ABSTRACT
Human liver plays a major role in metabolizing medications. One of its metabolic roles is to activate prodrugs into their active form enzymatically. The proposed structure below can be considered as a basic concept of utilizing a liver-on-a-chip for evaluating drug response. One example that of paramount interest to cardiologists is the variable response of their patients to an antiplatelet agent (Plavix®, clopidogrel) which is used after the implantation of a coronary stent to prevent stent clotting. It has been found that some patients, due to genetic variability, are slow metabolizers or resistant to clopidogrel which poses grave danger to them after coronary stenting. In this paper, a multi-layered microfluidic liver-on-a-chip is presented. The model consists of three processing steps: (1) mixing, (2) metabolizing, (3) and testing. Simulations in COMSOL® was performed and reported.

KEY WORDS
Liver-on-a-chip, Drug response, Microfluidic, Antiplatelet agent, Simulation.

1. Introduction
Microfluidics technology has been widely used in developing in-vitro systems to investigate drug response mimicking the in-vivo situation. Investigators translate organ functions into microfluidic culture devices incorporating human microtissues in an organ-like platform so-called organ-on-a-chip.


To more accurately re-create the in-vivo environment for hepatocytes, many scientists use “sandwich” configuration. Hepatocytes are cultured in between two layers of extracellular matrix. Three-dimensional cultures of hepatocytes can be achieved by culturing on an array of polydimethylsiloxane (PDMS) pillars, serving as a type of scaffold.

2. Device Design
2.1 Liver cell culture
The liver consists of four lobes with different sizes and shapes and is connected to two large blood vessels. The first is hepatic artery that carries blood rich in oxygen from the aorta. The second is the portal vein that carries blood with digested nutrients. These blood vessels subdivide into small capillaries to form liver sinusoids that lead to a lobule. Each lobule is roughly hexagonal (Figure 1), and made up of millions of hepatic cells so called hepatocytes. Between the hepatocytes are liver sinusoids. Excreted bile from the liver cells, is collected in bile canaliculi. The canaliculi are small grooves between the faces of those adjacent hepatocytes.

The typical technique used for culturing human liver cells involves perfusion of the whole liver or a biopsy sample (15-30 ml/min, depending on the size of the tissue sample). Complete isolation of hepatocytes into a single-cell suspension can be obtained by an additional incubation at 37°C with collagenase under gentle stirring for 10-20 min. For the purpose of this experimental procedure, a pre-purchased human liver PrimaCell™ kit; or similar, can be utilized.
2.2 Clopidogrel Metabolism

Clopidogrel is an antiplatelet agent used to prevent thrombosis (clotting) after placement of the intracoronary stent. One of its trade names is Plavix. Clopidogrel is an inactive pro-drug that the active metabolite is formed in the liver after two-step oxidative process (Figure 2).

![Clopidogrel Metabolism](image)

The first step of the activation process is the oxidation of the clopidogrel into 2-oxo-clopidogrel. The second phase is hydrolysis that produces the active metabolite of clopidogrel. In the first step, the CYP isozymes CYP1A2, CYP2B6, and CYP2C19 produce the intermediate 2-oxo-clopidogrel. The second phase is the CYP2B6, CYP2C19, and CYP3A4 oxidation to the clopidogrel active metabolite. 40% of the hepatic conversion of clopidogrel into the half-life active metabolite that binds to the platelet P2Y12 receptor is contributed to CYP2C19.

Some patients do not activate Plavix sufficiently. Each of eight different variants of the CYP2C19 gene significantly alters Plavix activation, if present in a patient. It is important for the physician to be aware of the presence of any of these alleles as anti-platelet therapy is being considered. It is estimated that 2% to 14% of the US population are poor metabolizers.

2.3 Design

The proposed design consists of three main parts: mixing, metabolizing, and testing. The first part introduces fluids through two inlets. Blood or plasma is pumped through the first inlet. The tested drug is inserted through the second inlet. The mixing process then takes place through a passive microfluidic micromixer.

The second part of the device has a layer of the cultured liver cells sandwiched in between upper and lower layers. The cells should be pre-cultured over a porous membrane prior to annealing and bonding of those two microfluidic layers.

Using splitting channels in the upper and lower layers is essential to fully immersing the cultured cells with the activated drug mixture; otherwise, the fluid will be expected to take a straight path. This will provide continuous nutrition for the cells to stay alive and active.

The third part is the drug testing where the activated drug in blood will be collected for testing. Initially, the idea was to use external commercially available devices for detection. While this could be tested by fabricating two-layer section that consists of pillars that allow for the activated drug adsorption in the stream.

The model was constructed in SolidWorks. The dimensions of the device are about 6mmx2mm. The final device consists of four layers; each layer is 100μm thick total thickness of 400μm.

![Schematic Configuration of the proposed device](image)
2.4 Fabrication process

The microfluidic liver-on-a-chip device is fabricated by casting PDMS against the SU-8 patterned master. The master can be created by transferring the computer aided design; such as SolidWorks, into a dark mask. SU-8 is a negative photoresist; a very viscous polymer that can be spun over a substrate to get the high aspect ratio of 100μm wall thickness, especially with the presence of a long pass filter. Soft lithography is performed to pattern the resist. The master is then used to cast PDMS (see Figure 4).

Channels made of PDMS are very popular because of the easy accessibility of material, rapid fabrication, and many desirable performance aspects of the material. The PDMS material can be obtained in viscous liquid precursor form. The precursor materials consist of two parts, the base, and curing agents. The two parts are mixed and then cured at room temperature, in a vacuum, with recommended mixing ratio (10:1), resulting in thermoses, transparent elastomeric solids.

The uncured PDMS can be poured over the master that has the negative image of the device structure. Curing of the PDMS can be performed by baking the master for a couple of hours, and then it can easily be peeled after cooling. Corona bonding technique is used to bond the PDMS to a glass substrate after punching holes for the inlets and the outlets’ specified points. Inlets can be connected to syringe pumps; one with the blood flow, and the other with the tested drug (Figure 6).

The rate of adsorption is

\[ r_{ads} = k_{ads}c_P \]  

where \( c_P \) is the concentration of \( P \) in the stream. The desorption rate is linear in the concentration of surface adsorbed species, \( c_{PS} \):

\[ r_{des} = k_{des}c_{PS} \]  

The simulation of the proposed model is done in COMSOL 4.3. The internal study of “Transport of Diluted Species,” “Laminar Flow,” and “Reaction Engineering” has been used.

The equations in the Transport of Diluted Species interface describe the transport of the species, \( P \), in the analyte stream according to

\[ \frac{\partial c_P}{\partial t} + \nabla (D_P \nabla c_P) + u \nabla c_P = 0 \]  

Here \( D_P \) denotes the diffusion coefficient (m²/s), \( c_P \) the species concentration (mol/m³), and \( u \) the velocity vector (m/s).

The sample pulse that enters the model is described by a Gaussian distribution at flow cell inlet with a maximum concentration of 80 mol/m³.

At the outlet, the Outflow condition is used:

\[ n \cdot (-D \nabla c) = 0 \]  

The adsorption and desorption of analyte at the active surfaces give rise to a net flux at the corresponding boundaries:

\[ N_p = -r_{ads} + r_{des} \]  

The mass flux due to desorption is dependent upon the local concentration of adsorbed surface species and is coupled to the equations in the Surface Reactions interface.
Transport of adsorbed species occurs in the tangential direction along the surface. The Surface Reactions interface models the tangential flux along the surface, the surface molar flux, \( N_{t,i} \) (mol/(m\( \cdot \)s)), according to

\[
N_{t,i} = -D_{s,i} \nabla c_{s,i}
\]

(7)

where \( D_{s,i} \) (m/s) is the surface diffusion coefficient for species \( i \).

The governing equation for the surface concentrations is written as

\[
\frac{\partial}{\partial t} c_{s,i} + \nabla \cdot (D_{s,i} \nabla c_{s,i}) = R_{s,i}
\]

(8)

where \( R_{s,i} \) (mol/(m\(^2\)·s)) is the sum of all sources due to surface reactions and adsorption/desorption phenomena.

The balanced equation for the surface species \( P \) and \( Q \) become:

\[
\frac{d c_{s,P}}{dt} = r_{ads} - r_{des}
\]

(9)

The rate of adsorption depends on the concentration of the \( P \) species in the analyte stream and is therefore coupled to the equations in the Surface Reactions interface to those provided by the Transport of Diluted Species interface.

The flow in the flow channels is laminar and given by the Navier-Stokes equations

\[
\rho \frac{d \mathbf{u}}{dt} = \mathbf{F} - \rho \mathbf{u} \times \nabla \mathbf{u} - \frac{2}{3} \eta (\mathbf{u} \cdot \nabla) \mathbf{u} - \frac{2}{3} \eta \nabla \mathbf{u} \cdot \nabla \mathbf{u}
\]

\[
\mathbf{V} \cdot \rho \mathbf{u} = 0
\]

(10)

where \( \rho \) denotes density (kg/m\(^3\)), \( \mathbf{u} \) represents the velocity (m/s), \( \eta \) denotes viscosity (kg/(m·s)), and \( \mathbf{F} \) equals the pressure in the tubes (Pa).

The calculated flow field serves as input to the Transport of Diluted Species interface, to describe the convective mass transport.

3. Results and Discussion

As described before, the proposed device consists of three main sections. The first part is the micromixer. The idea is to introduce regular blood with the ability to mix the drug needed to be activated through another inlet. The design of the mixer has been chosen to achieve “Dean Flow effect” and “Herringbone” advantage to maximize the effectiveness of the mixing.

The mixer channel width is 100μ with 100μ height. The flow rate used in the simulation was 0.1ml/hr. Figure 7 shows the concentration results and the mixing efficiency by the end of the mixer was above 90%.

The second stage is the metabolism of the drug. The top layer is where the mixture of blood and the tested drug is introduced to the liver cells. The liver cells are cultured in the second layer. The presence of the enzyme in those cells will activate the drug which will flow along with the blood for the last stage for examination.

![Figure 7](image1.png)

**Figure 7** left: COMSOL® surface concentration simulation of the mixing process of the tested drug (blue) and blood (red). right: mixing result in the actual fabricated device.

![Figure 8](image2.png)

**Figure 8** Simulation of the inactive drug concentration (blue) to active drug concentration (red) using COMSOL.

A reaction simulation for two species has been simulated. Figure 8 shows the result of the reaction. In the figure, it is clear that the concentration of the inactive drug will decrease once the time of the reaction and the production of the activated drug have begun. In the figure, the inactivated drug concentration seems to have some value (shown in blue), while the concentration of the active drug will elevate from zero to its maximum value. As discussed before, some patients have troubles with activating the drug due to the lack of the required enzyme. This test will determine whether the patient can use this drug depending on the final reaction test.

![Figure 9](image3.png)

**Figure 9** COMSOL simulation results of surface diffusion flux (top), and surface concentration (bottom).
In addition to the drug metabolism, the liver cells excrete bile into the intestine. The porous membrane shown in Figure 11, as the third layer, is the only way for the bile to flow. The choice of placing the membrane underneath the cells is due to the fact that liver cells excrete bile into the lower level and so it will not mix with blood and flow directly to the intestine.

Just like the way it is in liver cells, another fluidic channel has been placed beneath the membrane to collect the bile. This layer is shown as the fourth layer in Figure 3.

**4. Conclusion**

In this work, a Liver-on-a-Chip Microfluidic device has been discussed. The system consists of three functional parts: (1) mixing (2) metabolizing (3) and testing. The device is concise and easy to utilize which makes it suitable for portable use. PrimaCells™ has been adapted for cell culturing of human liver cells process. The fabrication process is simple and easy to align which makes it easily duplicate.

The proposed device is multi-layered with the ability to mimic the blood flow and bile excretion of actual cultured human liver cells. Drugs can be introduced into the system with the advantage of live observation to the cells reaction. This work focused on antiplatelet agent such as clopidogrel (Plavix™). The clopidogrel is introduced in its inactive form, and the liver cells enzymatically activate it. If the cells harbor the slower variant of the activating enzymes, then the drug will exit with poor activation.

The fluid structure interaction module in the MEMS module of COMSOL was used to simulate the system’s micro-channels. Simulation proves the validity of the designed liver-on-a-chip device.

Flow and concentration gradient simulations have been conducted in order to evaluate the performance of the devices prior to their fabrication. Also, simulation is beneficial in a way to confirm and explain the experimental results. This paper presented the idea behind membrane based culturing and the resulting designs, as well as the theoretical and experimental results.

Future work may focus on other functions of the liver. It can be easily integrated into other organ-on-a-chip devices. By increasingly employing Microfluidic devices in developing biomimicy devices, the use of animals in drug research will decrease and the process of drug investigations will accelerate.

**References**


