Stem Cell Differentiator-on-a-chip

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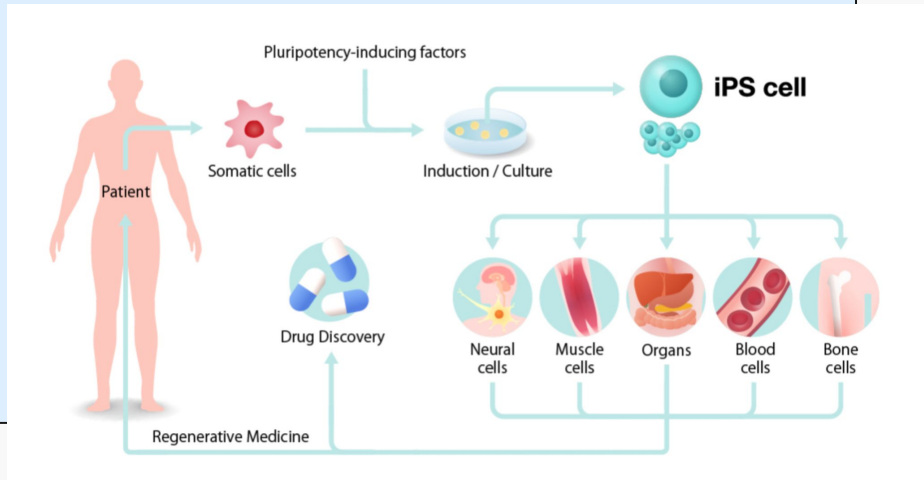
01

Background

iPSCs

- Our focus is on induced pluripotent stem cells (iPSCs)
- Recently created in 2012 by Professor Shinya Yamanaka
- Generated from adult somatic cells by introducing reprogramming factors via plasmids or viral vectors
    - Can differentiate into neurons, heart cells, cartilage, blood cells, T cells, liver cells, and insulin producing β cells
- Tissues derived from iPSCs are a nearly identical match to the cell donor
    - Enable production of diseased cells for patient specific drug screening, toxicity testing, and diagnostics
- Similar behavior to embryonic stem cells, but with fewer ethical implications



Methods of Differentiation

In Vivo

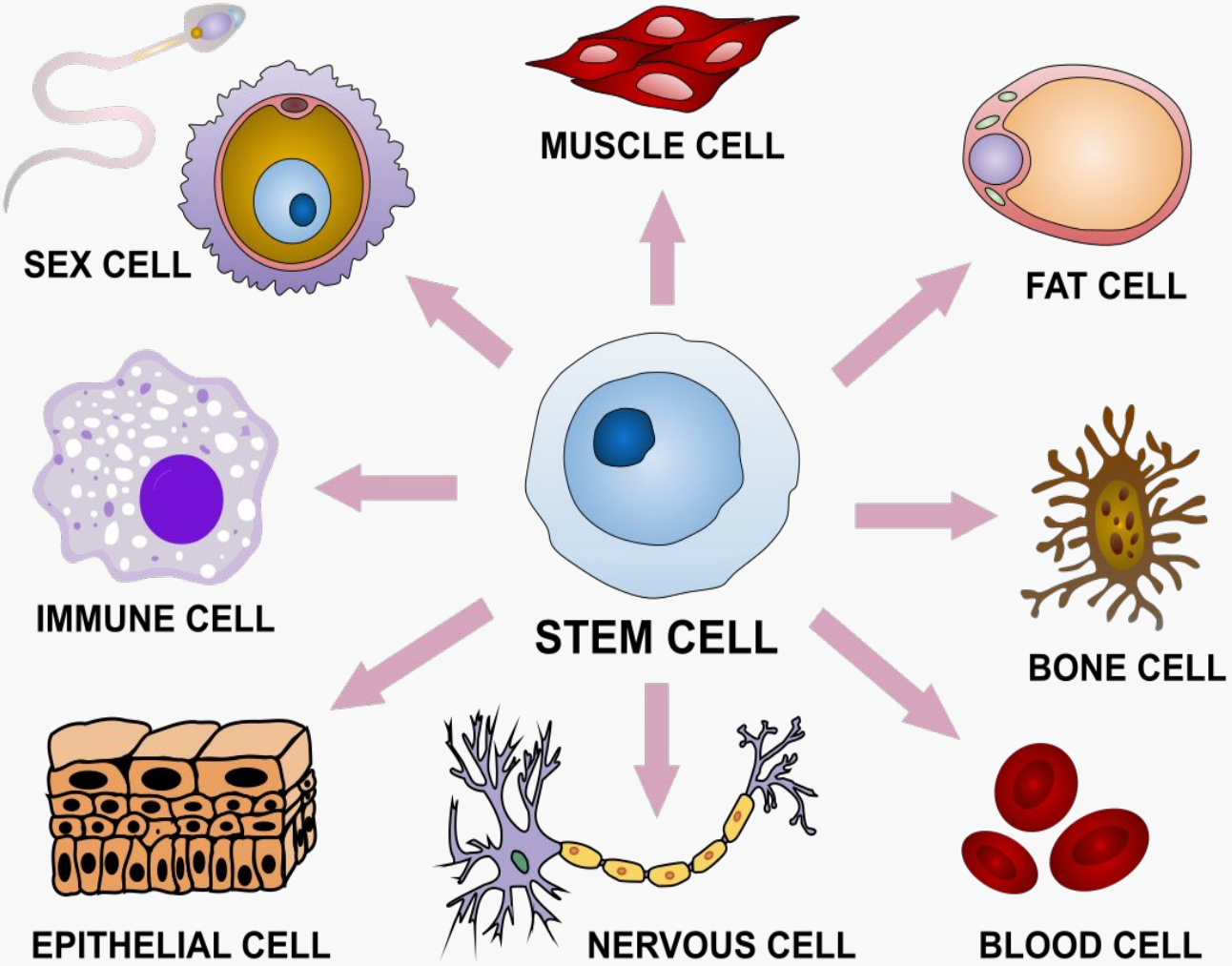
- Initiated by molecular signals transduced via either an extracellular or intracellular pathway
- More of a “hands off” method

Chemical Signaling

- Addition of extracellular matrix molecules within the culture media
- Co-culturing with other cell types
- Cell-conditioned media
- Exposure to cytoplasmic extracts from differentiated cells
- Stem cell hybridization with enucleated cytoplasts from differentiated somatic cells

Physical Stimuli

- Characteristics of biomaterial substrate can affect the process of differentiation
  - Composition
  - Adhesion
  - Stiffness
  - Shear stress
- Application of mechanical forces, heat treatment, or magnetic and electrical fields



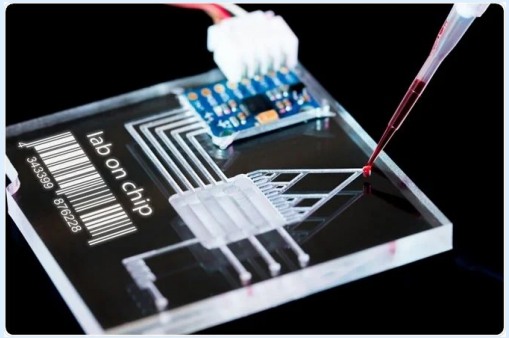
*As a whole, stem cell differentiation is a product of the microenvironment niche the cell is exposed to

Current Difficulties

- Most stem cell studies are done in vivo, which are very limited in scope and applicability to other situations and can lead to unwanted differentiation or even teratomas
    - Instead, in vitro differentiation avoids spontaneous differentiation into undesired lineages at the transplantation site as well as reduces risk of teratoma formation
- In vivo studies provide very little information about molecular mechanisms and signaling pathways
- Contact with other cells during differentiation can lead to pathogen transmission
- SC differentiation as a whole has low viability
- Sources of SCs are not always reliable and iPSCs are expensive to make
    - Experiments at a micro-scale could alleviate this issue

Lab-On-a-Chip

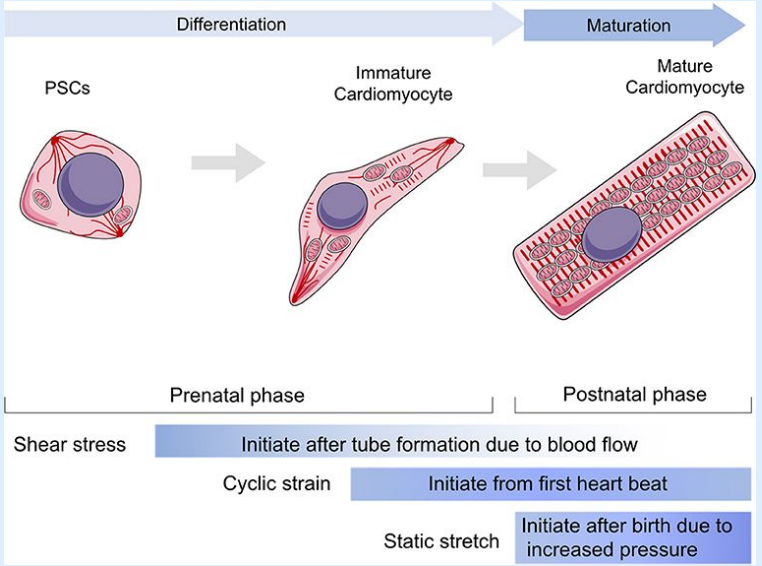
- Permits testing methods to manipulate products on the micro or nano scale
  - Optical, acoustic, electrical, centrifugal force, hydrodynamic, magnetic, surface modification
- Benefits include automation, portability and high sensitivity when testing
- Models are designed to recreate the microenvironment of tissues and organs as a way to study cell behavior and tissue development
  - Helps make strides in regenerative and personalized medicine
- Minimal material requirements
- Decreased cost



Cardiomyocytes

- Cannot proliferate on their own
  - However, iPSCs can proliferate and then differentiate, allowing for the creation of new cells
- Easily identifiable biomarkers
- Both contractile and excitable

All of these properties make them a great candidate for use in our stem cell differentiator!



02

Our Device

Lab on a chip - Directing and Optimizing Differentiation

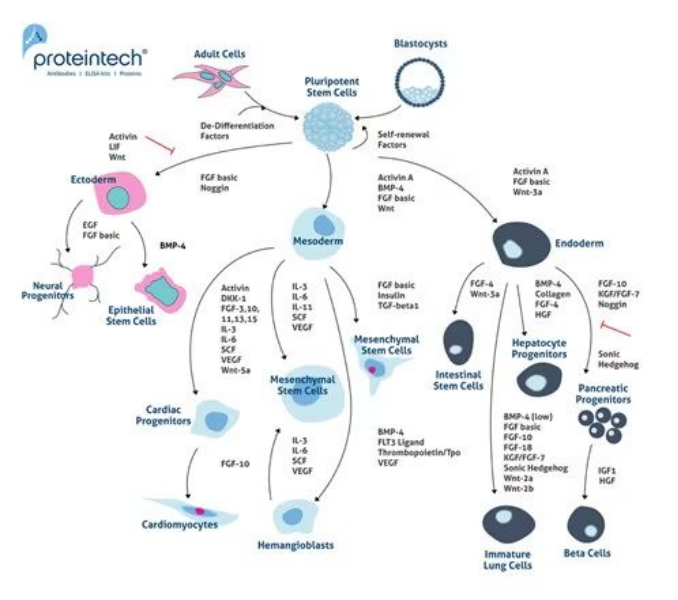
Device Purpose

- Stem cell differentiation is a lengthy and expensive process, so doing it at a micro scale on a lab-on-a-chip decreases the time frame, material requirements, and cost.

- This device will allow for multiple microenvironments for differentiation to be tested simultaneously and determine the best for generating excitable cells such as cardiomyocytes

Guiding Differentiation Pathways

- Transcription factors determine stem cell fate
  - Different cell types emerge from like stem cell population
- Our device would be designed for one pathway
  - Generalizable in concept



Optimizing Differentiation Efficiency

Goals

- Increase Viability
- Reduce number of cells mis-differentiated or non-differentiated
- Get all cells to maturity with functionality intact

Cell Environment Manipulation

- **Substrate Rigidity**
  - Adjust PDMS stiffness using underlying piezoelectric film
    - Substrate rigidity shown to impact cell viability
- **Constants**
  - Culture Media Composition (Transcription Factors)
  - Substrate material/surface characteristics
  - Culture Time
  - Desired Differentiation Pathway (Per Device)
  - Fluid shear stress

The Device

Microfluidic Function

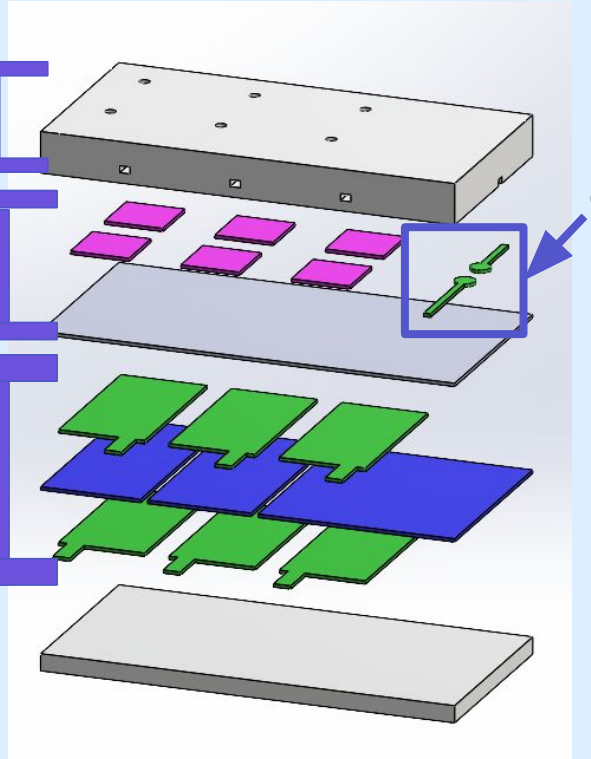
1. Allows for nutrient transport and washing of chambers
2. Transports old media to the sensors for cell and analyte detection
3. Provides suction to extract old media

Cell Growth Function

1. Provides an insulated and functionalized surface for growth
2. Allows for voltage detection through a microarray

Piezoelectric function

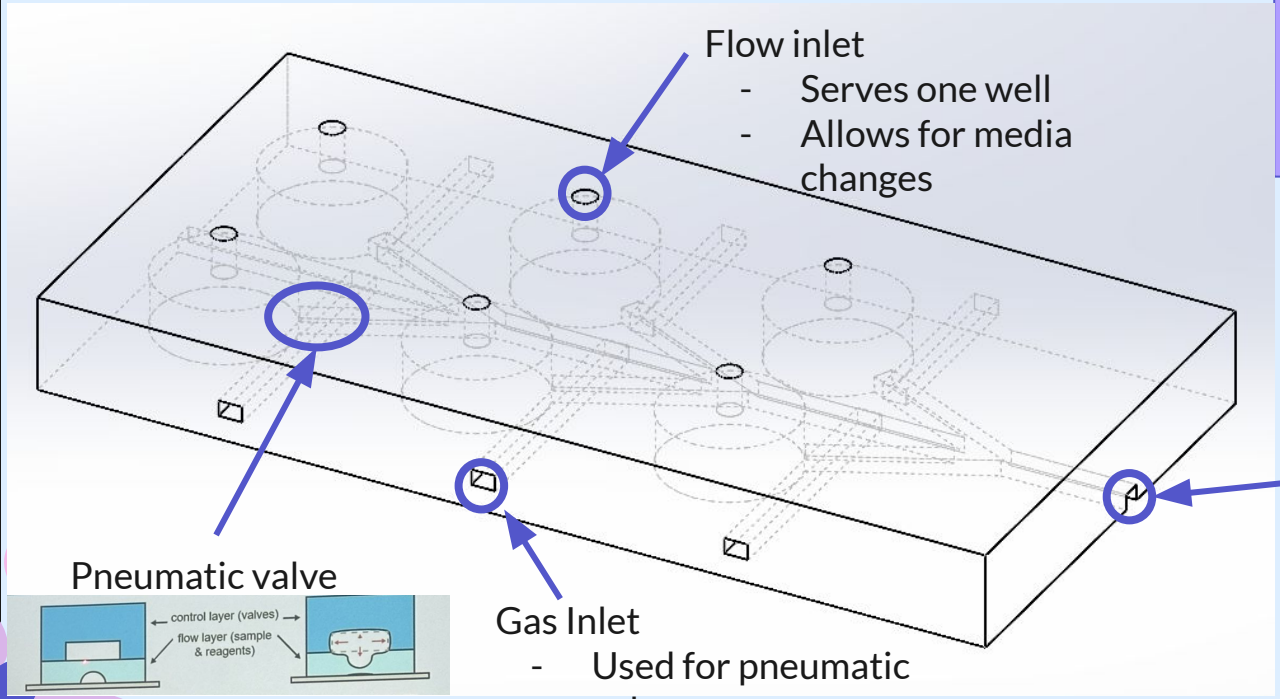
1. Allows for stiffness changes in the baseplate



Cell Count Function

1. Allows for time based cell death profile
2. Allows for final cell counts

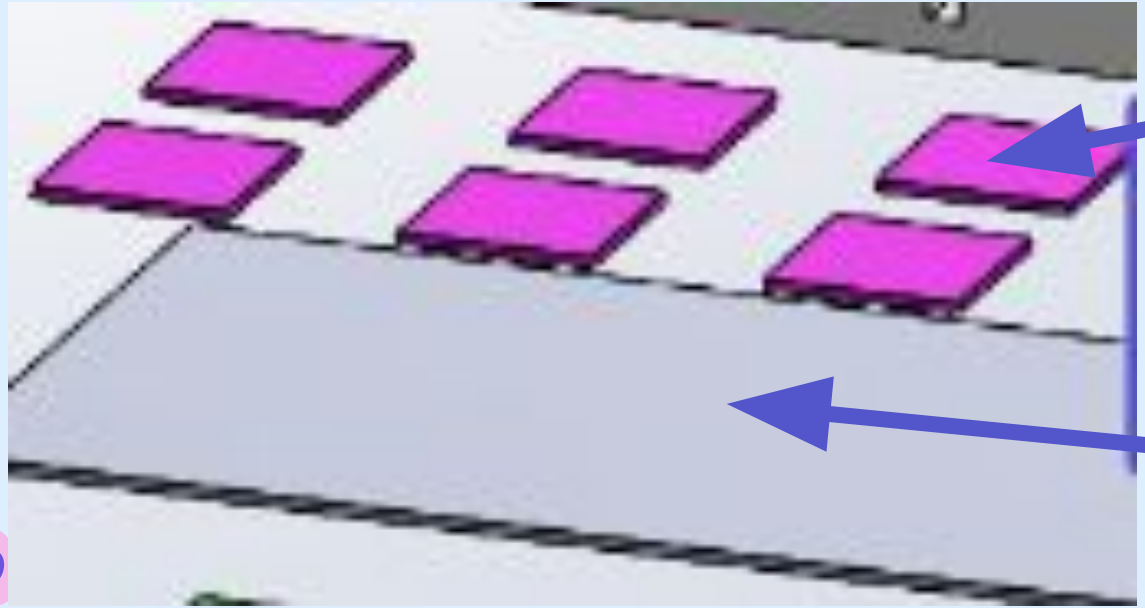
Microfluidic Functions



Fluid Mechanics:

- Laminar Flow regime
- Governed by poiseuille flow $Q=\Delta P\pi r^4/(8\eta L)$

Cell Growth Function



Microelectrode Array

- Functionalized for cell growth and voltage detection

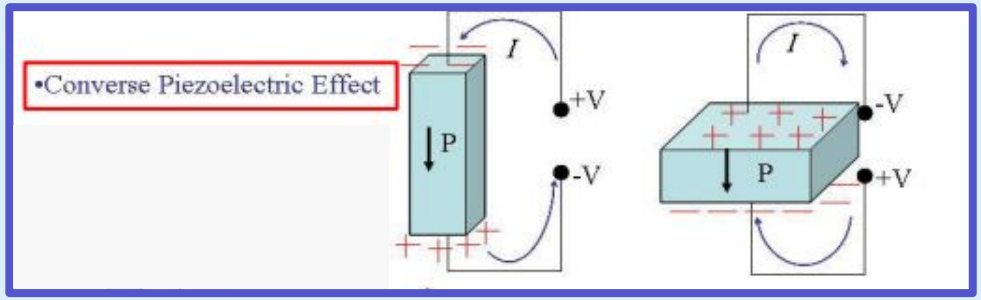
Insulating Layer

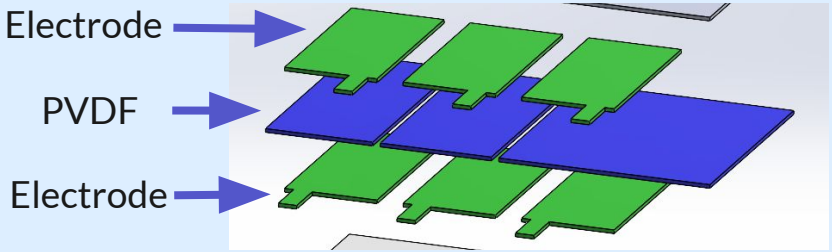
- Thin film to protect cells from electrodes

Piezoelectric function

- PVDF is piezoelectric polymer
- When a voltage is applied across it, PVDF can change experience a stiffness and shape change (Yang, Z)
- This effect can change substrate stiffness if the other materials sufficiently soft and thin

Converse Piezoelectric Effect

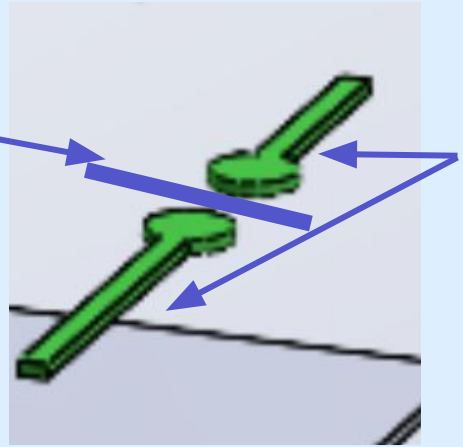




Cell Counter Function

Use coulter principle in the form of a resistive pulse sensor

- Detect and count dead cells each wash to get a time based cell death metric
- Detect and count cells that lasted the course of the experiment



Resistive Pulse Sensor

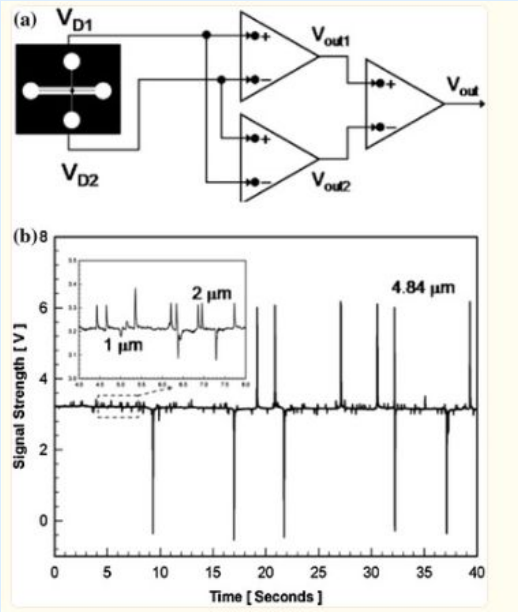


Figure from: Zhang, H, "Methods for counting particles in microfluidic applications"

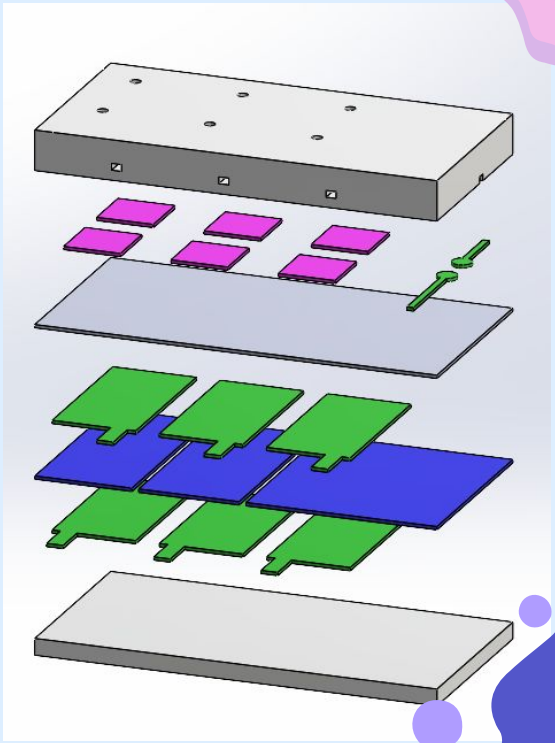
03

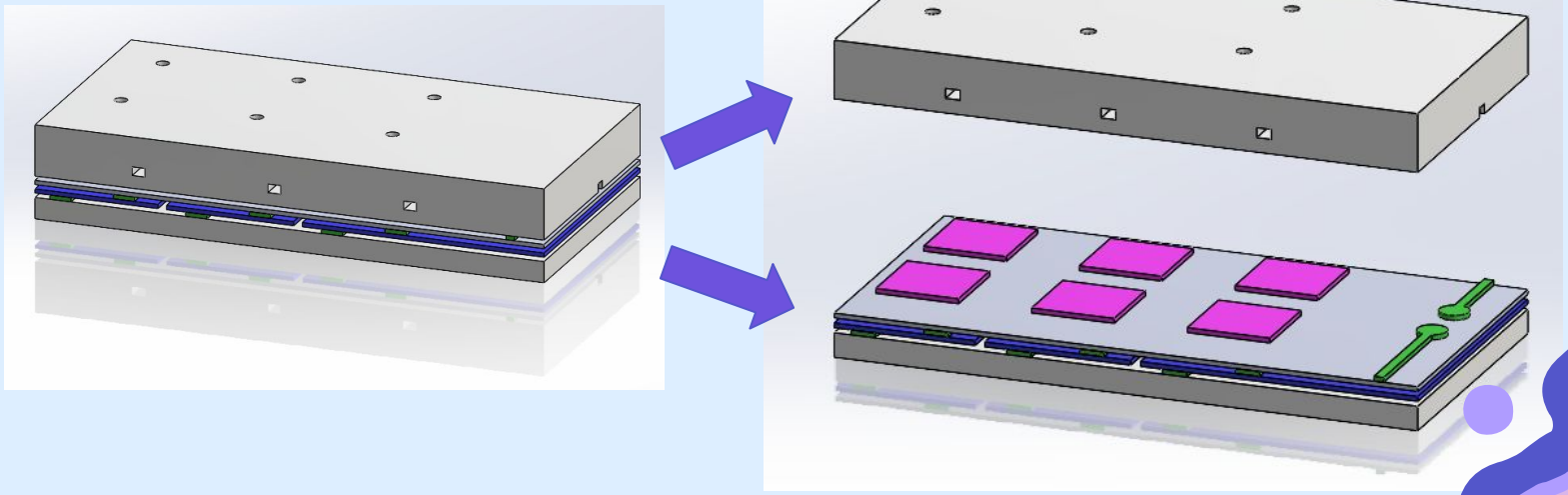
Fabrication

Proposed Layout

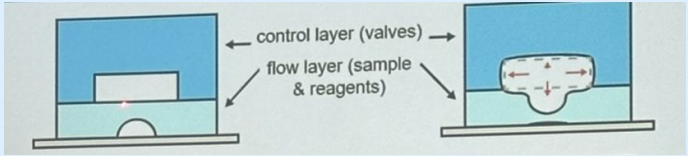
Made in two sections to be bonded together.

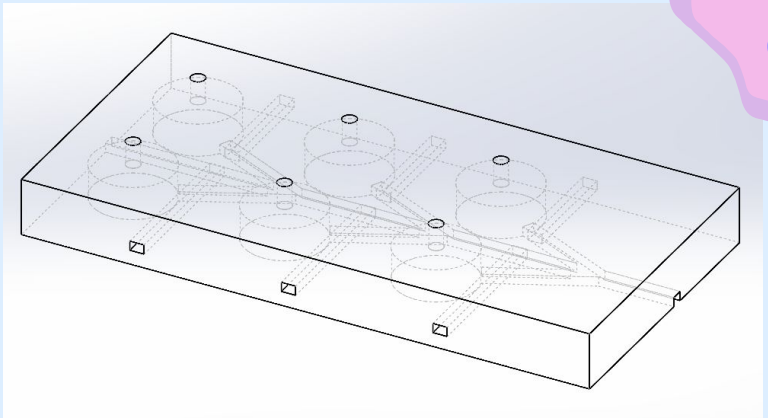
White -> PDMS
Pink -> Microelectrode array
Green -> Ti/Ni
Blue -> PVDF

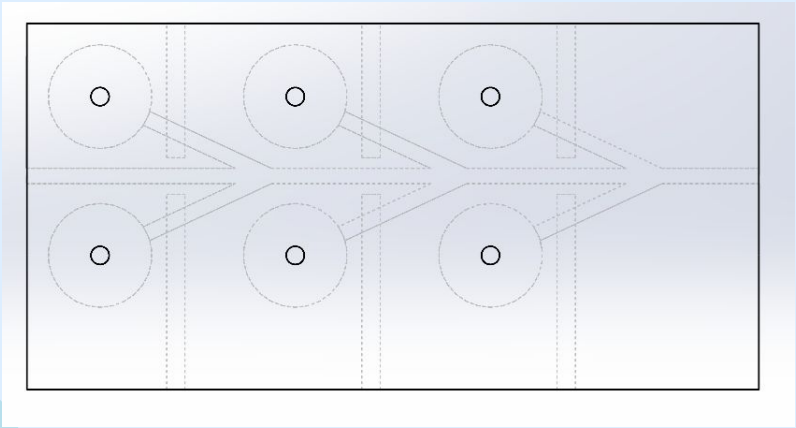




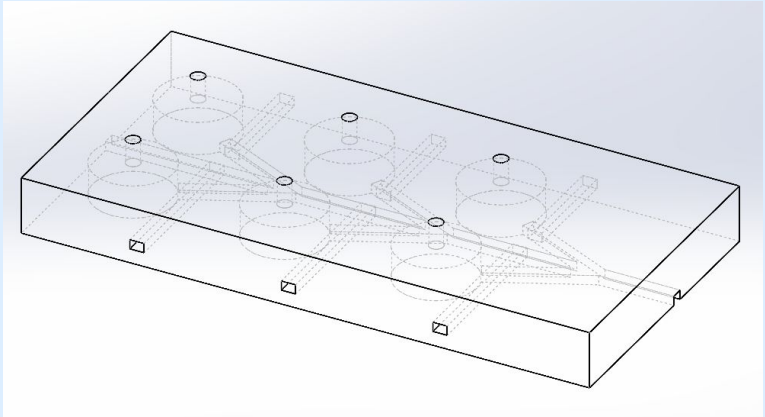
Part A







Part A Fabrication



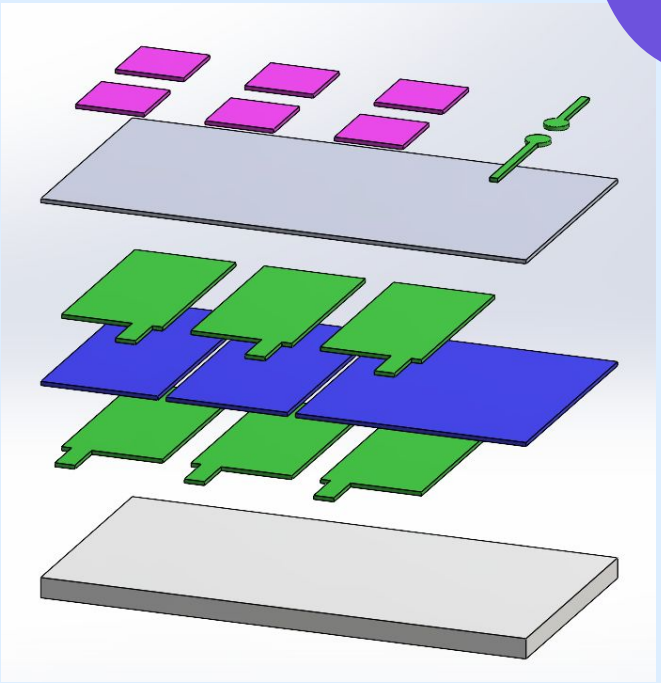
1. **Micromill** PMMA opposite of desired PDMA shape
2. Pour PDMS on top of PMMA mold, remove PDMS
3. Make side holes in PDMS using **micromilling**

- Carugo, D., Lee

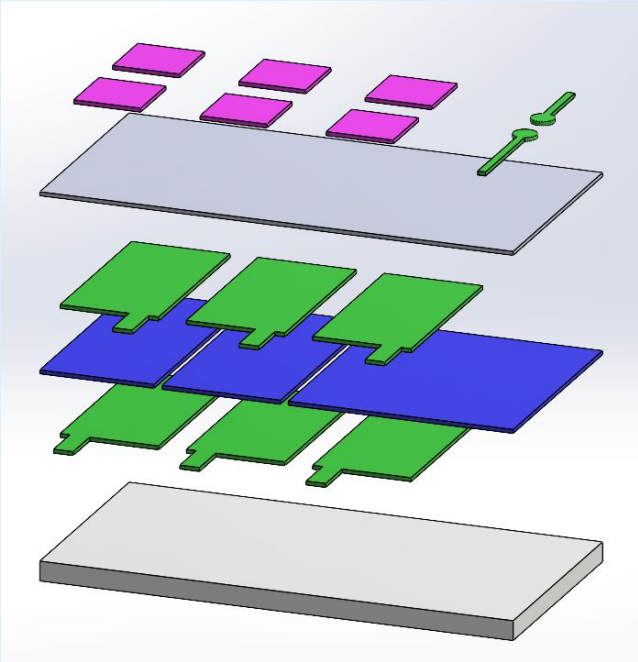
Part B

1. Ti/Ni (bottom green) connects to positive and negative charge
2. Charge activates movement in PVDF (blue) at differing intensities

1. Charge both ends of Ti/Ni(top green)
2. Measure impedance as cells pass through channel



Part B Fabrication

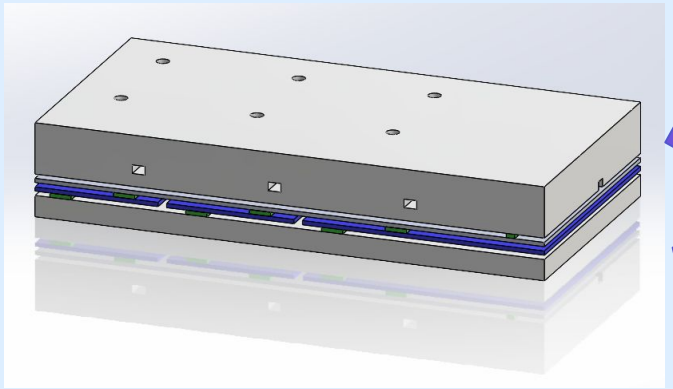


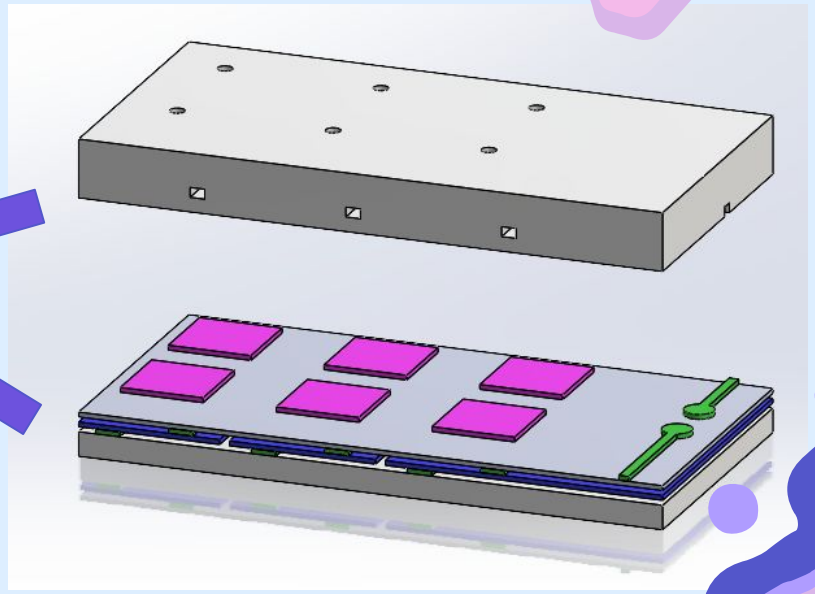
1. PDMS base
2. **Electron beam evaporation** to deposit Ti/Ni through mask
3. PVDF deposited through **Spin Coating**
4. **Etch** PVDF
5. Ti/Ni repeat with new mask
6. **Spin Coating** thin layer of PDMS
7. Final layer of Ti/Ni
8. Place microarrays on top

- Kim S
- Stefania Torino
- Ravi S Kane

Final Product

1. Oxygen plasma bonding





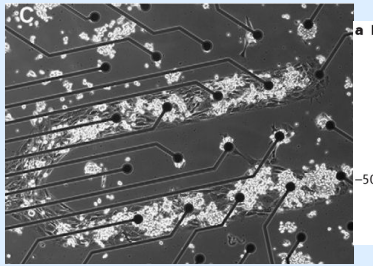
04

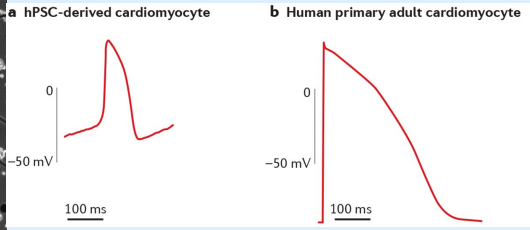
Testing

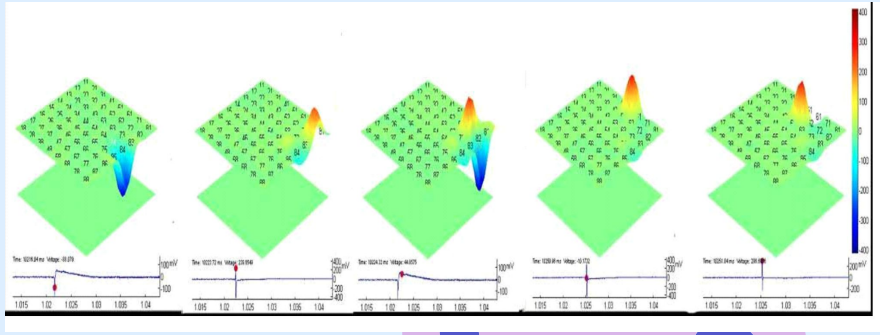
- Our intent is to differentiate stem cells into excitable cells, so we will implement the use of biosensors to deploy a voltage and verify that the cells have differentiated and matured properly.
- To determine the proper maturation of cardiomyocytes, the biosensor will detect the electrical signaling of the cells and use optical imaging for proper morphology.

Biosensing – Electrophysiology

- Electrical stimulation is applied **and** recorded via the microelectrode array (MEA)
- Multi channel amplifier can be used to record the electrical activity from the cells
- Cells will be stimulated using 500 mV at 2 Hz
- Action potentials can be recorded using same Multichannel software
- MEA can show the strength of the AP and temporal relationship

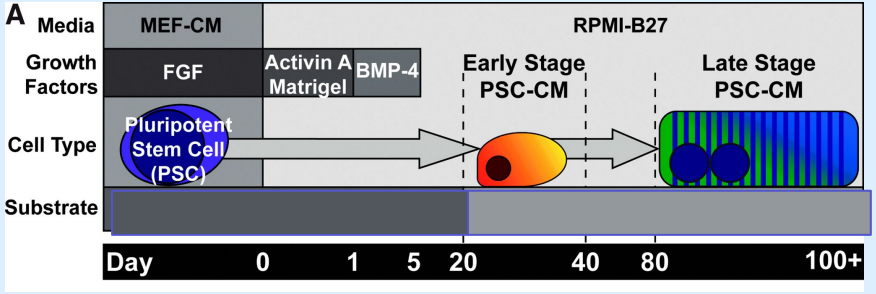


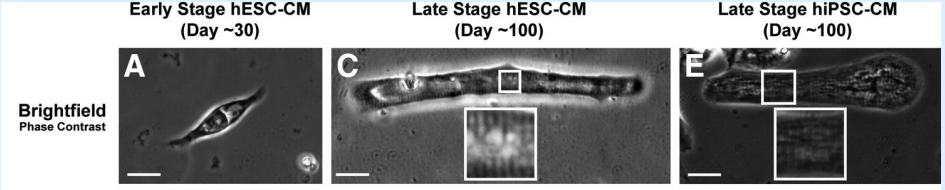




Cell Morphology

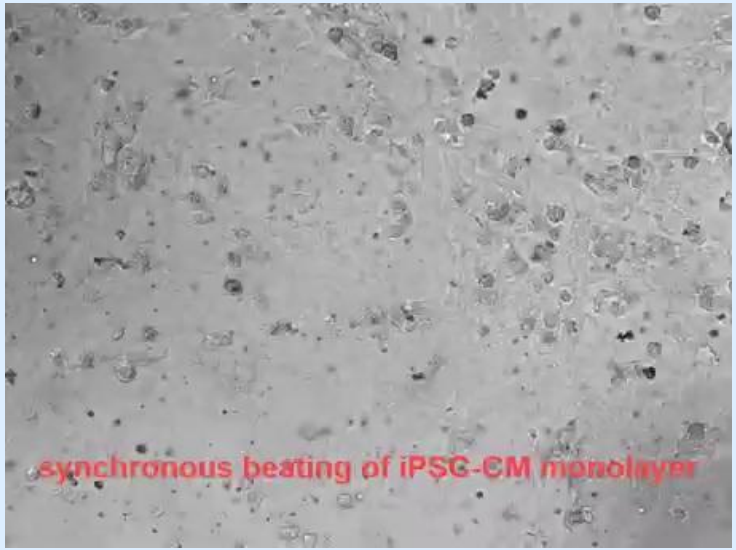
- As cells begin to differentiate, the cells function begins to change, therefore their shape and morphology must change to meet that function
- Cardiomyocytes are characterized by having a long, rectangular shape with a centrally located nucleus.
- Sarcomeres begin to form and organize





Cell Contractility

- Electrical stimulation is sent while observing the cells and recorded via microscopy
- The cells displacement can be tracked and then analyzed further to calculate their contractility
- The quantified contractility can be compared to native cardiomyocytes to confirm maturation



05

Biocompatibility

Material Biocompatibility

- PDMS & microelectrode array are the two materials in contact with cells
  - PDMS is widely accepted as biocompatible, so it would pose no risk to the cells

- It would be important to ensure that the microelectrode array does not deliver too high of a voltage
  - Just enough to excite the cells, not kill them

06

Limitations

Limitations

- **Design**
  - Only one parameter tested (substrate stiffness)
    - Room to add other signals, especially mechanical component.
  - Assume that PVDF stiffness change is detectable through other layers by cells
- **Testing**
  - Not an assessment of long term viability
  - Only one type of differentiation path can be tested at a time per device
- **Fabrication**
  - Microelectrode arrays are expensive
  - Microfluidic devices are not typically reused (cross contamination)
  - Sheet design using 5 different fabrication techniques require using 5 different expensive machines to achieve one product

Thanks!

Any Questions?

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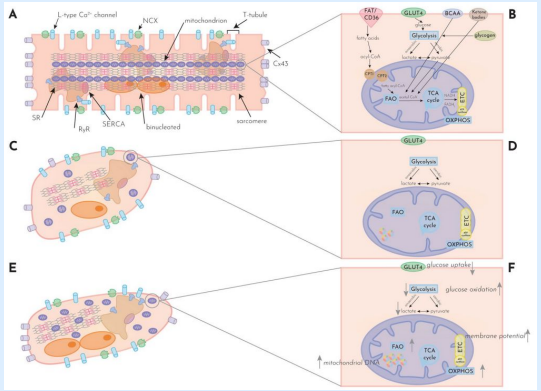
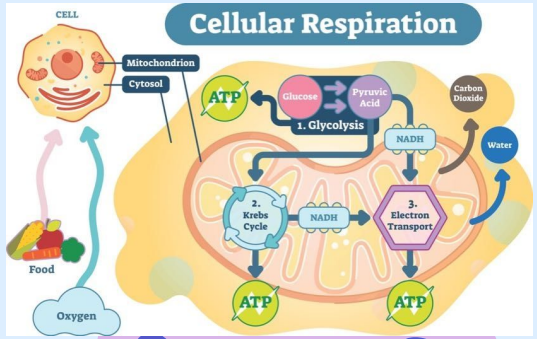
slidesgo

Biosensing – Metabolites

- As iPSCs are differentiating into cardiomyocytes (CM), they exhibit changes in their metabolic processes.
  - Immature CMs and mature CMs have different metabolic characteristics
  - From top to bottom: adult, immature iPSC-CM and mature iPSC-CM
- Immature iPSC-CM mainly rely on glycolysis, not fatty acid oxidation (FAO) for energy production
- As iPSC-CM mature, glucose uptake decreases and FAO increases.
- Oxygen consumption increases with the amount of mitochondria





Biosensing – Metabolites

- Cell are cultured in a different medium to test mitochondrial and glycolysis stress
- Wells are filled with respective medium and then incubated for 15 minutes to equilibrate under static conditions and then refreshed for multiple measurements