Organ on a Chip Device for Liver implants

Group 4

Outline

- Background
- Cell Type Detection
- Proposed Device Requirement
- Device Fabrication
- Device Operation Assessment

Background

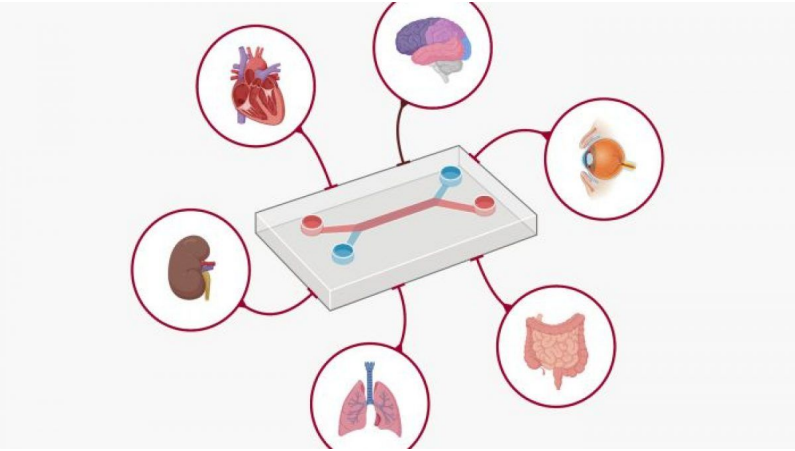
Main Goal:
Design a Liver-on-a-chip platform that can create a microfluidic cell culture system that mimics the structure and function of the liver.

Purposes:

- To determine immunocompatibility in liver transplantation
- To mitigate rejection risk of transplanted liver
- To make personalized medicine
- Introduce a cost-effective liver transplantation system

Immunocompatibility

Immunocompatibility in the context of liver transplantation refers to the degree to which the transplanted liver is accepted or tolerated by the recipient's immune system.



Organ-on-a-chip

Challenges Associated with Transplants

- **Donor Shortage:** limited availability of suitable donor organs
- **Immunological Rejection:** The recipient's immune system may recognize the donor liver as foreign tissue
- **Surgical Complexity:** The surgery involves complex procedures and complications can impact patients life
- **Device manufacturing complexity:** Accurately mimics the complex physiology of the human liver is difficult for liver-on-a-chip device

Approaches

- Design a microscale liver-on-a-chip device
- Cell culture and maintenance
- Device fabrication techniques
- Device operation assessment

Types of Cells

Cells and related factors important for donor/recipient compatibility:

- Cells must be healthy
  - Non-cancerous, no viral hepatitis (hepatitis C), no signs of alcohol/drug toxicity
- Blood type (A, B, AB, O)
- HLA (human leukocyte antigen) typing, aka tissue type
  - 6 important antigens, but not always necessary to completely match
  - 3 antigens inherited from each parent
  - Rare to get a 6-antigen match between two people (except with identical twins and some siblings)
- Low levels of certain liver enzymes
  - Alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) are elevated when injury is present

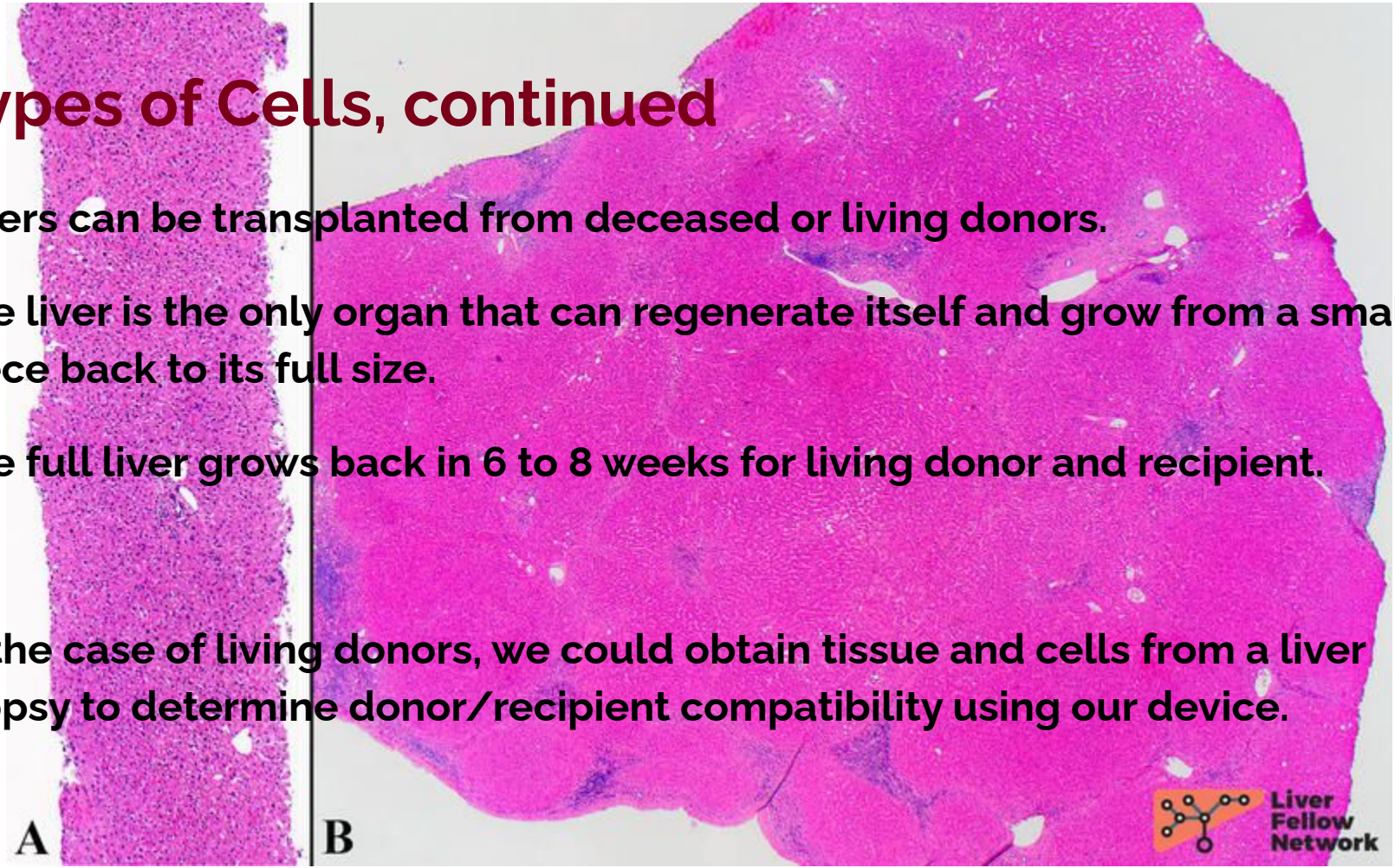
Types of Cells, continued

Livers can be transplanted from deceased or living donors.

The liver is the only organ that can regenerate itself and grow from a small piece back to its full size.

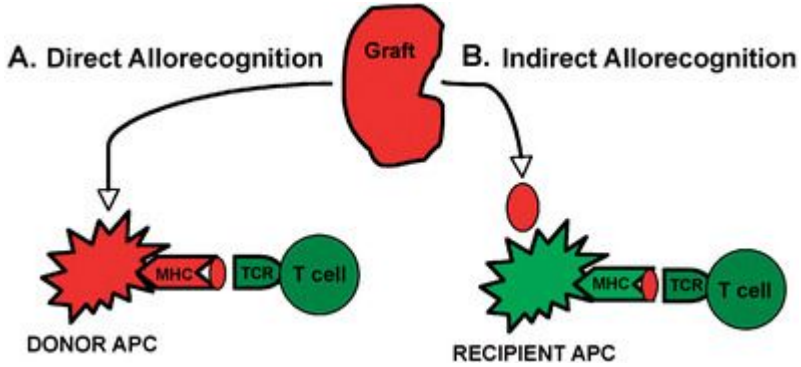
The full liver grows back in 6 to 8 weeks for living donor and recipient.

In the case of living donors, we could obtain tissue and cells from a liver biopsy to determine donor/recipient compatibility using our device.



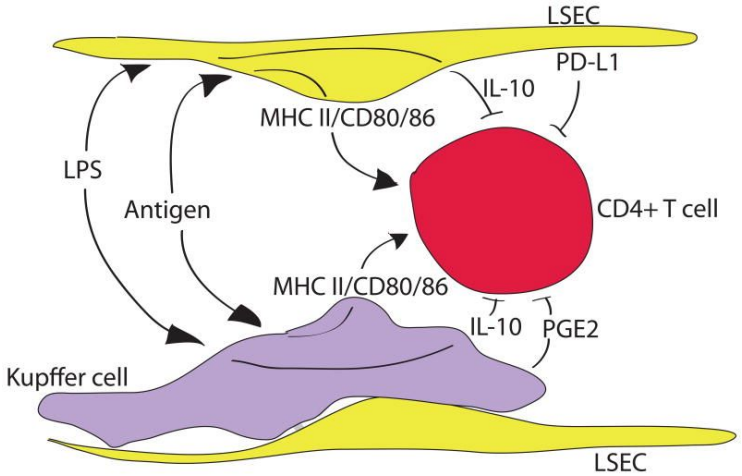
Biologics: How to Model the Immune Response

- T Cells and B Cells are the heart of the adaptive immune response, and are linked to both the Major and Minor Histocompatibility Complex in regards to transplant rejection
- Alloantigen responses can occur in two ways- unique to transplants. Either the non self MHC is recognized, or the processed proteins of the donor are



Biologics: How to Model the Immune Response

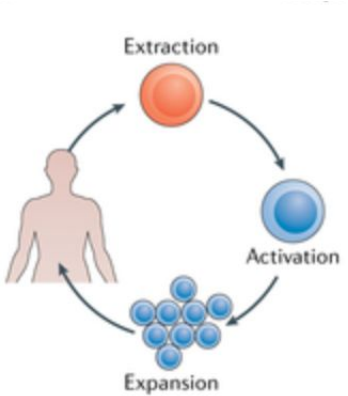
- To observe any sort of immune response following extraction, T Cells must be activated in the presence of an Antigen Presenting Cell (APC)
- For the liver, Kupffer Cells are a great candidate since they present both MHC Class 1 and 2, but come with the challenge of immunosuppression
- The relevant immunosuppressant agent is IL-10, an anti inflammatory cytokine
- Thus, to utilize Kupffer cells as our APC, litigating Toll-Like Receptor 3 or introducing a reactive oxygen species to amplify MHC expression is key



Biologics: What Cells and How to Obtain

T Cells

- An extraction in the form of a blood draw
- This will be taken from the organ recipient



Liver Cells

- Liver is from a donor, so a biopsy of the removed organ to sample Kupffer cells
- Liver sinusoids in literature are constructed using HUVECs (to mimic liver sinusoidal endothelial cells), and immortalized human hepatic stellate cells (LX-2) with HepaRG (immortalized hepatic cell line), and the donor Kupffer cells which are usually modeled using monocyte derived macrophages

Biologics: How to Keep Cells Alive

T Cells

- There is no pressing need to maintain T Cells for prolonged periods of time for our purposes
- However, to ensure their proliferation and efficacy, we have chosen to utilize the regulatory cytokine IL-2; a protein well known to aid in T Cell Growth

Liver Cells

- Liver portal veins need two blood supplies in vivo: oxygenated and nutrient rich. The microfluidics of our device must satisfy these nutritional requirements, i.e proper oxygenation
- Co-cultures that utilize both PCs and NPCs
- However, the most important factor is the microenvironment which is outlined in our design (PMMA)

Biologics: How to Model the Immune Response

We came up with a few different device designs to tackle this challenge
1 A/B. Take cells from the donor and deliver them to a liver on a chip device, administer recipient cells, and determine if immune cells from the recipient attack the donor cells (or vise versa).
2. Create a body on a chip device using the recipient cells and then administer the donor cells to the device and determine if the donor cells survive/ the recipient cells reject the donor cells.

- We decided to go with option 1A as this would significantly reduce the complexity of device and present

Current Liver on a Chip Devices

Strategies	Characteristics
Liver chip based on 2D planar culture	Pattern or capture hepatocytes in 2D form; co-culture with non-parenchymal cells.
Liver chip based on matrixless 3D spheroid culture	Hepatocytes form spheroid spontaneously, due to gravity or modification of material surface; also suitable for co-culture.
Liver chip based on matrix-dependent 3D culture	Encapsulate cells within a three-dimensional (3D) matrix, such as hydrogel, BME and collagen, which replicates the supportive functions of the extracellular matrix.
Liver chip based on layer-by-layer deposition	Pattern hepatocytes and nonparenchymal cells lay by lay by porous membrane or 3D printing technology, etc.
Liver chip based on 3D bioprinting	Cells and extracellular matrix are laid out according to a preset path through a 3D printer in the form of additive manufacturing.
Liver chip-based cell microarrays such as microwell systems	Seed cells in an array of well plates.
Liver chip-based hanging drops	Form 3D micro-tissues of cells (one type or multi-types) by hanging cells in drop.

[1]

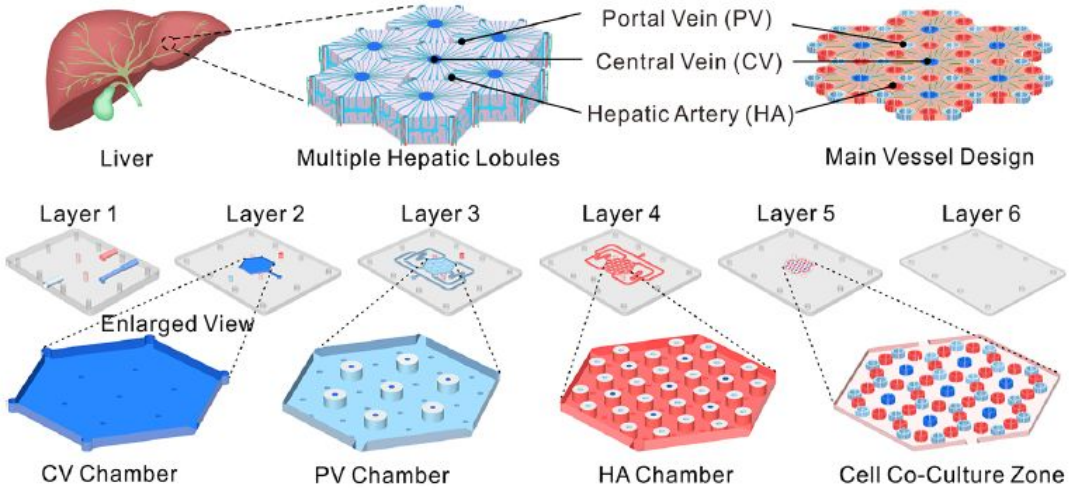
Requirements of Our Proposed Device

Our device must be able to complete the following:

- Receive and maintain primary cells
- Administer donor/recipient cells
- Detect immunocompatability

Baseline Device

- After considering the design requirements for our device, we concluded the device that seen in the figure would work best.
- This device was proposed by Ya et al and consists of multiple chambers to more accurately model the liver.



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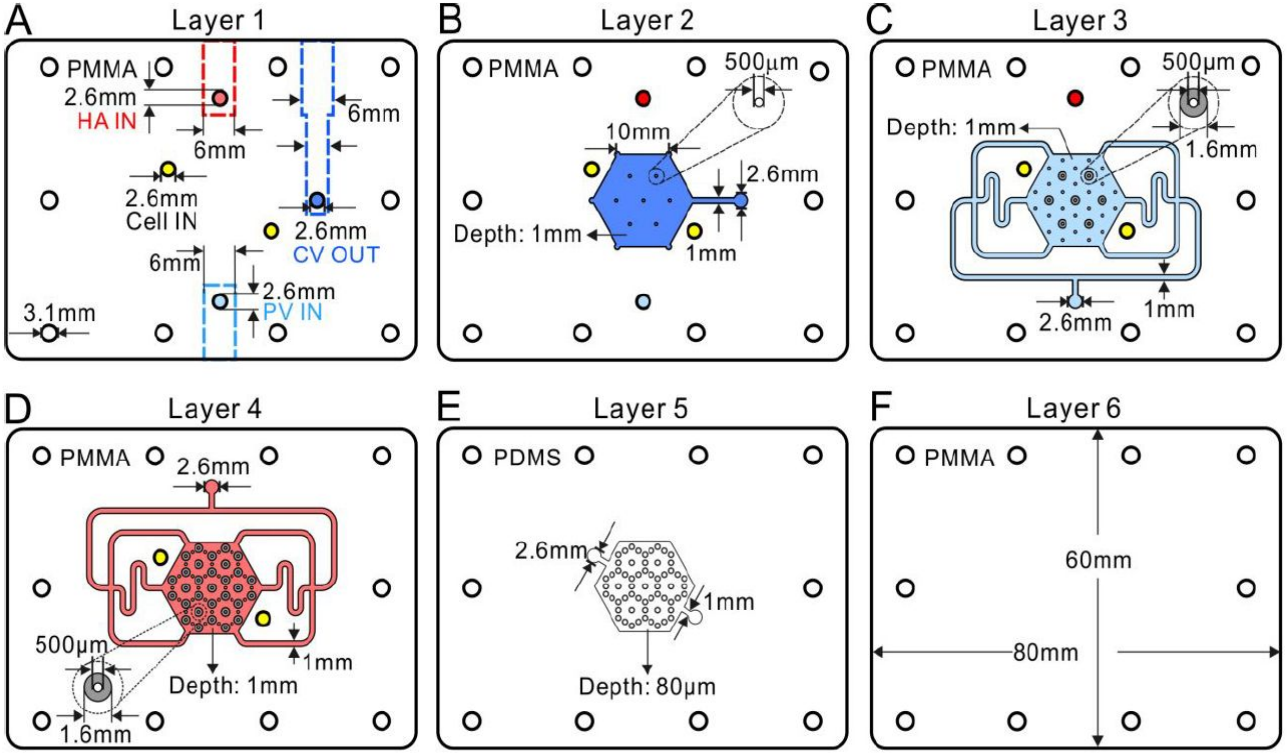
Device Fabrication

- The proposed device consists of one PDMS layer & 5 PMMA layers
- Layer 1: Support/ media biological fluid in/out
- Layer 2: central vein
- Layer 3: portal veins
- Layer 4: hepatic arteries
- Layer 5: Cell co-culture layer.
- Layer 6: Support layer



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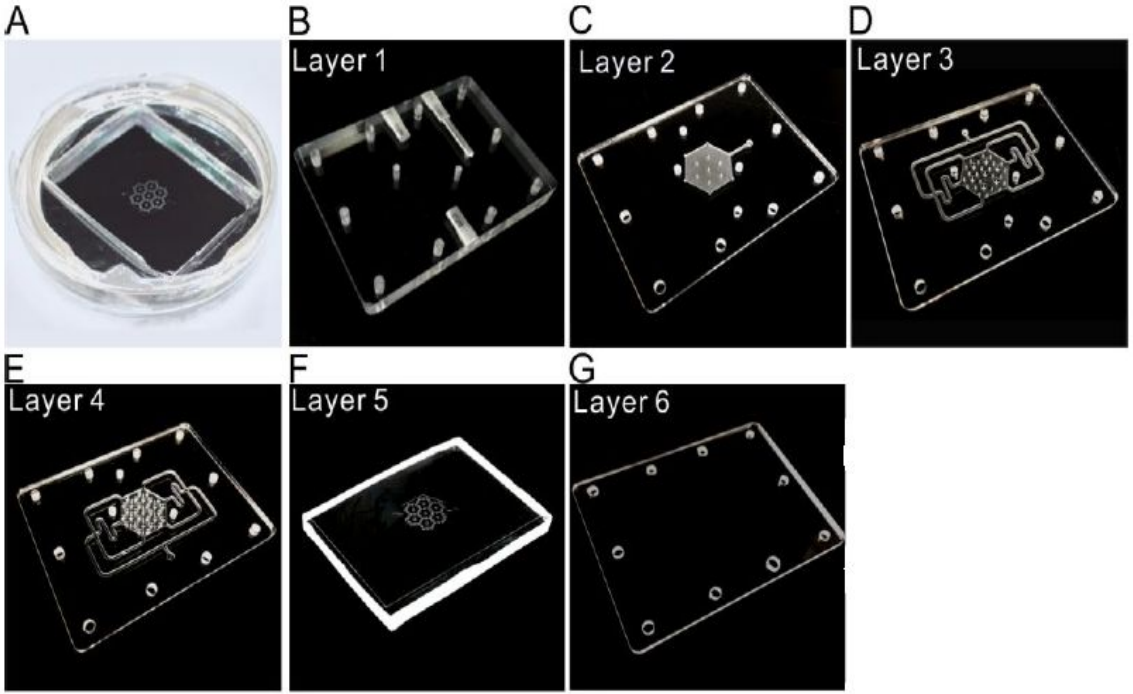
Realized Layers

A. Silicon template for the PDMS layer

B-E. 1st-4th PMMA Layer.

F. 5th PDMS

G. 6th and Final PMMA layer.



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PDMS Layer Fabrication Process

A. Spin-coating SU-8 on the silicon wafer.

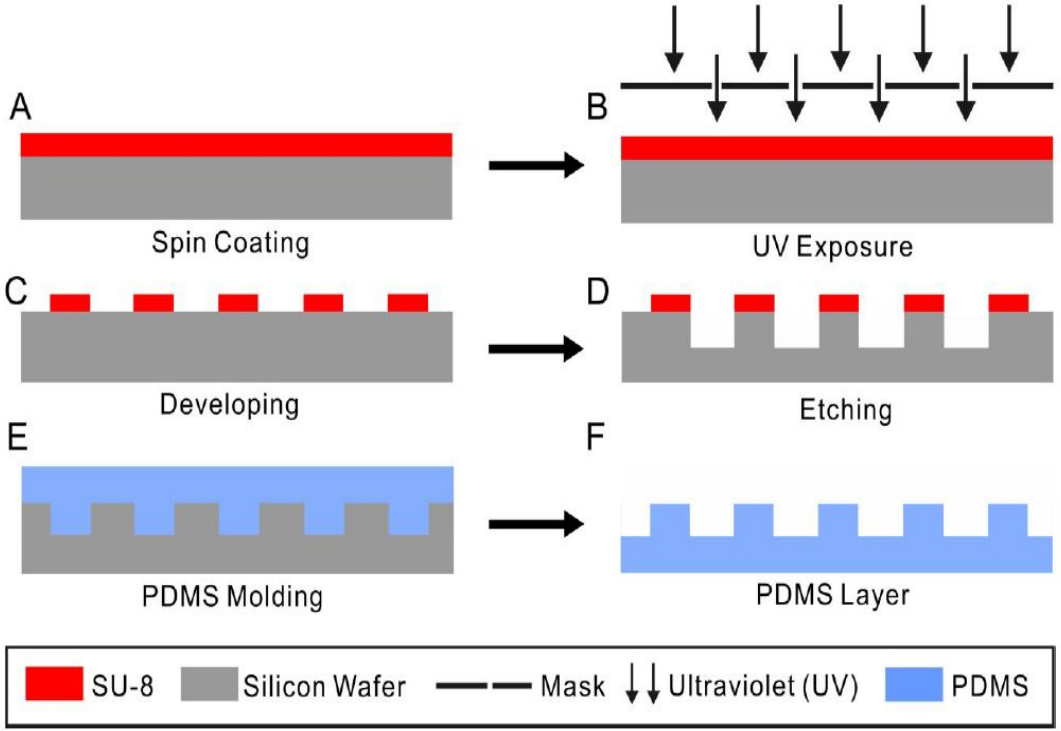
B. Patterning the SU-8 coated silicon wafer with UV exposure.

C. Developing the SU-8 coated silicon wafer with the developer.

D. Etching the silicon wafer

E. Molding PDMS on the patterned silicon wafer.

F. Release & formatting



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PMMA Layer Fabrication

- The PMMA layers could be manufactured using a variety of techniques.
- The finished product can be seen in the figure on the right.
- This could be accomplished by laser ablation, hot embossing, which can be seen in the figure below.

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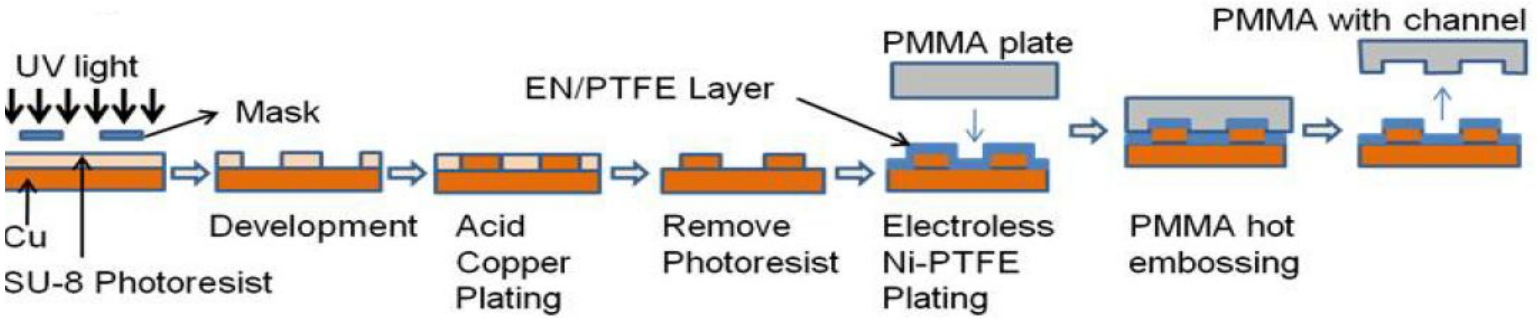

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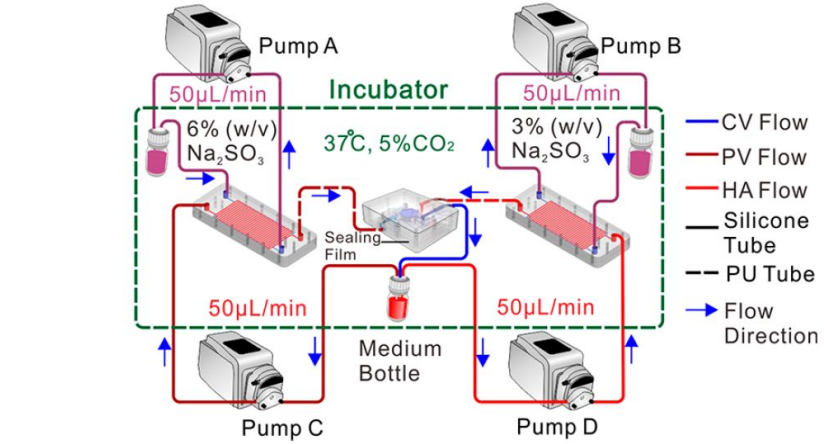
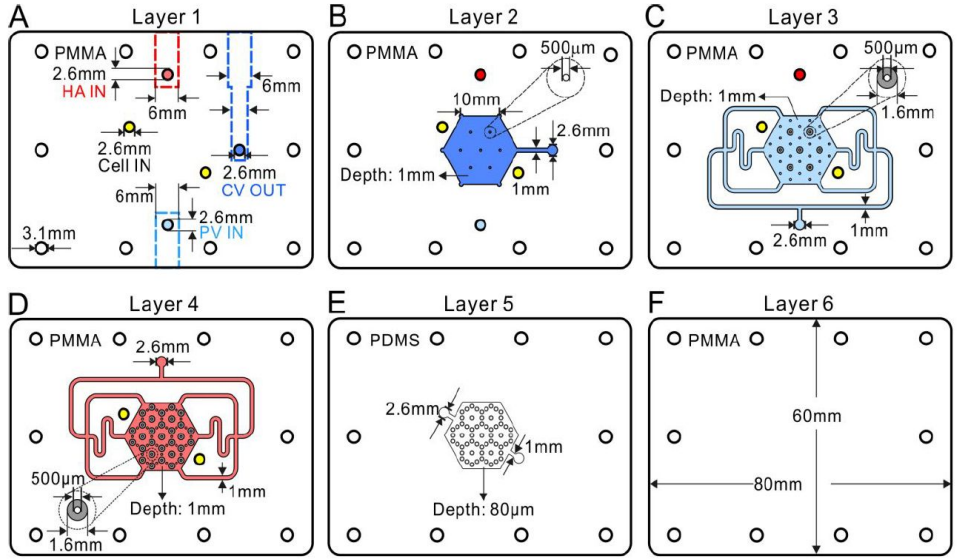
Device Operation

- Cells are loaded through the first layer and flowed through the through holes on the second, third, and fourth layers eventually reaching the culture zone.
- The culture media is then pumped through the HA and PV holes and pushed upwards towards the fifth layer.
- All components are then mixed together and the spent media is pushed back down through the CV outlet.





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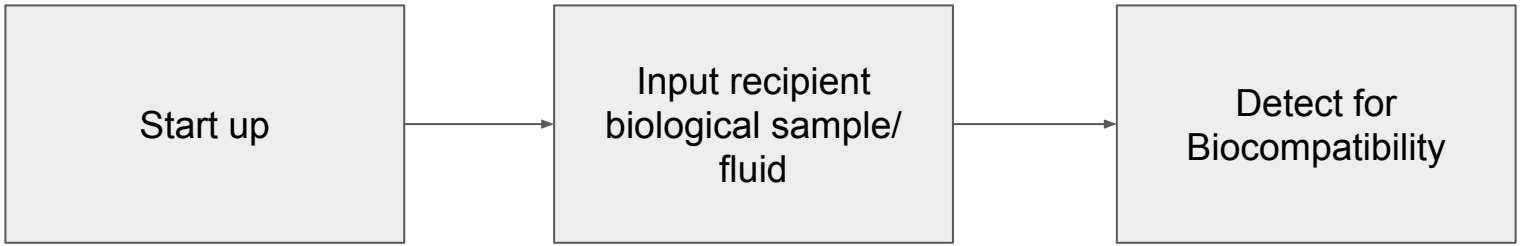
Applying the Device

- Our proposed device will utilize the previously discussed design but will switch to the recipients biological material once it's up and running using the inlet ports.
- Once the recipient biological material is administered, we will use inline sensors to detect for cell death/ biocompatibility.
- If the device is successful, it will accurately detect biological rejection prior of the recipient cells.



Determining Success

Our primary goal is that we are able to detect a rejection or a match prior to transplant

This goal can be measured by:

- Compatible or incompatible cell types/factors detected
- Verification/validation of physical device function

Future Work

- Tailor immunosuppressive therapies and optimize treatment strategies to minimize the risk of rejection
- Optimize organ preservation protocols and enhance the viability of donor organs for transplantation and donor-recipient matching
- Recreate the native microenvironment and study how immune responses are influenced by tissue-specific factors
- Assessment of immune cell activation, cytokine release, and tissue damage in real-time
- Modulate the flow rate and composition of the culture medium: mimic physiological processes such as blood perfusion and lymphatic drainage
- High-throughput screening of immunosuppressive drugs, cytokines, and other therapeutics to identify immunocompatibility profile of potential donors and tailor immunosuppressive therapies to minimize the risk of rejection
- Maintain long-term cultures and monitor immune responses over extended periods: study chronic immune reactions and assessment of late-stage complications associated with transplant rejection.

Future of Liver-on-a-Chip

- Closely replicate the complex architecture and function of the human liver including incorporating multiple cell types found in the liver, such as hepatocytes, Kupffer cells, stellate cells, and endothelial cells
- Mimic the intricate three-dimensional structure of liver tissue and the dynamic microenvironment, including nutrient gradients, shear stresses, and extracellular matrix composition
- Integrate into multi-organ-on-a-chip systems to study the interactions between the liver and other organs involved in immune responses and transplant rejection
- Investigate the systemic effects of immunosuppressive drugs and evaluate the cross-talk between different organ systems in the context of organ transplantation

Future of Liver-on-a-Chip

- Advanced Microfluidics: mimic physiological flow patterns, nutrient gradients, and shear stresses within the liver-on-a-chip device, oxygenation, and waste removal
- Microfabricated accurate sensors which can measure biomarkers of immune activation, cytokine release, and tissue damage within the chip in real time
- Integrate experimental data with mathematical models of immune cell behavior and tissue dynamics to simulate complex biological processes and predict the outcomes of liver transplantation experiments
- Patient-specific models: unique immunocompatibility profiles of individual patients and tailor immunosuppressive therapies, facilitate the screening of drug candidates and predict individual responses to treatment

Potential Organs-on-Chip for transplant immunocompatibility

- Key Components:
    - Immune cells such as T cells, B cells, macrophages, and dendritic cells
    - Human leukocyte antigen (HLA) matching
- Heart-on-a-chip: cardiomyocytes, endothelial cells, fibroblasts
- Kidney-on-a-chip: renal tubular epithelial cells, endothelial cells, mesangial cells
- Lung-on-a-Chip: alveolar epithelial cells, pulmonary endothelial cells, fibroblasts
- Intestine-on-a-Chip: goblet cells, intestinal epithelial cells, microbial cells
- Uterus-on-a-Chip: (hypothetical)

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