**Nanoscience Technology Assay Housing**

**Preliminary Report**

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**Client: Dr. James Hickman**

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**Need/Scope**

           For our senior design project this fall, we will be working with Dr. James Hickman and Balaji Srinivasan 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 complex system of drug screening is desired. Drugs don’t just affect the targeted systems, but can have a variety of effects on the other, not-targeted tissues of 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.

Our goal will be to combine different individual units, 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 will include the physical layout of the individual unit and the fluid dynamics in the system. The electrical/optical considerations have to do with the measurement of the functionality of organ tissues by bioMEMS devices. Each individual unit will contain a microelectrode array (MEA) or micro electrode cantilever array (MECA). Required for these devices are multiple electrical inputs and outputs, 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. Furthermore, the housing should be adaptable so that as new assays are created, subunits can be added to the original design.

We aim to first create an array that can house 4 units: motoneurons, hepatocytes, skeletal muscle, and cardiomyocytes. Although the end goal is to have a system that can house 10 or more subunits, it is unlikely that we will be able to complete this in the fall; however, our design could be used as a basis for such a system.

**Background**

Microfluidics is a field of science that began in the early 1980’s, and deals with the study, behavior, and controlling of minute amounts of fluid. The ability to manipulate such small volumes of fluid, either through active or passive microfluidics, allowed for the emergence of lab-on-a-chip technology. Active microfluidics is the manipulation of the fluid by active elements such as pumps or valves, while passive microfluidics relies on forces such as capillary action. At the extremely low volumes used in microfluidics, several unique properties emerge. The Reynold’s number in microfluids is extremely low, which means that fluids exhibit laminar rather than turbulent behavior when next to each other, making mixing difficult. Continued research has allowed for the development of technology to manipulate microfluids through the use of continuous flow through microfabricated channels [2]. It is through the implementation of these channels that microfluidic culture devices have been developed.

        Microfluidic culture devices, also known as organs-on-chips, are micrometer scale chambers that are continuously perfused with fluid. The most basic of these culture devices is a single microfluidic chamber containing only one cell line. More complex microfluidic culture devices can be made by connecting multiple chambers with porous membranes, with different types of cells in each chamber. This setup can accurately mimic physiological forces such as fluid shear stress due to the continuous fluid flow in the system. When microfluidic culture devices containing cells from multiple organs are used, it allows for the study of drug distribution *in vitro* or interactions between organ systems, without needing entire organs.

        Microfluidic culture devices allow for a great deal of control over the system. Because the channels are so small, viscous forces dominate over inertial ones, meaning that the fluid flow will be laminar if the diameter of the chamber is less than about 1 millimeter [3]. This laminar flow allows cells to grow and stay alive in the absence of blood, by supplying them with physiological nutrients in the microfluids, and also allows for the creation of gradients in the chambers, either chemical or physical. Fluid shear stress can also be manipulated in this system, by altering flow rates, channel dimensions, or using a porous membrane. This system also allows for a great degree of control over the plating of cells. Different cell types can be plated in the same chamber with the use of microposts, patterned plating surfaces, and the microprinting of ECM, which allows for the analysis of tissue barriers when two cell types are separated by a membrane in a channel. It is this ability to contain multiple cell types in a single microfluidic culture device that allows for the modeling of organ systems, and where current research is ongoing.

        Theoretical, physiological-based pharmacokinetic (PBPK) models are used to design the parameters of μCCAs. These models represent organs as chambers that can absorb, metabolize, and excrete different nutrients and substances, which can be modeled theoretically with differential equations. The μCCAs are physical representations of these models, with microfluidic chambers representing each organ, fluidic channels allowing for inflow and outflow through the system, and the plated cells of the various organs carrying out metabolic activities [4]. The parameters obtained from the PBPK model of the system are used in creating the μCCA to control the environment of the system. Changing the cross-sectional area of each channel leading into and out of a microfluidic chamber allows for control over the flow rate. The flow rate and size of the chambers then allows for the calculation of liquid residence time, an important parameter in the system that affects how the cells absorb and excrete substances in the fluid. The fluidic shear stress can also be calculated using the dimensions of the chambers as well as the flow rate[5]. By considering these parameters in light of physiological norms, a μCCA can be constructed to mimic the interactions of multiple organ systems.

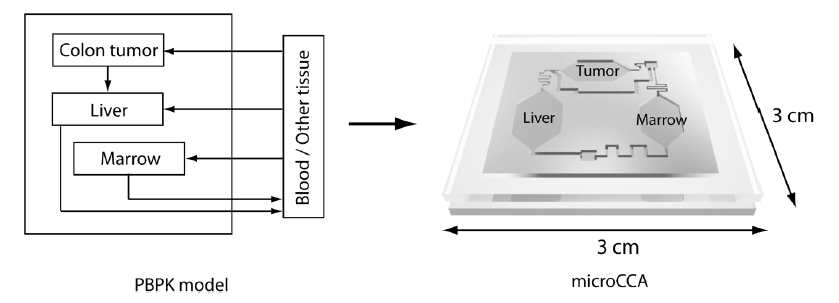


Figure : a multi-organ PBPK model and corresponding μCCA setup

**Specific 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 [6]. However, in order to create a multi-organ system that can be observed in great detail, certain design specifications must be met. Different organ models have different required parts and access in order to be properly interrogated and observed. In this report, we will focus 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 some sort of bioMEM 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. The MEAs have electrode contact pads along one edge – these pads must be easily accessible for both hepatocyte and motoneuron subunits. Although these MEAs must be able to measure currents on the cell surfaces, it is important that they are isolated from the fluid within the microfluidic chambers. Also, when housing multiple MEAs together, we want to maximize the electrical connection density. It is not feasible to have two MEAs directly next to each other, but it may be possible to have two MEAs in a box that is roughly 30mmx30mm, with only the corners touching. However, the actual connection density we achieve will be largely dependent on the size of the microfluidic chambers used.

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 (15mmx15mmx500µm), but require laser access in order to measure the force developed in the muscle [7]. These two subunits will need to be easily accessible by the laser measuring system. Furthermore, all four chambers will need to be accessible by optical microscope for monitoring cell viability during the cell lifetimes.

When designing the individual units and chambers themselves, 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. For the cell types we will be studying, physiological shear stresses are generally less than 2 dynes/cm2, and liquid:cell ratio is around 1:2. However, this liquid:cell ratio may be unrealistic given the two-dimensional nature of the cell culture – previous studies have yielded ratios closer to 5:1 [8]. Liquid residence times vary for different organ systems, but are dependent on the velocity of fluid flow and the length of the cell chambers.

Additionally, it is important to ensure that the different organ subunits interact in a physiologically meaningful way. In order to do this, the cellular components of the units should be constructed such that the relative number of cells matches the relative number of these cells present in the body. By using the average cell numbers of different organs, we can estimate that the cell ratios for this array should be approximately 1 motoneuron: 4167 skeletal muscle cells: cardiomyocytes: hepatocytes. Additionally, not all organ systems receive equivalent blood flow in the human body. 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). These calculations are discussed in greater detail in the preliminary calculation section of this report.

**Exploration of existing solutions**

The device proposed in this design is relatively novel, creating a system that doesn’t just provide metabolism for multiple cell type viability, but also measuring functionality of the included tissues in response to drugs using bioMEMS devices. Because of that many of the “existing solutions” are in concept form, or only deal with metabolic exchange. However, many of the design considerations of this device are analogous with those in other systems, or can be inferred from conceptual models.

        The first solution to be examined is a three-chamber µCCA designed by Sin et al. [6]. There are some notable design considerations from this device. The first is the use of silicon as a material for the array. The device was created in the silicon using lithography-etching. Silicon was used because of the ease with which the sidewalls of the channels could be fabricated with this technique. The silicon was housed in a Plexiglas (poly(methylmetacrylate); PMMA) casing made of two pieces with space cut into the bottom for the silicon chip and inlet and outlet holes for the fluid flow. The two pieces were held together with 4 stainless steel screws. Fluid flow was achieved using a peristaltic pump and debubbler, with flow rate chosen to achieve desired residence times based on the chamber dimensions.

        There are several features that can be taken note of in this setup. Silicon will be used as the cell culture substrate, as the MEA’s and MECA’s are fabricated from silicone. Plexiglas could also be used as the housing material for the chambers. The use of screws for assembly, peristaltic pump for flow, and a de-bubbler are also design considerations. The equivalence of the device to its physiological parameters is also addressed. Shear stresses, residence times, mass ratios, liquid to cell ratios, and flow ratios are all important considerations for our device, and were addressed by this paper in ways that could be used for the device proposed here, though this paper does not achieve the physiological values in a few cases. These parameters were set through the manipulation of the geometry of the system, and were unique to the types of tissues used. Because the tissues used in the proposed device are different, the exact measurements will not be the same as in the paper, but the techniques used to set the parameters of the system should still hold.

        This paper does not touch on certain considerations that our proposed array will need to implement, as they did not use bioMEMS devices to measure tissue functionality. Thus, they did not deal with isolating electrode pads from the system or managing electrical connections.

Another paper to consider deals with the culturing of skeletal muscle cells on cantilever chips for measurement of forces [7]. This paper is the basis for the skeletal muscle tissue unit in the proposed device, and thus has some design considerations to note. One of the most important requirements for this unit in the system is the optical and laser interrogation of the chamber. It needs optical access to determine cell viability and laser access from beneath to measure cantilever bending for force measurements -- see Figure 2 for a visual representation of this system. The optical access was achieved as it is in most of these units, by leaving the upper surface of the chamber medium exposed for microscope access. The laser access was achieved by using a glass coverslip to seal the bottom of the cantilever chip and provide a transparent surface to access the undersides of the cantilevers. The ways in which optical and laser access were achieved could be used in the array proposed in this report.

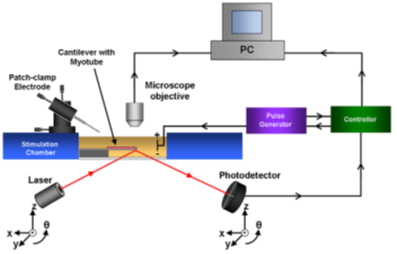


Figure : Schematic representation of cantilever access, both with microscope and laser [6]

The layout of a device such as this was theorized in another paper by Shuler’s group [4]. In this paper, a PBPK model of the body was proposed, which lends some conceptual value to this design. Since a µCCA is a physical representation of a PBPK model, the PBPK model can serve as a physical layout for the final system. Figure 3 shows the PBPK model from the paper. As this report is only concerned with 4 tissue types, an obvious physical layout for the chambers would have them all in parallel, since no lung, gut, or spleen tissues will be used.

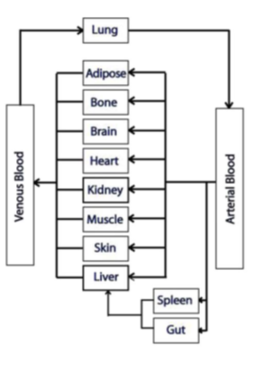
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Figure : Full-body PBPK, as developed by Shuler et al

**Preliminary Calculations**

In designing this system, there are several variables that must be calculated for each chamber. Although the physiological parameters for each type of cell are different, the equations that will be used are the same, derived by Oh et al. [16].

Fluid flows are almost always at low Reynolds numbers, so it can be assumed the flow in the system is laminar and that turbulence is absent and flow is laminar. Thus, the hydraulic resistance of the channel can be determined. Hydraulic resistance is dependent upon the geometry of the channel, described by these equations:

Where *η* is the viscosity of the fluid, *w h* and *l* are the dimensions of the rectangular channel, and *r* is the radius of the cylindrical channel. It is assumed that the microchamber has a high aspect ratio (*l<*r). The hydraulic resistance can then be used to calculate the fluidic flow rate in the microchamber, analogous to an electrical circuit current:

In which *Q* is the steady state flow rate, Δ*p* is the pressure drop across the channel, and R is hydraulic resistance.

The fluidic shear stress seen by a microchannel wall with laminar flow can be derived from Newton’s law of viscosity. Assuming that the channels have a rectangular shape and a high aspect ratio (*h<w*), the wall sheer stress *τ* can be approximated by the following equation:

In which *η* is the viscosity, *Q* is the flow rate, and *w* and *h* are the width and height of the channel.

The liquid residence time is defined generally as the amount of time a particle spends in a system. When there is continuous flow in a channel, the residence time of the chamber can be described as:

Where *l* is the length of the chamber, and *v* is the velocity of the fluid.

Previous clinical studies have characterized the average number of cells present in different organ systems. From these studies can be obtained the following cell counts for the average person [8,13]:

Normalizing the above values to motor neurons, it can be found that the ratio of cells present in the final array should be:

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The average blood flows to human organs *in vitro* can be used to estimate the flow rate ratios of the corresponding microchambers that will be present in the final design. The average blood flow to these organ systems is as follows [10,11,12]:

Normalizing the above values to motor neurons, it can be found that the flow rate ratios in the final design should be:

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**Anticipated Progress**

In continuing this project, the work will be divided into three sections. One section will be researching the parameters and making the calculations to determine the dimensions of the microfluidic chambers, while another section requires the same for the microfluidic channels. That work will be combined to model the equations of the system and solve for the required parameters of flow rate, shear stress, and residence times. The final section will be working on the physical layout of the system, ensuring that the microfluidic chambers all have access to MEA’s and electrical outputs, so that data can be obtained from the system. After these design concerns have been addressed, the overall design of the system will be analyzed and then a model of the system in CAD will be created.

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| Task/Milestones | 21-Sep | 28-Sep | 5-Oct | 12-Oct | 19-Oct | 26-Oct | 2-Nov | 9-Nov | 16-Nov | 23-Nov | 30-Nov | 7-Dec |
| Oral Report |  |  |  |  |  |  |  |  |  |  |  |  |
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| Oral Report |  |  |  |  |  |  |  |  |  |  |  |  |

Figure : GANTT chart detailing our project development schedule

**Citations**

1. Smith, Alec ST, et al. "Microphysiological systems and low-cost microfluidic 7latform with analytics." *Stem cell research & therapy* 4.Suppl 1 (2013): S9.
2. Bhatia, Sangeeta, et al. “Microfluidic organs-on-chips” *Nature Biotechnology Vol 32 No. 8* (2014): 760-772
3. Esch, Mandy et al. “How multi-organ microdevices can help foster drug development” *Advanced Drug Delivery Reviews* 69-70 (2014) 158-169
4. Shuler, Michael et al. “Body-on-a chip: Using microfluidic systems to predict human responses to drugs” *Pure Appl. Chem.* Vol. 82 No. 8 (2010) 1635-1645
5. Sung, Jong et al. “A micro cell culture analog (mCCA) with 3-D hydrogel culture of multiple cell lines to assess metabolism-dependent cytotoxicity of anti-cancer drugs” *Lab chip 9* (2009) 1385-1394
6. Esch, Mandy et al. “Promises, challenges and future directions of mCCAs” *Journal of Biotechnology 148* (2010) 64-69
7. Wilson, Kerry, et al. "Measurement of contractile stress generated by cultured rat muscle on silicon cantilevers for toxin detection and muscle performance enhancement." *PloS one* 5.6 (2010): e11042.
8. Sin, Aaron, et al. "The design and fabrication of three‐chamber microscale cell culture analog devices with integrated dissolved oxygen sensors."*Biotechnology progress* 20.1 (2004): 338-345.
9. Bianconi, Eva, et al. "An estimation of the number of cells in the human body."*Annals of human biology* 40.6 (2013): 463-471.
10. DOBSON, ERNEST L., et al. "The Measurement of Liver Circulation by Means of the Colloid Disappearance Rate I. Liver Blood Flow in Normal Young Men."*Circulation* 7.5 (1953): 690-695.
11. Klabunde, Richard. *Cardiovascular physiology concepts*. Lippincott Williams & Wilkins, 2011: 164-168.
12. GUYTON, Arthur C. – HALL, John E.. *Textbook of medical physiology.* 11th edition. Philadelphia : Elsevier Saunders, 2006. Chapter 21: Coronary Circulation.
13. Tomlinson, B. E., and Dorothy Irving. "The numbers of limb motor neurons in the human lumbosacral cord throughout life." *Journal of the neurological sciences* 34.2 (1977): 213-219.
14. Oh, Kwang et al. “Design of pressure-driven microfluidic networks using electric circuit analogy” *Lab Chip* 12 (2012) 515-545