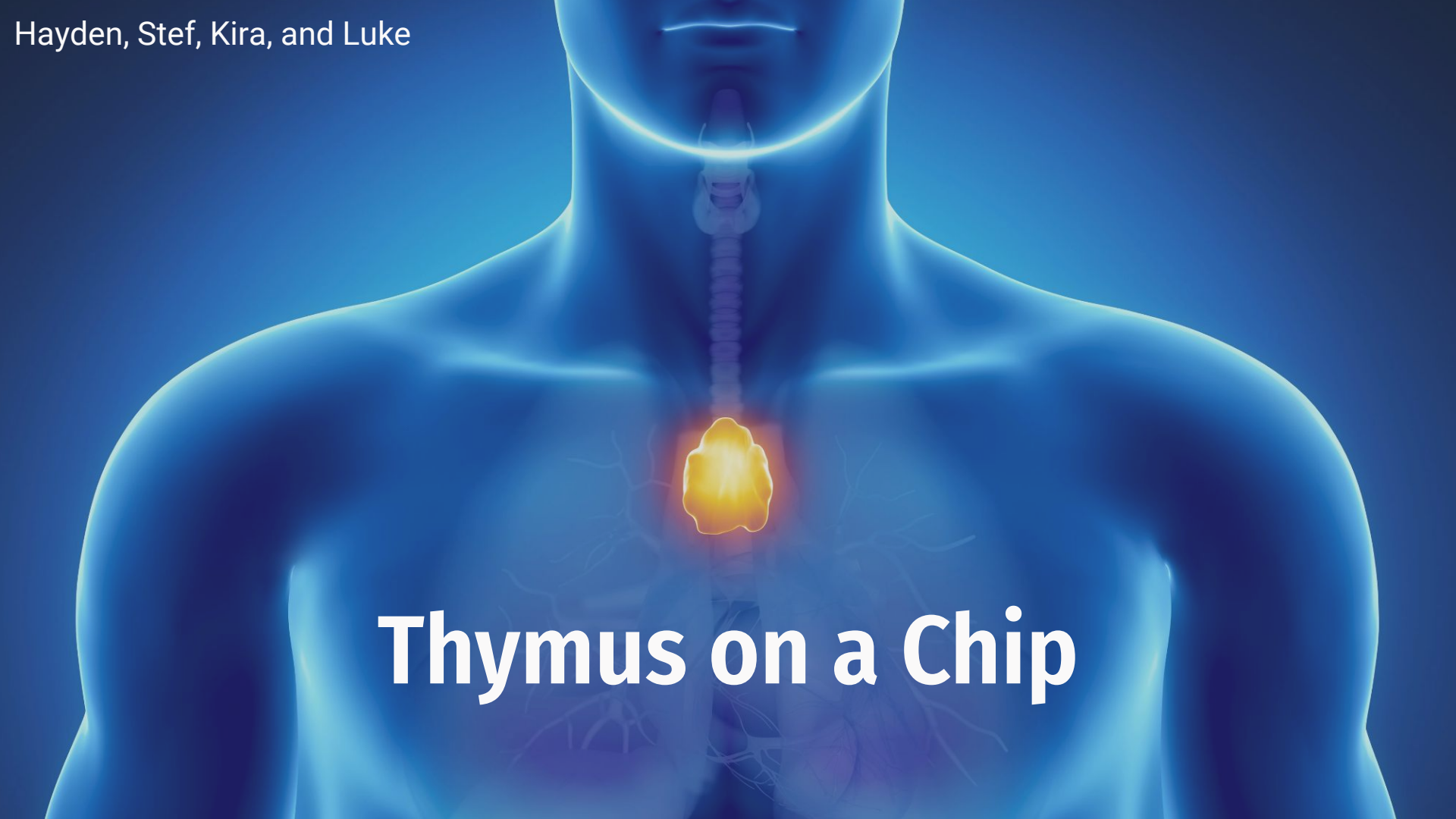
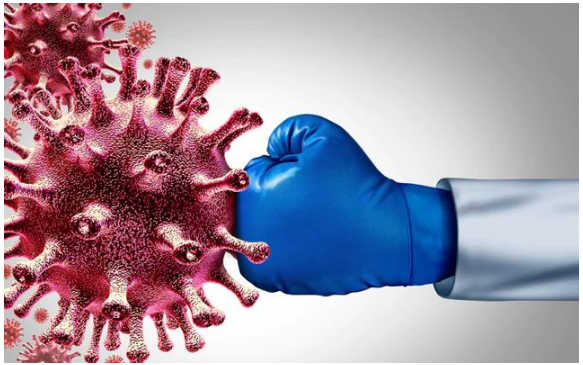
Hayden, Stef, Kira, and Luke

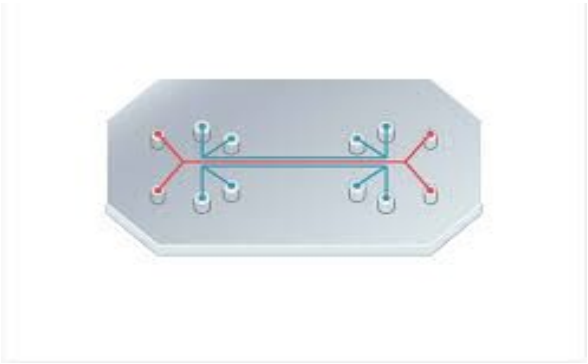


Thymus on a Chip

Background



Immune System



Organ on a Chip

Immune System

Immune Organs

Primary

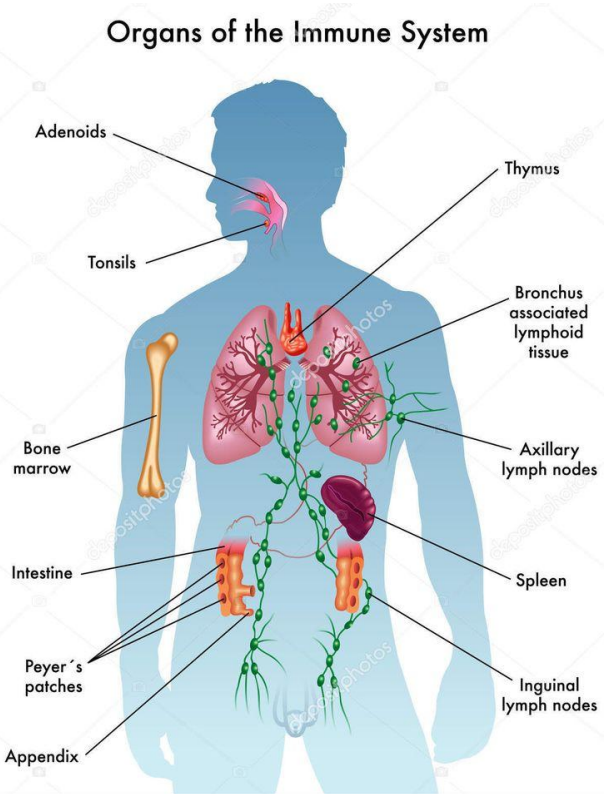
Generate Immune Cells

- **-Bone Marrow**
- **-Thymus**

Secondary

Cell programming

- **-Tonsils**
- **-Lymph Nodes**
- **-Spleen**



Immune Cells

Myeloid Precursor

- **Eosinophils**
- **Basophils**
- **Neutrophils**

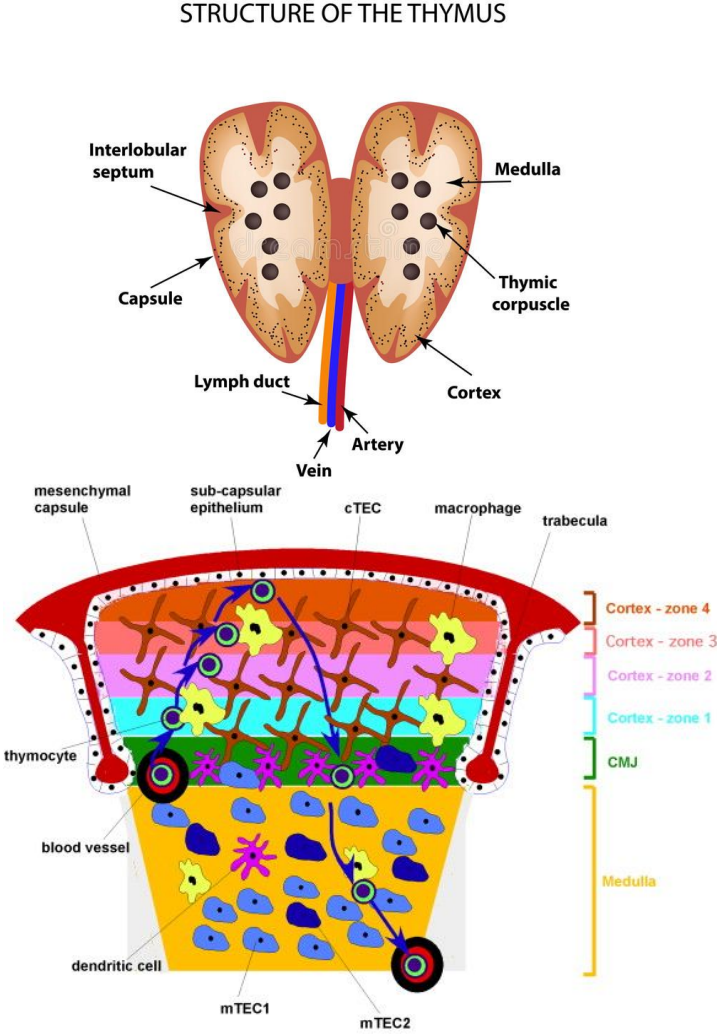
Lymphoid Precursor

- **T and B Lymphocytes**

Thymus

Generates a diverse army of T cells essential to the efficient function of the adaptive immune system.

- Components:
  - Cortex and medulla in which two epithelial cell types reside:
    - cTECs and mTECs
    - Stromal cells: ECs, fibroblasts, and APCs.

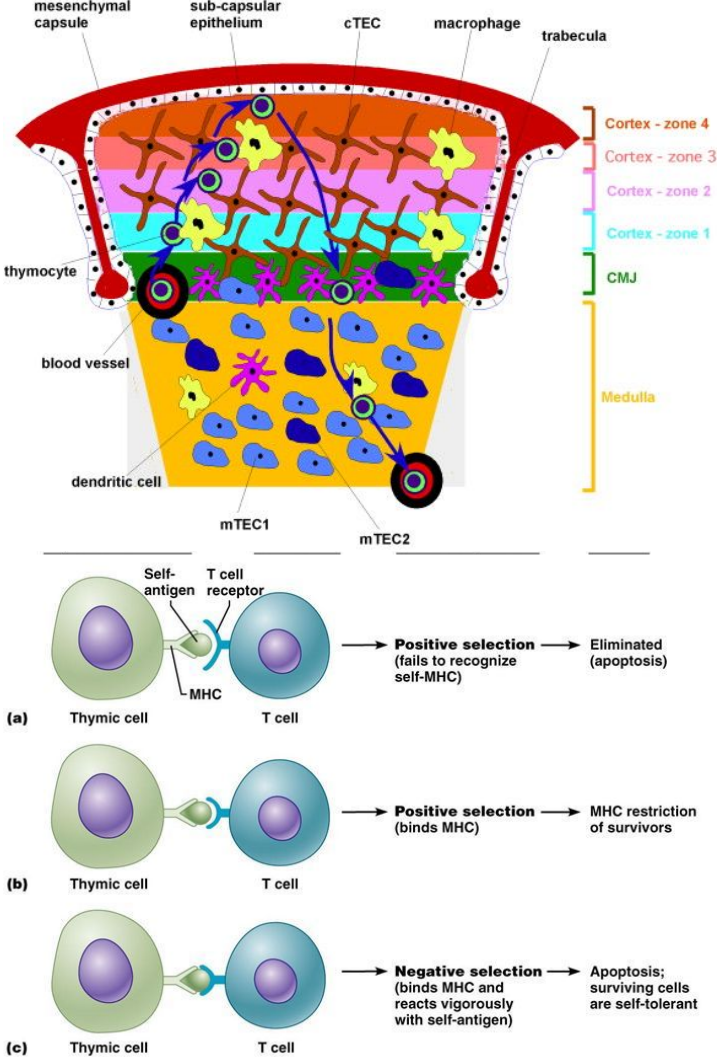


Thymus

Main function: T cell selection and maturation

- Positive selection:
  - Ensure stable TCR interactions with cTECs.
  - Occurs within cortex
- Negative selection:
  - Ensure T-cells with TCRs for self-antigens are eliminated.
  - Occurs within medulla

These events require interactions with the TECs in the 3D stromal environment.



Current Challenges in Studying the Thymus

Scale

Limits Architectural Complexity

Accurate Representation of All Functions

Dependent on Architecture

Limited TEC Regenerative Capacity

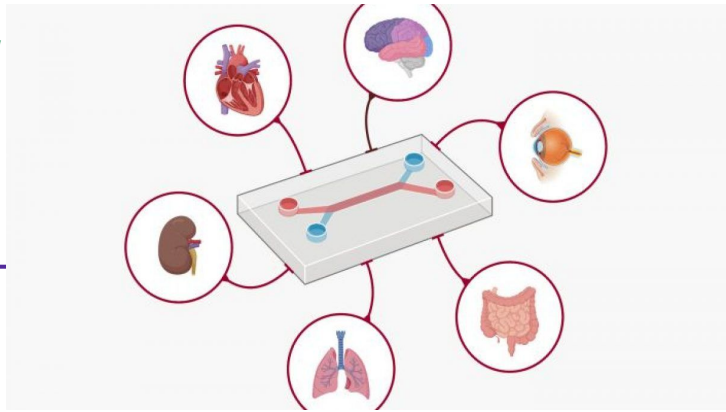
Current TEC Seeding Methods

Poor Cell Viability

Organ-on-a-chip (OOAC)

Drug discovery and cancer modeling

ECM engineering for healthy and disease states

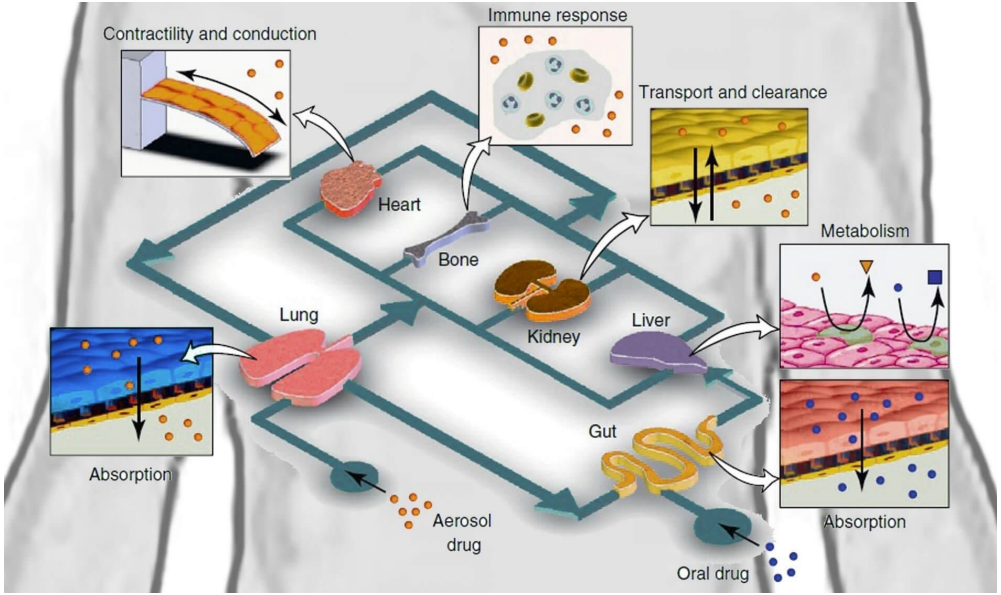


Pathophysiology replication

Assessment of environmental effects

Understanding drug sensitivities in patient subsets

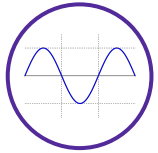
OOAC Components



Geometric confinement and patterning

Control of flow

Inlets and outlets



Environmental control

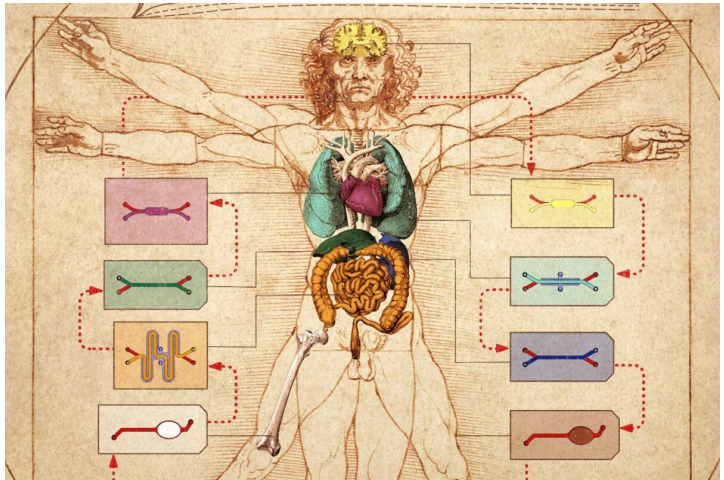
Mechanical/electrical stimulation

Sensors

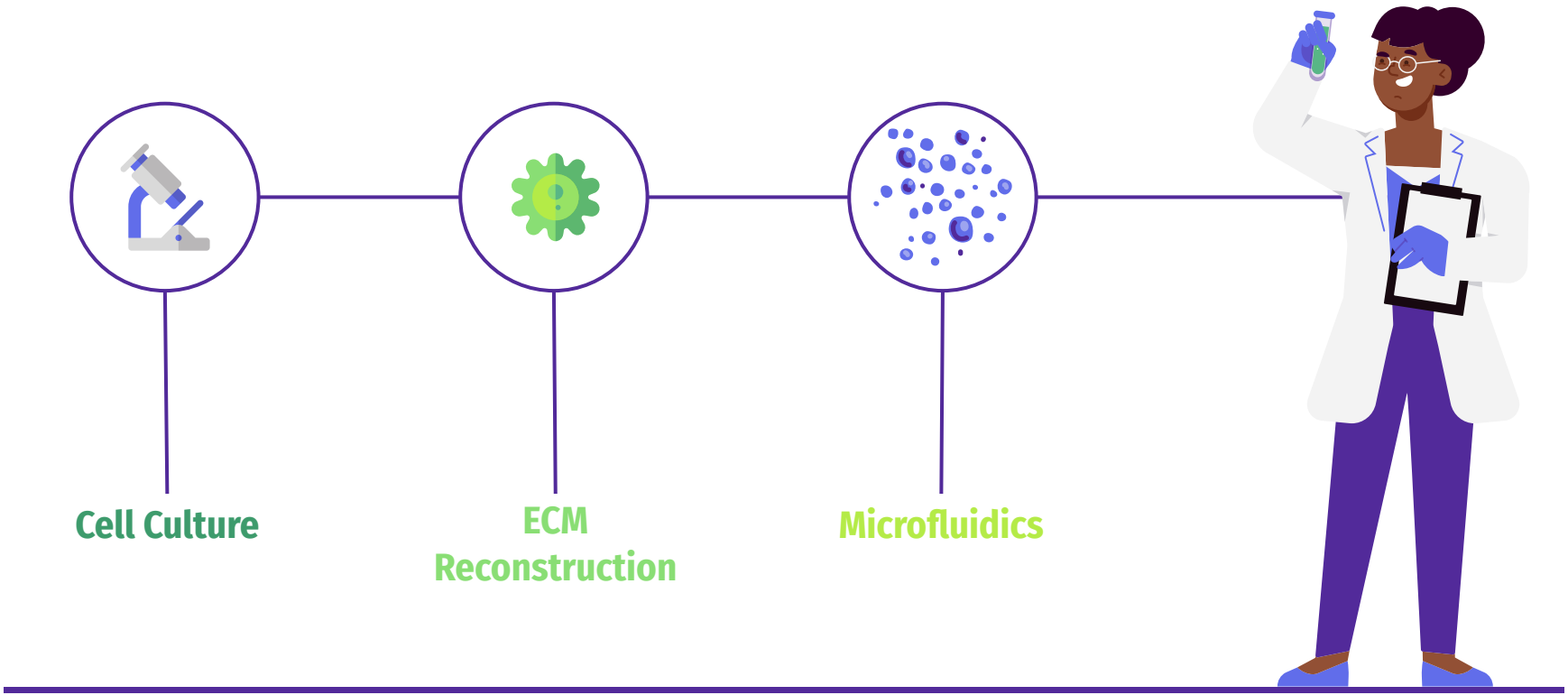
Need for Immune Response in OOAC

“So far, the development of OoC systems to emulate the immune system has lagged behind”

- Necessary for complete body-on-a-chip
- Critical to fully characterize/test new drugs
- Complete pathophysiologies cannot be replicated without immune response



Theory and Principles



Cell Culture

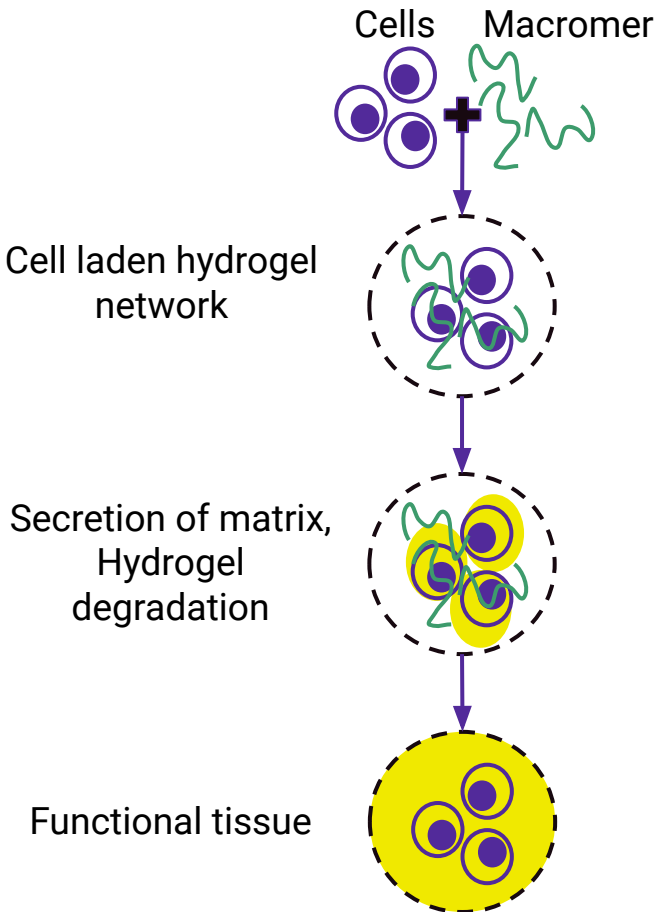
Options for Labs

1. Purchase a cell line (VECs)
2. Harvest cells from animal (TECs)

Reaggregate Thymic Organ Cultures

- Harvest thymic lobes→ cut into small pieces → filter thymic remnants → select and separate desired cells (TECs)
- Continue to culture and split cells as needed

ECM Reconstruction



ECM Function:

- Cell adhesion
- Cell growth
- Cell signaling

ECM Reconstruction

- Hydrogel

Microfluidics

- Features less than 1 mm & internal volumes less than 100 µL

Pros:

1. Less volume and analyte required
2. Gravity and inertia become less relevant at this scale

$$\mathrm{Re} = \frac{\rho V D}{\mu}$$

Small diameters → Small Reynolds numbers
→ ***Laminar flow*** ✅

$$\bar{x}^2 = 2Dt$$

In laminar flow, mixing is predominately by ***diffusion***

Requirements of Modeling the Thymus

1. Utilization of functional cell types of the Thymus

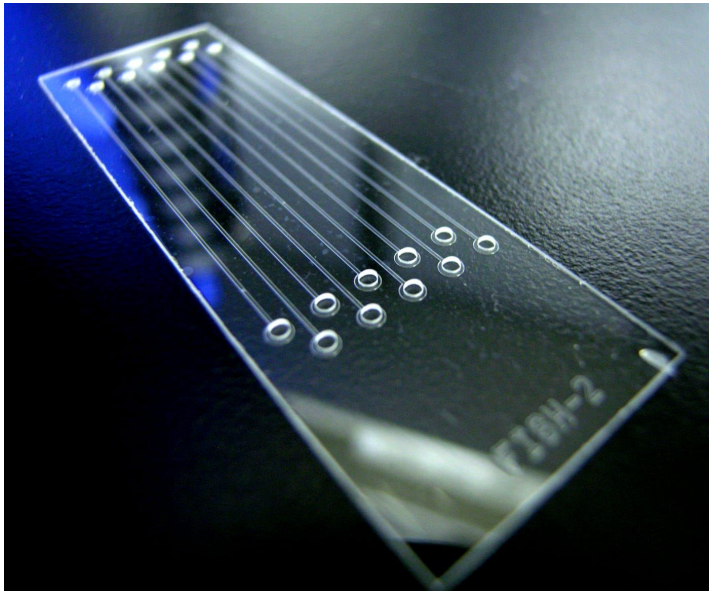
2. Suitable biomaterials for cell adhesion/cell interactions

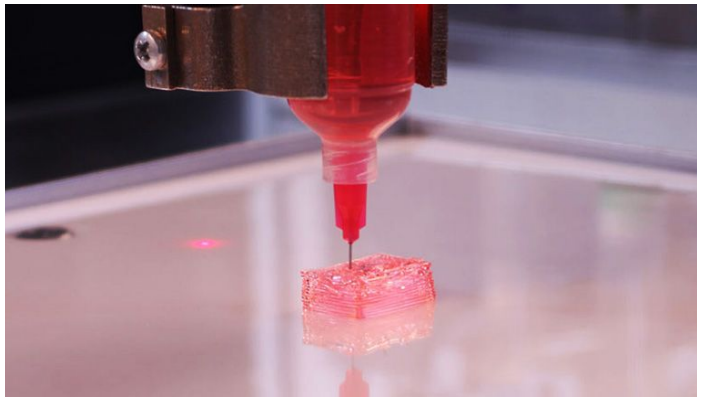
3. Cell viable environment

4. Monitoring of T-cell selection

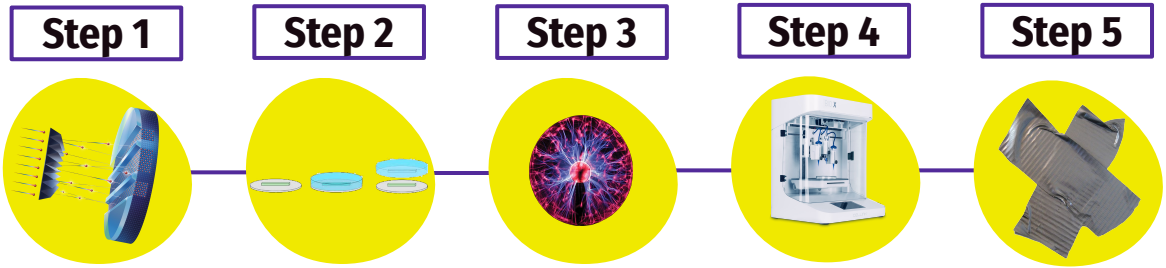
Device Fabrication

Microfabrication
+
3D Bioprinting

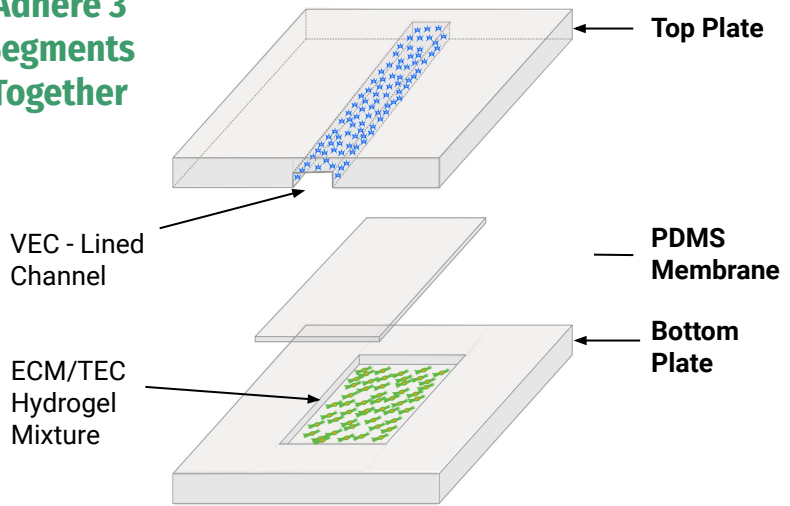




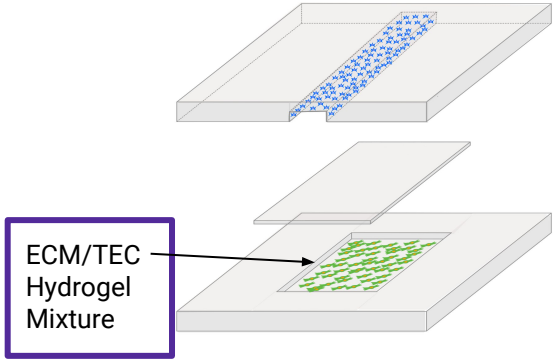
Microfluidic Fabrication

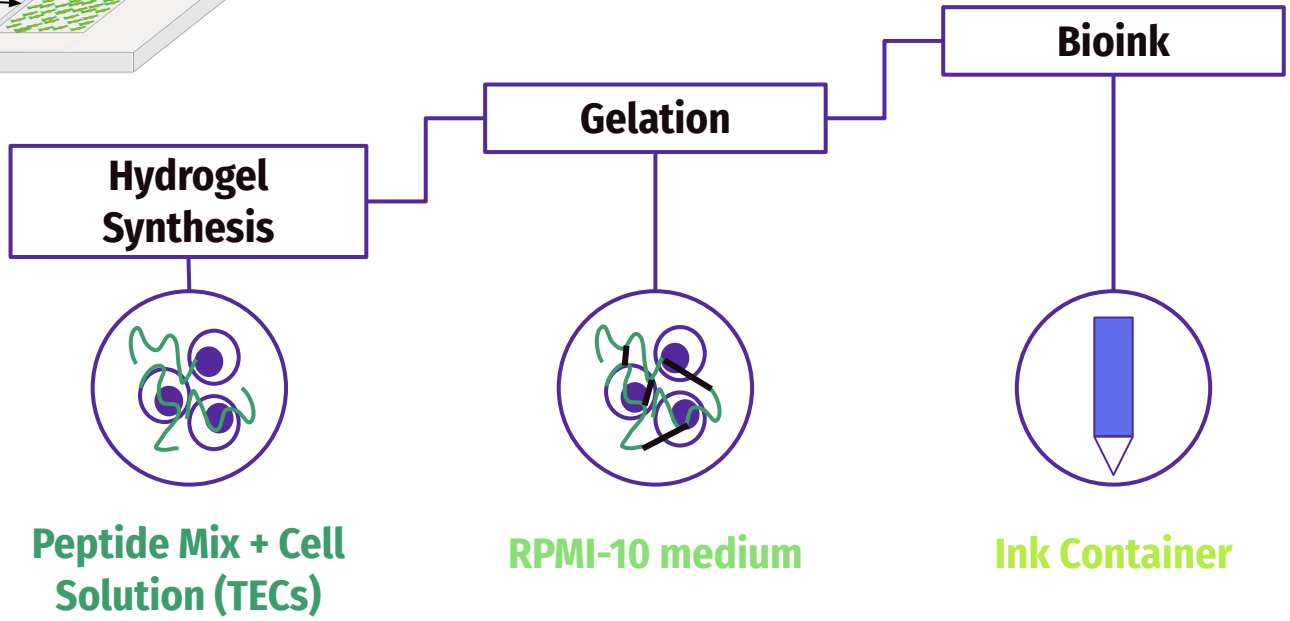


1) Top Microfluidic Channel
2) Thin Porous Membrane
3) Bottom Functional Unit Pocket



Bioink Construction





Endothelialization of Channels w/ VECs

Why is this component needed?

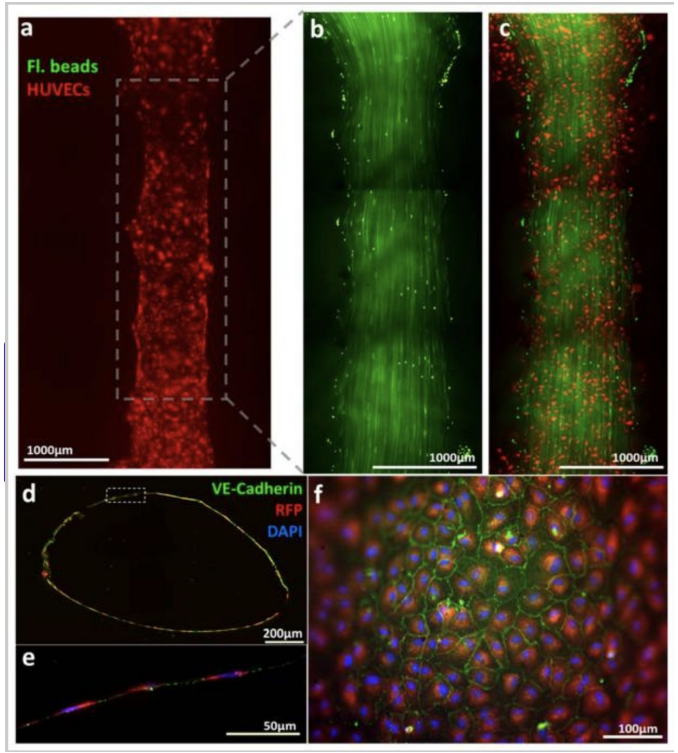
- Current thymus organoids do not include vasculature

Deposition of Adhesive ECM Components

- Fibronectin coating

Cell Suspension

- Connect tubes to channel inlet and outlet
- Flow VEC solution through
- Place on rocker for 5 minutes
- Flip upside down



Testing & Validation

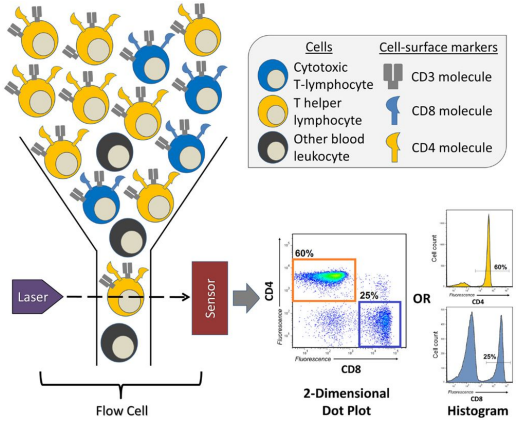
Biosensing

Flow Cytometry

1) (+) Selection and Successful Maturation

Stain appropriate cell surface markers indicative of T cell maturation

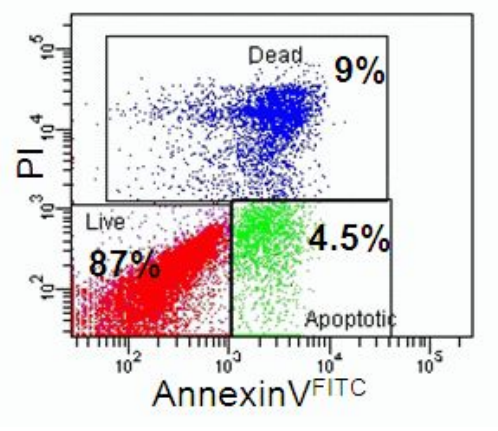
- CD3+: All T Cells
  - CD8+: Cytotoxic
  - CD4+: Helper
  - CD25+: Regulatory



2) (-) Selection

Cell Viability: Annexin V (Stains PLs) and Propidium Iodide (Membrane Integrity)

- Live Cells => PL within cell
- Apoptotic => PL exposed
- Other Death => PI stained



Validation

Cell Viability

Are the cells alive post-implantation?

Testing loss of TECs

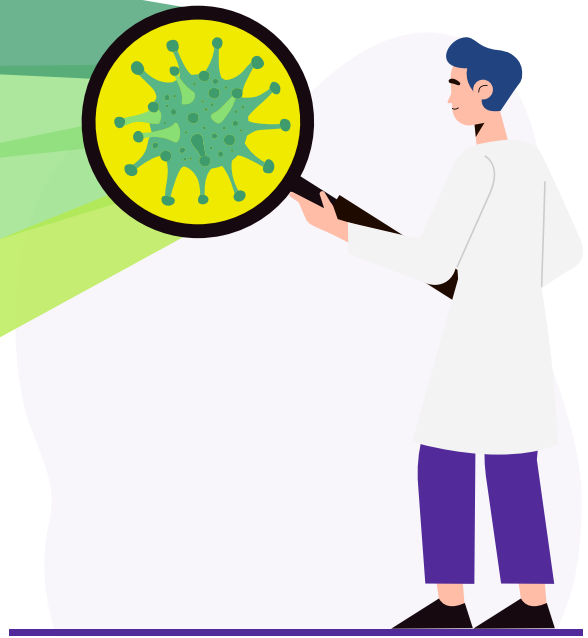
Are epithelial cells being lost when solution is flowed?

Expected Function

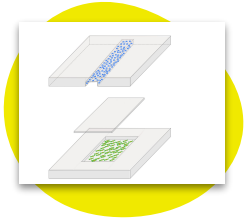
Are the T-cells being “killed” as expected?

User Interviews

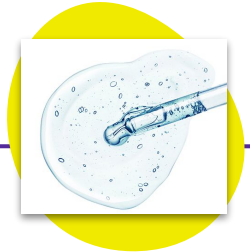
Will the device be useful?



Cell Viability

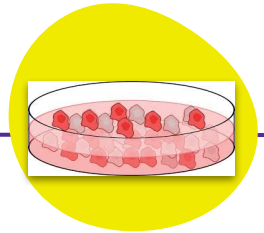


TOAC (Thymus on a chip)



Hydrogel Cell Culture

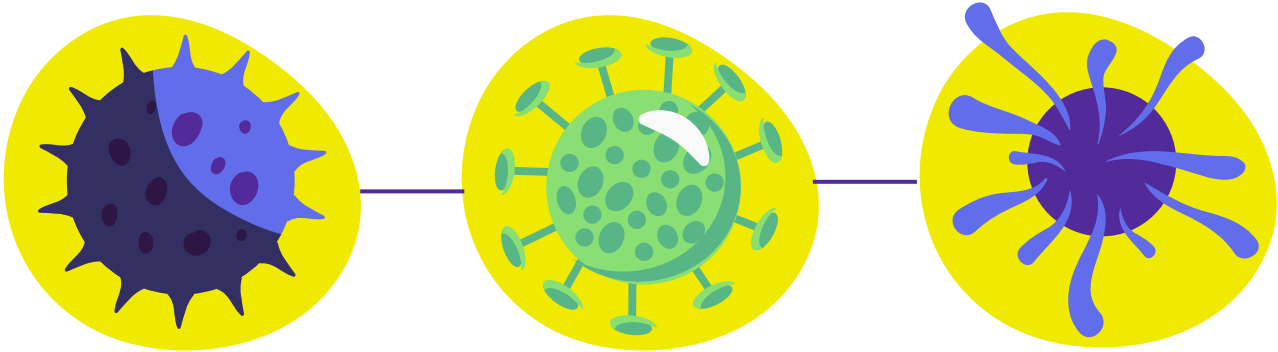
Fluoroprobe CMFDA
Bind fluoroprobe CMFDA to cells
Place cells into hydrogel
Use computer software to measure the amount of viable cells with an image



Liquid Cell Culture

Trypan blue staining
Stain cells with trypan blue
Implant
Collect sample after 1 hour, 1 day, 1 week, and 2 weeks
Use a hemocytometer and measure amount of viable cells

Quantifying Loss of TECs



Finalize Prototype → **Flow T cell solution** → **Flow cytometry**

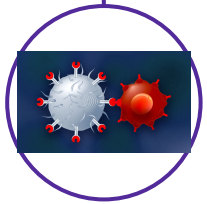
Finalize hydrogel and complete prototype

Flow solution of T cells through device

Add stain to solution to use flow cytometry and ensure no stained CD1a leaves the device

Expected Function

Step 1



Insert T cells

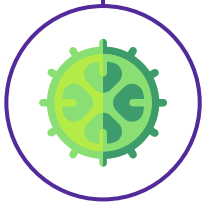
Flow solution of T cells through TOAC

Step 2

Collect Solution

Collect the solution after flowing through TOAC

Step 3



Measure cell viability

Use flow cytometry to find % viability of solution (i.e., how many cells were killed)

Step 4

Evaluate

Does the cell viability match what was expected?

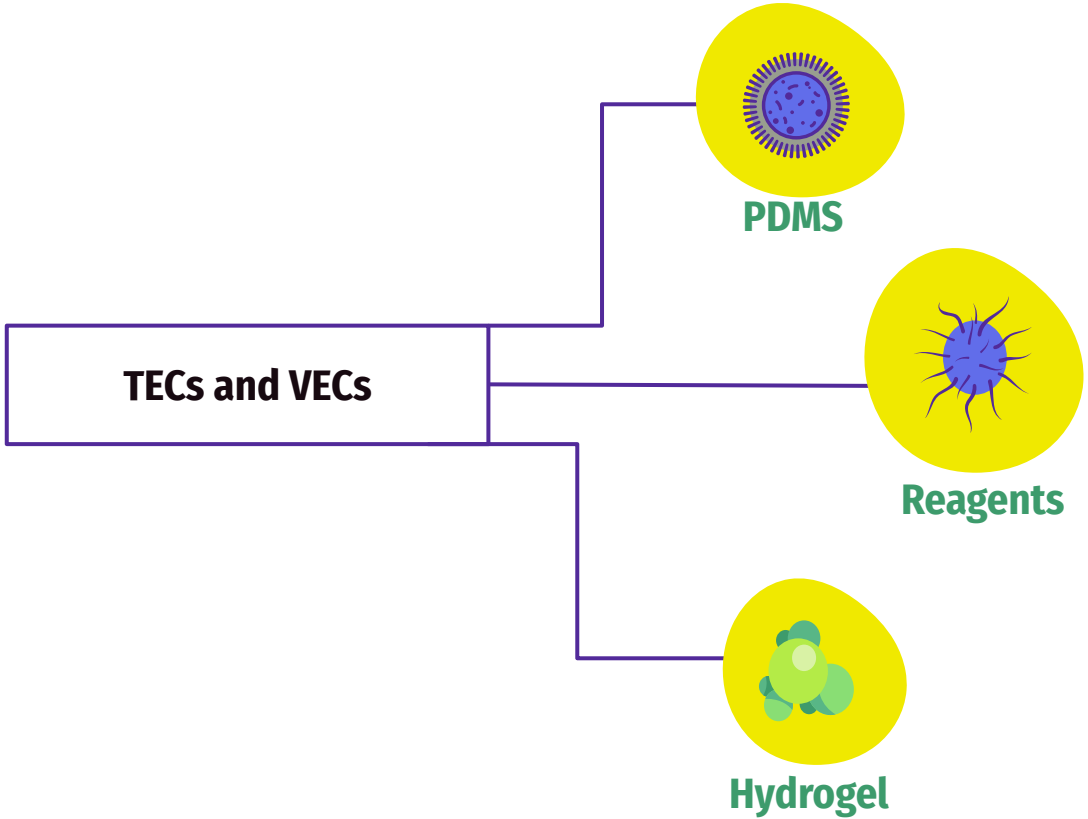
User Interviews

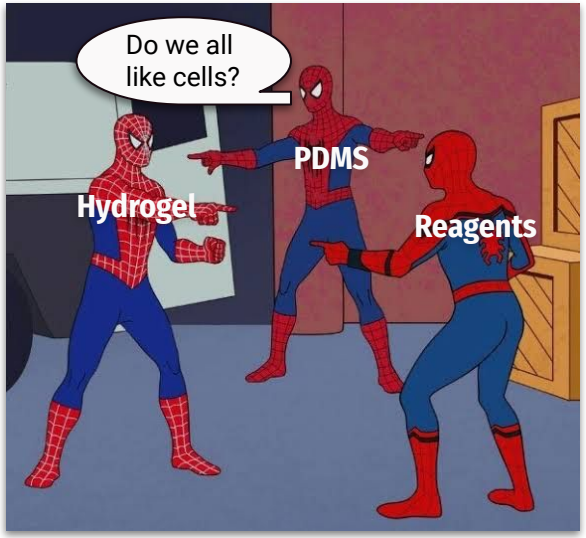
Rate the usefulness

Rate the usability

What is missing that you would like to see?

Biocompatibility





Limitations → Future Directions

1	**Scale**	3D Organoid via new fabrication methods
2	**Organ Complexity**	Incorporate vasculature to generate large 3D organoids capable of cell and ECM spatial organization
3	**Unable to Differentiate Origin of Apoptosis**	New Methods
4	**Frequency of Interactions**	Diffuse T cells through a 3D organoid
5	**Timescale for Processes**	Diffuse T cells through a 3D organoid

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Questions?