attaching the cap, which can be done following normal cellular culture protocols.

## Chamber Shape

Another set of design alternatives considered for this device is the shape of the chambers that contain the cells. The chamber needs to accomplish the following tasks: culture cells, house the silicon chips used for cellular functional measurement, and direct fluid flow uniformly across the cells for drug metabolism. The solution chosen for this design element is a rectangular chamber, which has a rectangular cross section, and also has multiple inlet and outlet sub-channels.

This chamber shape, proposed by Sung et al., has four sub-branch points before and after each cell chamber evenly spaced across its width, which serve to spread the fluid flow much more evenly across the entire chamber [10]. This rectangular chamber shape, seen in Figure 4, provides the most even flow over the cell area, a critical component in emulating physiological conditions. In this design, because of its fully laminar flow, all cells should receive flow such that the variance in different parts of the chamber is negligible. However, while multiple inlets and outlets to each chamber allows for more even flow, it also makes resistance calculations more difficult.

Cell chamber

Inlet sub-branches

Outlet sub-branches

Figure - Diagram of chambers with multiple inlet and outlet sub-branches

## Laser Access

Another design element considered for this device is the method of access by laser for two of the four chambers. The laser needs to interrogate the underside of the MECA’s, located in the skeletal and cardiac muscle cell chambers, for functional analysis. This is achieved by reflecting the laser off the bottom of the silicon cantilevers and then onto a detector. When the muscle cells contract, they produce a bending moment on the cantilever that deflects the laser, and this deflection is then recorded by the detector. Thus, there must be a pathway for the laser to travel so that it can reach the underside of the MECA’s unimpeded. This has been done previously by sealing the bottom of the MECA to a glass coverslip with a silicon gasket, providing a clear material that the laser could travel through [11]. In other designs, the MECA was not subjected to microfluidic flow and was not contained in a device with other chambers.

The solution selected in this design is to cover the entirety of the underside of the cell array housing with a thin sheet of glass. The chambers will be etched so that the silicon chip is flush with the bottom of the chamber, with free space underneath the MECA. The laser will shine through the glass, through the empty cavity below the silicon chip, and reflect back onto the detector. This allows access by laser to every chamber in the design, though only the two chambers containing the MECAs require it. This design is the most simplistic in fabrication and setup, and was chosen for these reasons. However, it uses more glass than the other designs and requires the entire housing to be sealed against the glass, rather than the individual chambers.

The PDMS material used in the assembly does not preserve the integrity of the laser light, which is why glass must be placed under the PDMS assembly. Having one large glass slide that the entire PDMS assembly can be bonded to allows for much simplified assembly, as well as creating a cavity underneath the MECA’s to allow the electrical leads access to the chips. Having a single glass slip also means that the cell chambers can be filled after the PDMS assembly has already been bonded to the system housing, simplifying the assembly process and decreasing possible danger to the cells by having to bond the PDMS assembly after plating the cells. In this design, when calculating the deflection of the laser, both the refractive index of the glass as well as the cavity below the MECA (i.e. air) must be taken into account when programming the detector.

## Optical Access

One of the most important design requirements that must be met is the ability to optically image the cells, mainly for assessing cell viability. The cells will be imaged by microscopes in reflective mode, meaning light must be able to penetrate the chambers, reflect off the cells/chip, and bounce back to the receiver. In order for the light to enter the chamber, the lid must either be transparent or must be easily removable. Since PDMS is transparent to white light, and PDMS is the material being used for the housing, the cells will be able to be imaged through the housing without having to remove the lid. This prevents possible contamination of the cell cultures. Having a lid made of PDMS means that once sealed, the system can remain in a closed state while cell viability is assessed, a critical requirement in providing for system longevity.

**CAD Design**

The housing array (i.e. the PDMS housing) was designed using Autodesk Inventor CAD software. Figures 5 and 6 show a 3D rendering of this design, which includes the specific design components discussed previously. Due to the relative scales of the chambers and channels, it is difficult to visualize certain components of the design in the CAD model; this is why general outlines such as Figures 2, 3, and 4 are included in this report.

 

Figure 5 - 3D rendering of the bottom layer of the PDMS housing assembly

 Additionally, the PDMS cap contains the inlet and outlet for the system. Shown in Figure 6, the lid is a solid piece of PDMS with lofted inlet/outlet tubing.



Figure 6 - 3D rendering of the top layer of the PDMS housing assembly

# Specific Parts, Materials, and Manufacturing

**PDMS Creation**

The chip in this design will be constructed out of polydimethylsiloxane, or PDMS. This material is a mineral-organic polymer commonly used in microfluidic chip design. The specific type of PDMS material that will be used is PDMS Sylgard 184. The creation of the PDMS assembly is a multi-step process, seen in Figure 7, from McDonald et al. [12].



Figure 7- PDMS fabrication process, from McDonald et al.

Creating a microchip using PDMS molding is an established process. We will be closely following the process explained by Friend, James, and Leslie Yeo [13]. Care must be taken throughout the fabrication process to avoid surface contamination. If fabrication cannot be done in a clean environment, rinsing tools and substrates with deionized water is an acceptable alternative. Initially, photolithography is used to create a negative mold for the desired chip, using the desired dimensions and layout. Because the channels and chambers in this design are located at the top of the assembly, the mold will be created upside down. This mold can be created out of a variety of materials, but SU-8, an epoxy-based negative photoresist will be used in this design, as it is the most common molding media used in PDMS microchips. The SU-8 polymer will be poured onto a silicon wafer substrate, rotating at 500 rpm for 15 seconds, and then 30 seconds rotating at 1250 rpm. This will yield the desired housing thickness of 500 um. This creates a photoresist, which must then be degassed to remove any bubbles. This is achieved by heating the photoresist to 50⁰ C. Next, the SU-8 will be prebaked to evaporate the solvent. This is done by heating the photoresist to 90⁰ C for 75 minutes. The photoresist will then be covered with a laser printed overhead transparency (250 um resolution) and exposed to 350 um UV light to achieve the required film thickness. The final step in creating the mold is a post bake, at 90⁰ C for 15 minutes. The completed mold will then be placed in a heat tolerant plastic tray and the PDMS will then be poured over it. A silicon overlay will be placed on top of the poured PDMS and a 1 kg weight will be placed on top of the overlay in order to squeeze out any excess PDMS. Finally, this assembly will be placed inside an oven at 70⁰ C for 1 hour in order to cure the PDMS, which can then be cut away from the mold.

**System Assembly**

 Once the PDMS assembly has been created, the full microfluidic system can be assembled. The first step in this process is the drilling of 4 small holes in the lower side of the PDMS assembly, connecting to the cavities below the bioMEMS chips and large enough to allow the electrical leads of the chips to be fed through to the outside of the assembly. The BioMEMS chips are then sealed flush to the bottom of the cell chambers through oxygen plasma bonding. In this process, the surface to be bonded is exposed to oxygen plasma for 10 minutes. The PDMS is then placed immediately against the glass or BioMEMS surface, forming a permanent bond. Once the BioMEMS chips are bonded to the PDMS assembly, the entire assembly, with attached chips, is bonded in the same way to the top of the glass microscope slide. The next step in the assembly process is the plating of the cells, to the densities and heights determined in the calculations above. After the cells have been plated, grown, and assessed for initial viability, the PDMS cap can be placed upon the housing and assembly. The top and bottom pieces of the PDMS housing are sealed through the use of silicon gaskets, which provide a flush seal between the layers, creating a closed system. The cap is secured to the main housing by 4 titanium screws on the corners. The next step in the assembly process is to attach to the PDMS cap the inflow and outflow tubes, and then to connect the tubes to the fluid reservoir/pump system. Fluid can then be pumped into the housing to create a closed fluidic flow system, completing the assembly process.

**Material Specifications and Costs**

In this system, there are a variety of components which must be assembled into the final system, and each component must fit several requirements. The creation of a photoresist mold and the PDMS fabrication process is done in the Hickman lab, and the specific costs of production can be neglected in this report. The specific material used in the PDMS cell array and assembly cap is Sylgard 184 [14]. This is the cleaner variant of PDMS, and is transparent at optical frequencies. This material is most commonly used in mammalian cell cultures. This elastomer is prepared in a 10:1 mix, and some key properties of the material are minimal shrinkage and no exotherm during cure, as well as no solvents or cure byproducts. This material costs $60.48 for 0.5 kg of material, which is more than sufficient to create the designed assembly [15].

 The BioMEMS silicon chips utilized in the housing assembly are a proprietary invention of the Hickman Lab, and thus their cost is disregarded in the report. Each chip is formed out of silicon, and their dimensions are 15mm x15 mm, with a thickness of 500 µm. The bonding process which attaches the silicon chips and the glass slip to the PDMS is another step done in the lab by the Hickman group, and can be neglected for the purposes of this report.

 The PDMS cell array and assembly cap are sealed through the use of a silicon gasket. This gasket must cover the top of all channels and chambers in the cell array in order to maintain a closed fluidic system. This gasket will be a flat sheet of transparent silicone clear rubber, a high performance elastomer with exceptional resistance to temperature, remaining functional at temperatures up to 180⁰ F. This high temperature resistivity means that the microfluidic system can be placed in an autoclave for decontamination without adverse effects to the system seal. Additionally, silicone has outstanding tensile strength, elongation, tear strength, and compression set. These physical properties, in addition to the material’s low temperature flexibility, allow for the creation of a tight seal of the system when compressed. This material can be purchased as a sheet, and trimmed in lab to fit the specific microfluidic system. This material costs $40.03 for a sheet that is 30.48 cm x 30.48 cm and is .397 mm thick [16].

The PDMS cap and cell array are attached with 4 ProTek R/C Titanium Screws. Titanium has an extremely high strength-to-density ratio, is a very strong material, and is much lighter than steel. When tightened properly, these screws will compress the silicon gasket, sealing the channels in the cell array to create a closed system. The dimensions of these screws are 4 mm radius, and 14 mm length. These screws are flat head hex screws, and will require a screwdriver to match. These screws cost $8.00 for 8 screws [17].

 The PDMS housing is placed on top of a large glass slip to allow for laser access to the MECA’s from the underside of the housing. This glass slip is made from optical grade soda lime glass, which has a refractive index of 1.515 for calculating laser deflection [18]. The dimensions of this slide are 178 mm x 127 mm, with a thickness of 1.2 mm. The glass slide costs $178.35 for a package of 36, and can therefore be bought in bulk to supply slides for many microfluidic devices [19].

 As discussed previously, the success of the system depends on a consistent and precise fluid flow rate. There are a large variety of pumps that can be used to attain this flow rate. This pump is connected to the fluid reservoir and the inflow to the cell array, and is an integral part to the design. The pump selected is a Masterflex C/L Variable-Speed Tubing Pump, which can run between 50 and 300 rpm and requires 90 to 260 VAC of power. This is a peristaltic pump which can support flow rates between 0.002 and 43.0 mL/min, meeting the required flow rate of 0.1395 mL/min, and also allowing room for adjustment if certain design specifications are altered. Peristaltic pumps are widely used in microfluidics due to their ability to achieve relatively steady, low flow rates [20]. The Masterflex pump is also quite compact, with dimensions of 17.8 cm x 9.5 cm x 8.9 cm, and comes with 1.5 m of 0.89 mm ID Tygon LFL tubing, which can connect the pump to the fluid reservoir and the inflow of the cell array. The pump also comes with a dual voltage (115/230 VAC) power supply with a 1.8 m power cord, allowing flexibility in the placement of the pump relative to the system. The pump is the most expensive component of the system, as it costs $645.00, but it can also be reused through multiple cell array experiments, making it a long-term investment [21].

**Conclusion**

**Summary of Solution**

 Over the course of this project, several key concepts and ideas have become apparent. Initially, not enough preparation and research was done on the topic, and preliminary brainstorming sessions suffered as a result. More time invested in planning and research in the early stages of the project would have been much more efficient. Additionally, the scope at the start of the project was extremely broad, with too many variables and considerations to realistically balance. Initially the project was to design a 10-organ cell array, with considerations on every aspect of the system. This scope was later narrowed to a 4-organ cell array, with several proprietary system constraints allowing for more focus on other variables. More detailed conversations with the client would have served to narrow the scope down quickly, allowing more time to focus on the key elements of the design.

**Future Applications**

 The system designed here is useful on its own for doing experiments on cell viability and interaction between the 4 organ systems selected, but there are numerous directions that this project could be applied to in the future. Different organ systems could be selected using the same basic system, adjusting chamber dimensions to suit physiological values. This could allow for greater variability in cell types being examined. Another future application of this project is the expansion to more than 4 organ systems. Theoretically, 5 or 6 or even as many as 10 organ systems could be interconnected on the same housing, which would go a long way towards the eventual goal of an entire human body system modeled with a single array. Additionally, because this design allows laser access for all of the chambers, one or both of the MEAs could be replaced by MECAs without altering the design. Another possible future direction of this project is the application of such a microfluidic system in the testing of potential drug candidates on human organs. This is a safer, cheaper alternative to either animal testing or human trials, and if such a device were proven to work consistently in predicting drug interactions, it could tap a massive market in pharmaceutical development companies.

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**Appendix A: Product Specification Sheets/Company Contact Information**

Dow Corning Sylgard 184 Silicone Encapsulant Clear 0.5 kg Kit

* Product # 184 SIL ELAST KIT 0.5KG
* Technical Data: <file://warehouse2.seasad.wustl.edu/home/e.galles/My%20Documents/Dow-Corning-Silicone-Encapsulant-Product-Information.pdf>
* Ordering Information: <http://www.ellsworth.com/dow-corning-sylgard-184-silicone-encapsulant-0-5kg-kit-clear/>
* Ellsworth Adhesives
W129 N10825 Washington Dr.
Germantown, WI 53022
* **Phone:** 1-800-888-0698

**Transparent Silicone Clear Rubber**

* **Technical Data:** <http://www.rubbersheetroll.com/techdata/transparent_silicone_clear_rubber.pdf>
* Ordering Information: <http://www.rubbersheetroll.com/silicone-rubber-clear.html>
* P.O. Box 171

Shippensburg, PA 17257

* **Phone:** 866-832-6006

ProTek R/C 4x14 mm Titanium Flat Head Hex Screw

* Product # PTK-T-5058
* Technical Data: <http://www.northamericanalloys.com/CPTi%20props.htm>
* Ordering Information: <http://www.amain.com/protek-r-c-4x14mm-titanium-flat-head-hex-screw-8/p300749?utm_source=GoogleBase&utm_medium=cpc&utm_campaign=ProductFeeds&gclid=CIeZl_aApMICFeRzMgodTgwAVg>
* A Main Hobbies

424 Otterson Drive, Suite 160

Chica, CA 95928

* **Phone:** 1-800-705-2215

Large Microscope Slides

* Product # 260234
* Technical Data: <http://www.tedpella.com/technote_html/0215%20corning%20glass.pdf>
	+ Ordering Information: <http://www.tedpella.com/histo_html/slides-large.htm>
* P.O. Box 492477

Redding, CA 96049

* **Phone:** 800-237-3526

Masterflex C/L Variable-Speed Tubing Pump; 50-300 rpm, 115/230 VAC

* Product # HV-77122-24
* Instruction Manuel: [http://www.masterflex.com/Assets/manual\_pdfs/77122-04,-06,-14,-16,-,24,-26.pdf](http://www.masterflex.com/Assets/manual_pdfs/77122-04%2C-06%2C-14%2C-16%2C-%2C24%2C-26.pdf)
* Ordering Information: <http://www.masterflex.com/Product/Masterflex_C_L_Variable_Speed_Tubing_Pump_50_to_300_rpm_115_230_VAC/HV-77122-24>
* 625 East Bunker Court

Vernon Hills, IL 60061

* **Phone:** 1-800-323-4340

**Appendix B: CAD Design**



## Appendix C: Designsafe 6 Report

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Application: | NanoScience Technology Assay Housing | Description: | A housing for a micro cell culture ananlog device to screen drugs on a multi-organ system. | Analyst Name(s): | Chase Walker, Eric Galles, Joe Marmerstein |
|  |  |  |  | Company: |  |  |  |
|  |  |  |  |  |  |  |  |  |
| Assessment Type: | Detailed |  |  |  |  |  |  |  |
| Limits: |  |  |  |  |  |  |  |  |
| Risk Scoring System: | ANSI B11.0 (TR3) Two Factor |  |  |  |  |  |  |
|   |   |   | **Initial Assessment** |   |   | **Final Assessment** |   | **Status /**  |
|   | **User /** | **Hazard /** | **Severity /** |   | **Risk Reduction Methods /** | **Severity /** |   | **Responsible/** |
| **Item Id** | **Task** | **Failure Mode** | **Probability** | **Risk Level** | **Comments** | **Probability** | **Risk Level** | **Reference** |
|   |   |   |   |   |   |   |   |   |
| 1-1-1 | Equipment Servicer  | biological / health : hazardous biological waste  | Serious  | High  | safety glasses, gloves, special clothing,  | Serious  | Low  | On-going [Daily]  |
|   | install / setup equipment  | Contact with Cells  | Likely  |   | good lab practice, clean environment | Remote  |   | Equipment Servicer  |
|   |   |   |   |   |   |   |   |   |
| 1-1-2 | Equipment Servicer  | biological / health : bacterial  | Serious  | High  | safety glasses, gloves, special clothing, | Serious  | Low  | On-going [Daily]  |
|   | install / setup equipment  | Contact with Cells  | Likely  |   | good lab practice, clean environment | Remote  |   | Equipment Servicer  |
|   |   |   |   |   |   |   |   |   |
| 1-1-3 | Equipment Servicer  | mechanical : pinch point  | Minor  | Negligible  | extra care when assembling system  | Minor  | Negligible  | On-going [Daily]  |
|   | install / setup equipment  | Careless Assembly of parts  | Unlikely  |   |   | Remote  |   | Equipment Servicer  |
|   |   |   |   |   |   |   |   |   |
| 1-1-4 | Equipment Servicer  | electrical / electronic : water / wet locations  | Moderate  | Low  | extra care when bonding BioMEMS to PDMS housing  | Moderate  | Negligible  | On-going [Daily]  |
|   | install / setup equipment  | Poor electrical isolation from fluid  | Unlikely  |   |   | Remote  |   | Equipment Servicer  |
|   |   |   |   |   |   |   |   |   |
| 1-1-5 | Equipment Servicer  | chemical : reaction to / with irritant chemicals  | Serious  | High  | safety glasses, gloves, special clothing, | Serious  | Low  | On-going [Daily]  |
|   | install / setup equipment  | Exposure to drugs used on cells  | Likely  |   | good lab practice, clean environment | Remote  |   | Equipment Servicer  |
|   |   |   |   |   |   |   |   |   |
| 1-1-6 | Equipment Servicer  | chemical : skin exposed to toxic chemical  | Serious  | High  | safety glasses, gloves, special clothing, | Serious  | Low  | On-going [Daily]  |
|   | install / setup equipment  | Exposure to drugs used on cells  | Likely  |   | good lab practice, care while handling drugs | Remote  |   | Equipment Servicer  |
|   |   |   |   |   |   |   |   |   |
| 1-1-7 | Equipment Servicer  | chemical : failure at key points and trouble spots  | Serious  | Medium  | test system integrity with non-toxic fluids before  | Serious  | Low  | On-going [Daily]  |
|   | install / setup equipment  | Failure of device to contain drugs  | Unlikely  |   | experimentation begins with the system | Remote  |   | Equipment Servicer  |
|   |   |   |   |   |   |   |   |   |
| 1-1-8 | Equipment Servicer  | chemical : chemical / toxicity effects felt at distant time / place  | Serious  | High  | safety glasses, gloves, special clothing,  | Serious  | Medium  | On-going [Daily]  |
|   | install / setup equipment  | Exposure to drugs with long term effects  | Likely  |   | good lab practice, clean environment | Unlikely  |   | Equipment Servicer  |
|   |   |   |   |   |   |   |   |   |
| 1-1-9 | Equipment Servicer  | fluid / pressure : hydraulics rupture  | Serious  | High  | test system integrity with non-toxic fluids prior | Serious  | Medium  | On-going [Daily]  |
|   | install / setup equipment  | Pressure build-up in the system resulting in rupture  | Likely  |   | to experimentation | Unlikely  |   | Equipment Servicer  |
|   |   |   |   |   |   |   |   |   |
| 1-1-10 | Equipment Servicer  | fluid / pressure : fluid leakage / ejection  | Serious  | High  | test system integrity with non-toxic fluids prior | Serious  | Medium  | On-going [Daily]  |
|   | install / setup equipment  | hydraulic rupture would result in the expulsion of potentially toxic fluid  | Likely  |   | to experimentation | Unlikely  |   | Equipment Servicer  |
|   |   |   |   |   |   |   |   |   |
| 1-2-1 | Equipment Servicer  | chemical : reaction to / with irritant chemicals  | Serious  | High  | safety glasses, gloves, special clothing,  | Serious  | Medium  | On-going [Daily]  |
|   | equipment maintenance  | Exposure to irritating drugs upon accidental contact, | Likely  |   | good lab practice, clean environment | Unlikely  |   | Equipment Servicer  |
|   |   |  leakage, or ejection of fluid  |   |   |   |  |   |   |
|   |   |   |   |   |   |   |   |   |
| 1-2-2 | Equipment Servicer  | chemical : skin exposed to toxic chemical  | Serious  | High  | safety glasses, gloves, special clothing, | Serious  | Medium  | On-going [Daily]  |
|   | equipment maintenance  | Exposure to toxic drugs upon accidental contact, | Likely  |   | good lab practice, clean environment | Unlikely  |   | Equipment Servicer  |
|   |   | leakage, or ejection of fluid  |   |   |   |  |   |   |
|   |   |   |   |   |   |   |   |   |
| 1-2-3 | Equipment Servicer  | chemical : failure at key points and trouble spots  | Serious  | Medium  | test system integrity with non-toxic fluids  | Serious  | Low  | On-going [Daily]  |
|   | equipment maintenance  | Key points for failure are the inlets and outlets of the PDMS housing,  | Unlikely  |   | prior to experimentation, special clothing when | Remote  |   | Equipment Servicer  |
|   |   | where pressure can build up and break connections  |   |   | running tests |  |   |   |
|   |   |   |   |   |   |   |   |   |
| 1-2-4 | Equipment Servicer  | chemical : chemical / toxicity effects felt at distant time / place  | Serious  | High  | test system integrity with non-toxic fluids  | Serious  | Medium  | On-going [Daily]  |
|   | equipment maintenance  | Exposure to drugs with long term effects upon accidental contact,  | Likely  |   | prior to experimentation, special clothing when | Unlikely  |   | Equipment Servicer  |
|   |   | leakage, or ejection of fluid  |   |   | running tests |  |   |   |
|   |   |   |   |   |   |   |   |   |
| 1-2-5 | Equipment Servicer  | fluid / pressure : hydraulics rupture  | Serious  | High  | test system integrity with non-toxic fluids  | Serious  | Medium  | On-going [Daily]  |
|   | equipment maintenance  | Pressure build-up in the system resulting in rupture  | Likely  |   | prior to experimentation, special clothing when | Unlikely  |   | Equipment Servicer  |
|   |   |   |   |   | running tests |   |   |   |
| 1-2-6 | Equipment Servicer  | fluid / pressure : fluid leakage / ejection  | Serious  | High  | test system integrity with non-toxic fluids  | Serious  | Medium  | On-going [Daily]  |
|   | equipment maintenance  | hydraulic rupture would result in the expulsion of potentially toxic fluid  | Likely  |   | prior to experimentation, special clothing when | Unlikely  |   | Equipment Servicer  |
|   |   |   |   |   | running tests |   |   |   |
| 1-3-1 | Equipment Servicer  | fluid / pressure : hydraulics rupture  | Serious  | High  | test system integrity with non-toxic fluids  | Serious  | Medium  | On-going [Daily]  |
|   | adjust controls  | Pressure build-up in the system resulting in rupture  | Likely  |   | prior to experimentation, special clothing when | Unlikely  |   | Equipment Servicer  |
|   |   |   |   |   | running tests |   |   |   |
| 1-3-2 | Equipment Servicer  | fluid / pressure : fluid leakage / ejection  | Serious  | High  | test system integrity with non-toxic fluids  | Serious  | Medium  | On-going [Daily]  |
|   | adjust controls  | hydraulic rupture would result in the expulsion of potentially toxic fluid  | Likely  |   | prior to experimentation, special clothing when | Unlikely  |   | Equipment Servicer  |
|   |   |   |   |   | running tests |   |   |   |
| 1-4-1 | Equipment Servicer  | electrical / electronic : energized equipment / live parts  | Serious  | Low  | extra care when plugging in power source  | Serious  | Low  | On-going [Daily]  |
|   | conduct tests  | Handling of power supply  | Remote  |   |   | Remote  |   | Equipment Servicer  |
|   |   |   |   |   |   |   |   |   |
| 1-4-2 | Equipment Servicer  | electrical / electronic : overvoltage /overcurrent  | Serious  | Medium  | voltage control through circuit , care when plugging | Serious  | Low  | On-going [Daily]  |
|   | conduct tests  | Accidental over-application of voltage to MEA's and MECA's.  | Unlikely  |   | in power sources | Remote  |   | Equipment Servicer  |
|   |   |   |   |   |   |   |   |   |
| 1-4-3 | Equipment Servicer  | lasers : eye exposure  | Serious  | Low  | safety goggle usage, care when activating | Serious  | Low  | On-going [Daily]  |
|   | conduct tests  | Accidental exposure  | Remote  |   | laser testing | Remote  |   | Equipment Servicer  |
|   |   |   |   |   |   |   |   |   |
| 1-4-4 | Equipment Servicer  | lasers : UV skin exposure  | Serious  | Low  | proper safety attire and clothing when running | Serious  | Low  | On-going [Daily]  |
|   | conduct tests  | Accidental exposure  | Remote  |   | experiments | Remote  |   | Equipment Servicer  |
|   |   |   |   |   |   |   |   |   |
|  |  |  |  |  |  |  |  |  |