Atherosclerosis-on-a-Chip

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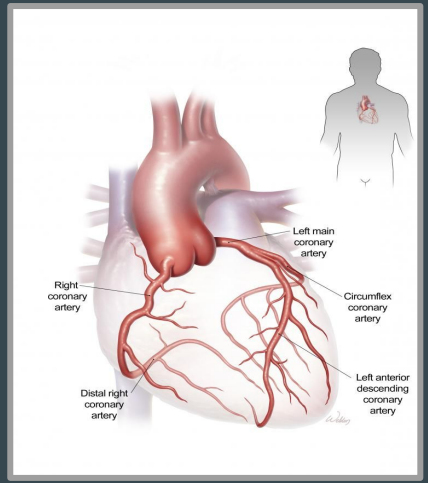
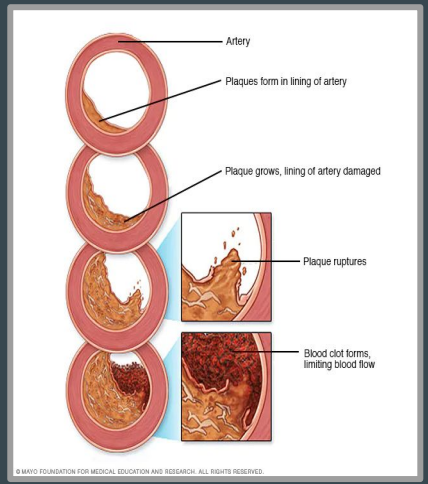
Introduction & Background

Disease Background

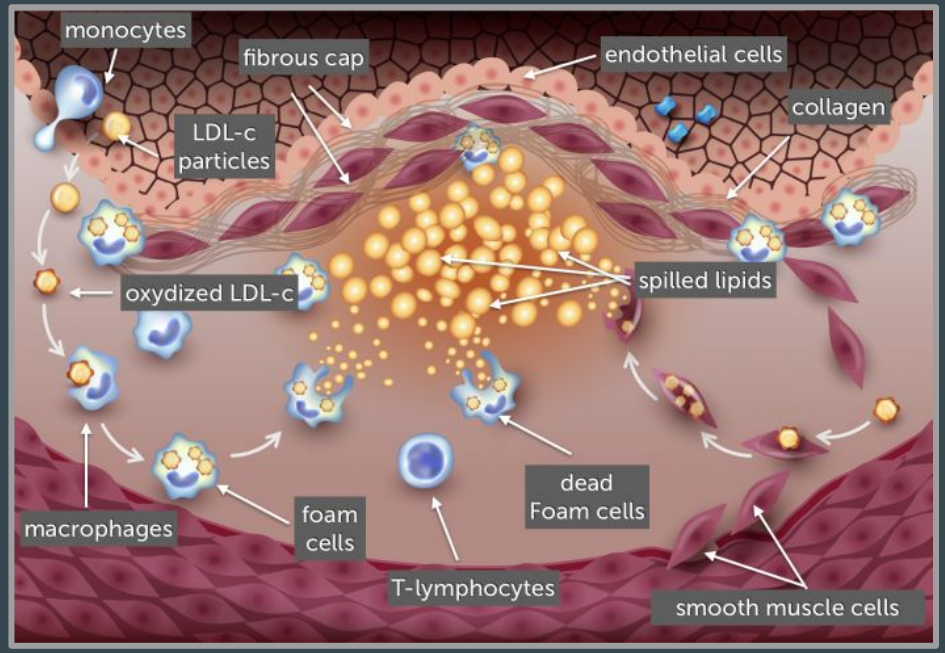
- ***Atherosclerosis*** - Plaque buildup in the arteries that perfuse the heart
  - Diabetes, diet, and lack of exercise are major contributors
- ***Complications***
  - Arrhythmias
  - Heart Failure
  - Heart Attack / Stroke
- ***Disease Prevalence***
  - 18.2 million adults > 20 years old have CAD
  - 360,900 deaths in 2019 from CAD
- ***Symptoms***
  - Angina
  - Shortness of Breath
  - Arm/Shoulder Pain





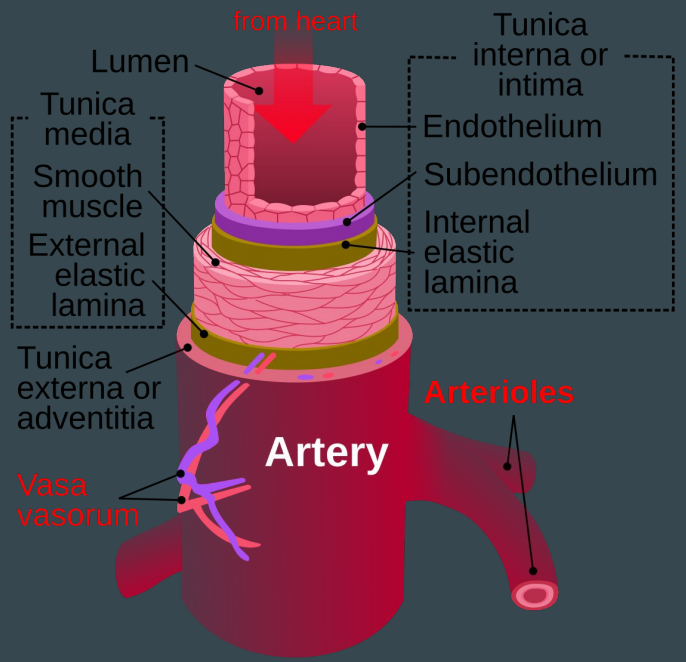
Atherosclerosis: Formation of Subendothelial Fatty Plaques

1. **Vascular insult** occurs to the tunica intima
2. **Monocytes migrate** to damaged subendothelial space
3. **Monocytes take up oxidized LDL** becoming foam cells
4. **T cells become activated** releasing cytokines
5. **Growth factors** activate smooth muscle cells
6. **Smooth muscle cells** take up LDL and deposit at the injury site resulting in subendothelial plaque



Target Physiology for OOC: Coronary Arteries

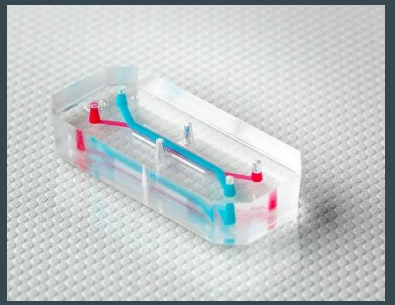
- *Three Key Layers*
  - Tunica Intima
  - Tunica Media
  - Tunica Externa/Adventitia
- *Multiple Different Cell Types*
  - Epithelial Cells
  - Smooth Muscle Cells
  - Perivascular Adipose Tissue Cells
  - Fibroblasts
- *Physiological Flow Through Lumen*
  - Flow of Cells Across Tunica Intima for Disease Pathophysiology
- *Expansion of Tunica Intima and Narrowing of Vessel Lumen under Disease State*



Organ-on-a-Chip (OOC)

- Multi-channel 3-D microfluidic cell culture
- Microdevices engineered to contain (human) cells and tissues and to model or mimic organ structures for *in vitro* study
- Highly used in drug discovery and development process to bring the gap between cell study and clinical study closer
- Three main characteristics:
  - 3D nature and arrangements of the tissues on the platforms
  - Presence and integration of multiple cell types to reflect a more physiological balance of cells
  - Presence of biomechanical forces relevant to the tissue being modelled

Advantages and Limitations of OOC Technology



Pros:

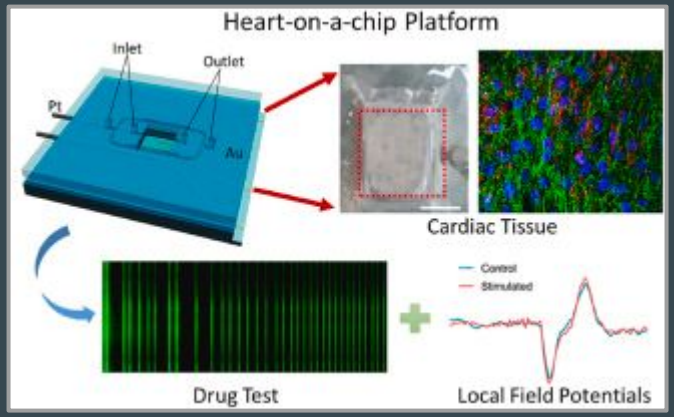
- Cellular and specific tissue architecture is well maintained to emulate chemical gradients and biomechanical forces
- Can vascularized or perfuse tissues to bring nutrients and fluidic flow to cells
- Can incorporate real-time tissue function sensors

Cons:

- Air bubbles can form easily in the channels of devices and interrupt the physiological response of the cells
- Extracellular matrix tend to degrade as time goes on
- PDMS can absorb organic compounds and cause a nutritional imbalance
- Organ volume versus organ area imbalance effect on mimicking function of original organ is controversial

Current Technology

- Comparable Heart-on-a-Chip (HOC) Technology
  - Goal: Reproduce mechanisms of the heart; mimicking mechanics of the heart as a whole
  - Technology exists; more work needed to reproduce highly controlled microfluidic environment
  - Limitations:
    - Material Selection
    - Interface Development
    - Improper Vascularization and Perfusion
- Only a few **specific applications** to **localized heart mechanics**



Many current applications attempt to mimic contractility and electrophysiology of the heart

Purpose of Our Atherosclerosis-on-a-Chip

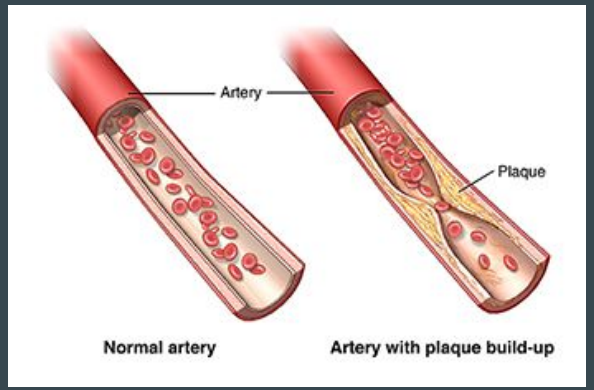
1. **Improvement on Current Fabrication Techniques**
    a. Lower Cost
    b. Increased Production Efficiency
    c. Increased Scalability > Increased Accessibility
2. **Drug and Toxicology Studies for CAD**
    a. Test New Drugs Meant for Other Diseases
        i. Ensure No Effect on Patient's Pre-Existing CAD
    b. Test Newly Developed Drugs for CAD
3. **Highly Patient-Specific Model of the Coronary Arteries**
    a. Investigate/Quantify Effects of Other Risk Factors
        i. Smoking
        ii. Increased Blood Sugar from Diabetes
        iii. Diet

Design of Atherosclerosis-on-a-Chip (AOC)

Overall Chip Design

- **Bioprinting**
  - Techniques to combine cells and various biomaterials to fabricate biomedical parts
    - Aim of imitating natural tissue characteristics
  - **In our application, we will use bioprinting to create the structure of the coronary arteries for incorporation into OOC**
- **Microfluidic Chip Development**
  - Module-Based
    - Including active and passive mixing modules
  - Fabricated from PDMS

- **Inducing Atherosclerosis**
  - Low-density lipoproteins into the endothelial cell layer
  - Increasing heart rate with pulsatile flow
  - Observe plaque buildup within vessel walls



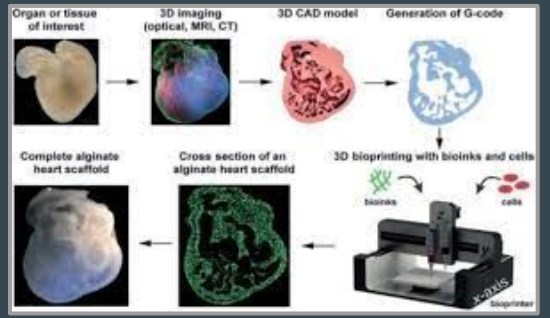
3D Bioprinting

Design Parameters for 3D Bioprinting

	3D Bioprinting Method	3D Bioprinting Bioink	3D Bioprinting Cells/Materials

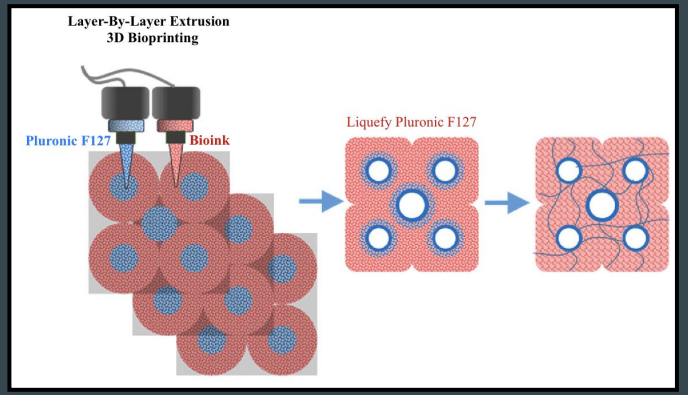
Component(s)	• Pneumatic Microextrusion 3D Bioprinter
	  ○ Coaxial Nozzles for Simultaneous Printing and Crosslinking Material
	  ○ UV Light Source for Photocrosslinking
		• Pluronic® F127 (Sacrificial Material)
		• Methacrylated Gelatin (GelMA), Alginate, PEGTA Blend (Cell-Seeded Layer)
		• Methacrylated Gelatin (GelMA), Collagen I, PEGTA Blend
			• Human Umbilical Cord-Derived Vein Cells
			• Human Umbilical-Cord Derived Arterial Cells
			• Mesenchymal Stem Cells
			• Vascular Smooth Muscle Cells
			• NIH 3T3 Fibroblast Cells

Reasoning	Layer-by-layer microextrusion bioprinting methods, components will be simultaneously printed and crosslinked through multiple nozzles	Pluronic® F127 as a sacrificial material for inner lumen; reverse gelation properties Bioink blends for cell growth and proliferation	Different cell types embedded after co-culture with blends to facilitate development of target physiology



Bioprinting Methods

- **Three Key Layers with Separate Bioinks and Nozzles**
  - Tunica Intima (Endothelial Layer)
    - GelMA/Hyaluronic Acid/PEGTA (UV Light & $CaCl_2$)
    - HUAEC/HUVEC Cells
    - Mesenchymal Stem Cells
  - Tunica Media (Smooth Muscle Layer)
    - GelMA/Alginate/PEGTA (UV Light & $CaCl_2$)
    - Vascular Smooth Muscle Cells
    - Mesenchymal Stem Cells
  - Tunica Externa/Adventitia (Fibroblasts & Collagen Layer)
    - GelMA/Collagen I (UV Light & $CaCl_2$)
    - NIH 3T3 Fibroblast Cells
- **Vessel Lumen**
  - Pluronic® F127



Tunica Intima (Endothelial Layer)

GelMA/Hyaluronic Acid (UV Light & $CaCl_2$):

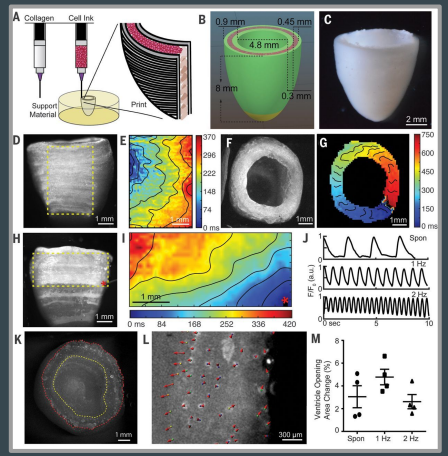
- GelMA
  - Biocompatible Biomaterial
  - High Mechanical Strength
  - Can Blend with Other Gels to Increase Cell Viability
- Hyaluronic Acid
  - Favorable Rheological Performance (Si et *al.*)
    - Following modification with methacrylic anhydride and crosslinking with 3,3′-dithiobis(propionylhydrazide)

HUAEC/HUVEC Cells

- Co-Culture of HUVECs and HUAECs (Lau et *al.*)
  - Demonstrates optimal development of endothelium
- Mesenchymal Stem Cells (MSCs)
  - Substantial capacity in facilitating cardiac and endothelial repair

Tunica Media & Externa/Adventitia

- Smooth Muscle Layer
  - GelMA/Alginate/PEGTA (UV Light & $CaCl_2$)
    - Alginate is bioactive; tunable properties
    - PEGTA encapsulate cells
    - Vascular Smooth Muscle Cells
      - Characteristic of Layer
  - Mesenchymal Stem Cells
  - PEGTA
    - Cell Encapsulation
      - Prevents damage to cells in the process of printing
- Tunica Externa/Adventitia
  - GelMA/Collagen I (UV Light & $CaCl_2$)
    - Collagen I has demonstrated use
    - NIH 3T3 Fibroblast Cells
      - Characteristic of Layer



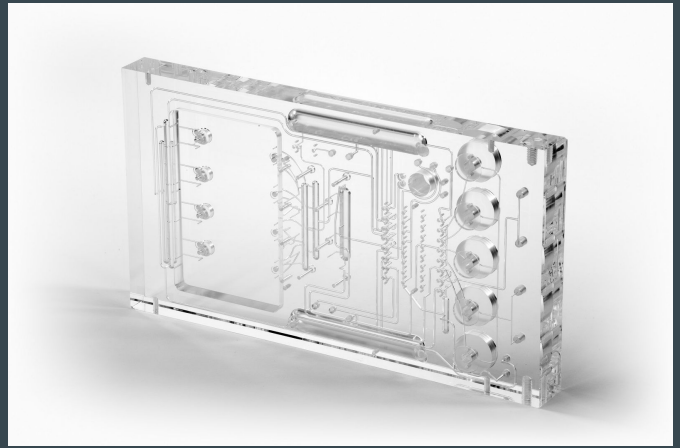
Microfluidics

Design Parameters for Microfluidic Chip

	Microfluidic Chip Material	Microfluidic Chip Design

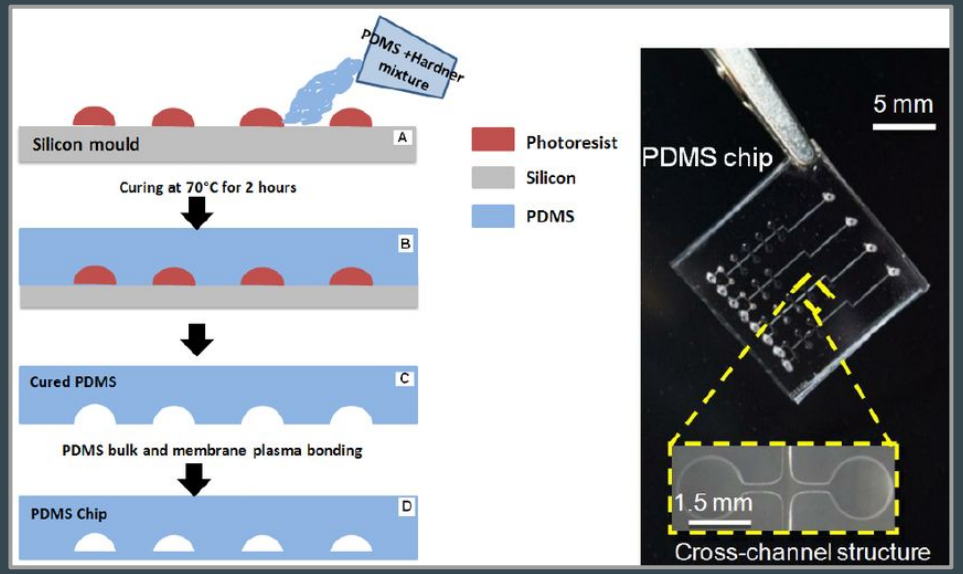
Component(s)	• PDMS (Polydimethylsiloxane)
	  ○ Transparent
	  ○ Forms Tight Seals with Plasma Treatment
	  ○ Easy to Mold
	  ○ Inexpensive
	  ○ Gas Permeable
		• Combination of Continuous and Droplet-Based Flow
		• Incorporate 3D Hydrogels
		• Bubble Trapper

Reasoning	Susceptibility of bubble formation and slight evaporation avoided through bubble traps	Allows for the visualization of flow through fluorescence



Microfluidics: Size and Fabrication

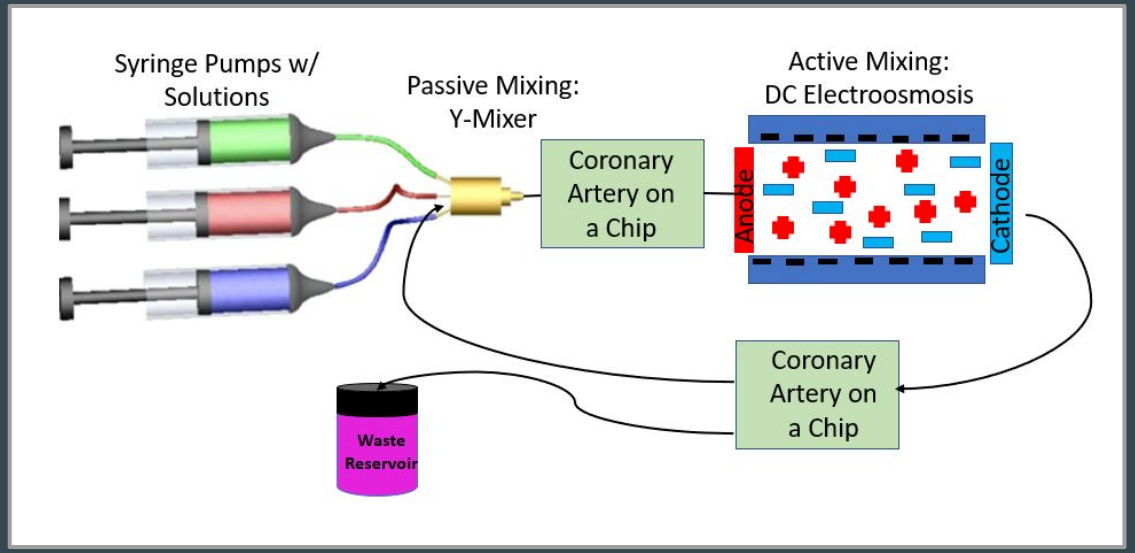
- **Size**
  - At least one dimension <500 µm
  - Volume scale: 0.01 nL - 1 L
- **Fabricate with PDMS**
  - Mold Master with CAD software
  - Contact Photolithography
  - Glass Posts Placed Upright
  - PDMS is Cast Against Master
  - Plasma Oxidation for Sealing



Microfluidics: Module-Based Design

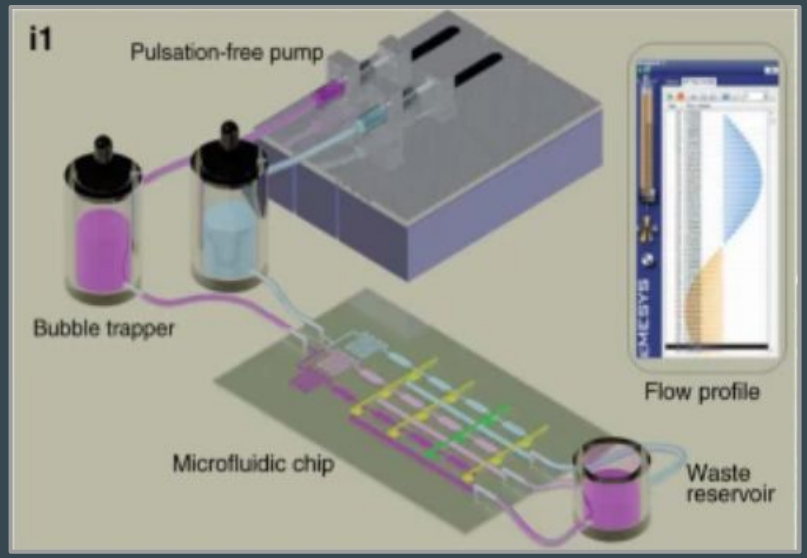
Four Modules:

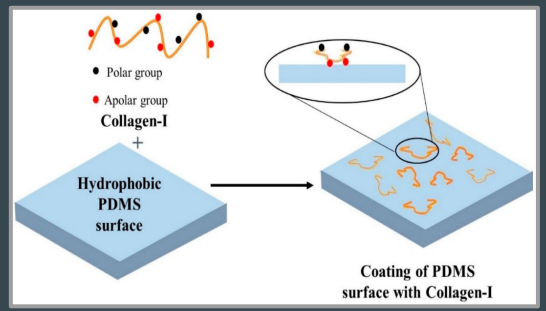
1. **Solutions**
   a. Syringe Pumps with Blood and Nutrients
   b. Drop-Based
2. **Passive Mixing**
   a. Diffusion
   b. Combo Y-Mixers
   c. Laminar Flow
   d. Connected to Syringes Supplying Solutions
3. **Active Mixing**
   a. DC Electroosmosis Pump
   b. Pulsatile Flow
      - Goes to Arteries
4. **Waste Reservoir**



Microfluidics: Integration

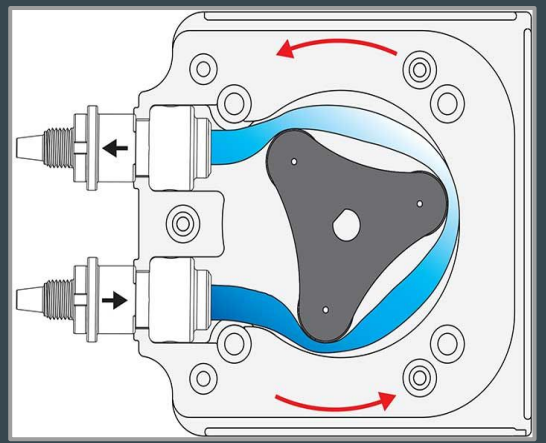
- **Incorporation of 3D Hydrogels**
  - Collagen mimics cardiac or vascular tissue
  - Correlation between decreased lymphatic barrier function and increased collagen density (Lugo-Cintron et *al.*)
- **Bubble Trapper**
  - In-Line
    - Bubbles float to the top when they enter the network





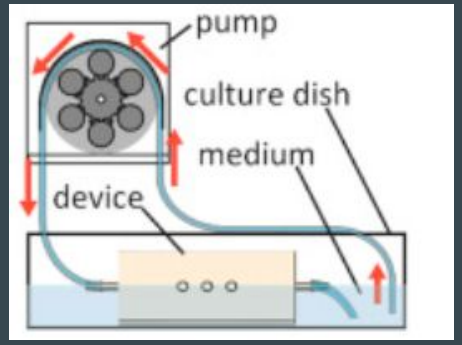
Microfluidics: Pulsatile Flow

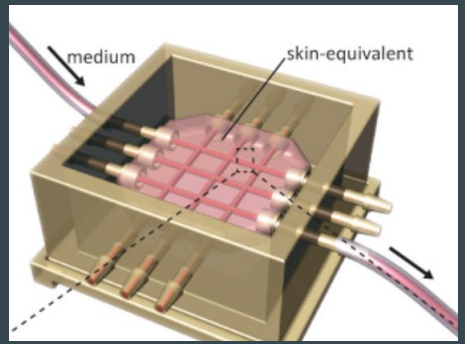
- Pulsatile Flow: Simulate cycles of contraction and relaxation in cardiovascular system
- Two Potential Methods
  - Varied Diameter Channels
    - 600 µm wide (healthy human artery) gradually narrows to 300 µm
    - Pulsatile shear stress applied: flow rate 1.41 µl/s at a pulse rate 70 bpm
    - Results in shear stress 15 dyn/cm^2 in 600 um channel (typical in healthy human arteries)
  - Peristaltic Pumps
    - Allows for continuous circulation of fluid and recirculations
    - Mechanism: pinches fluidic tube with roller on a rotor in a circular pump
    - Generate strong pulses
    - Non-fluidic contact operation

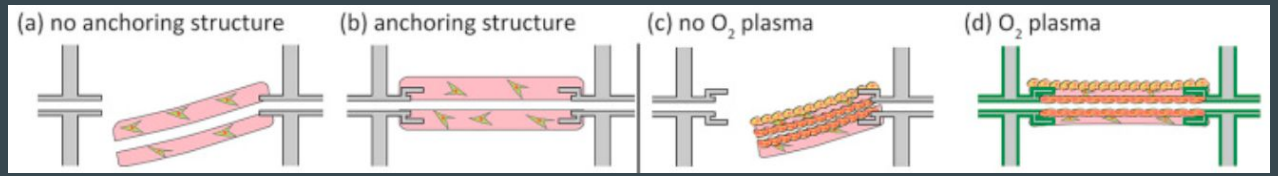


OOC: Integration of Microfluidic Chip & Bioprinted Coronary Arteries

- Bioprinted coronary arteries within culture device (Mori et *al.*)
  - Both edges of the vascular channels fixed to connectors of culture device
  - Attached to a perfusion system composed of a peristaltic pumps and silicone tubes connected to passive Y-mixer
  - Culture device attached to anchoring structures with oxygen plasma treatment

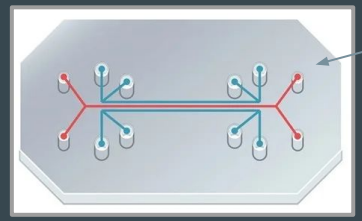






Testing and Validation: Endothelial & Smooth Muscle Cell Function

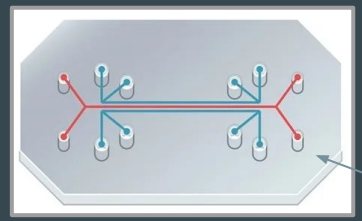
- Vasodilation and vasoconstriction are important functions of these cells
- Caused by the release of several factors (nitric oxide (NO), endothelin (ET-1))
- Commonly used test is to inject acetylcholine and angiotensin into the arteries



Pump in Acetylcholine

Release of NO

Measure NO Through Direct Chemiluminescence



Release of ET-1

Measure ET-1 Using ELISA

Pump in Angiotensin II

Testing and Validation: Flow, Permeability, Fibroblast Function

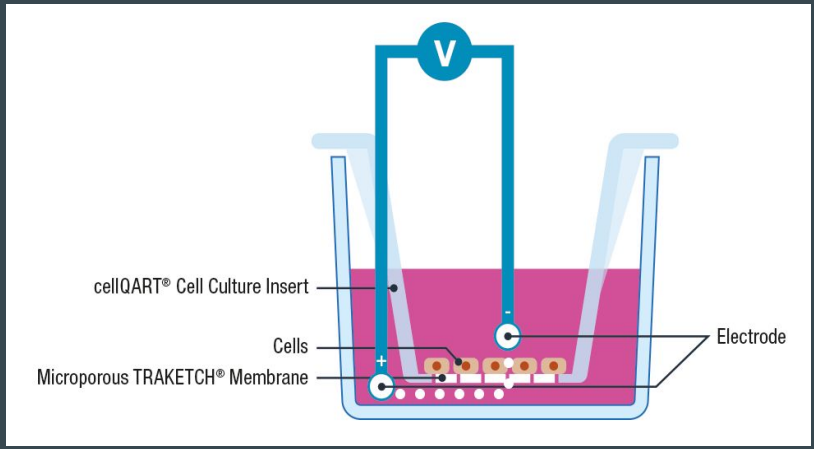
Fibroblast: Angiotensin II (Ang II) has shown to causes fibroblasts to produce inflammatory cytokines

- Pump in Ang II and measure cytokine production using multiplex ELISA

Pulsatile Flow: Use embedded sensors

- Average Flow: 1.41 µL/s
- Pressure > 1.5 Pa
- Average Shear Stress: 15 dyn/cm^2

Permeability: Use transendothelial electrical resistance (TEER) to make sure there is no leakage



Biocompatibility

- PDMS: Biocompatible material made from the repetition of silicon and oxygen bonds and methyl groups with low cost.
- GelMA has great biocompatibility and mechanical properties. It is developed by gelatin, the product of hydrolysis and collagen denaturation at high temperatures
- Hyaluronic Acid, Pluronic®F127, polyethylene glycol polyacrylamide copolymers (PEGTA) as hydrogels are all biocompatible material

Application: Drug Testing and Efficacy

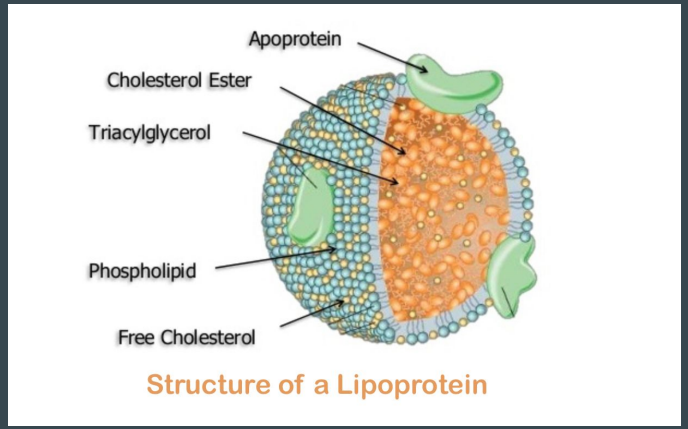
This device could be a way of testing drug efficacy in treating atherosclerosis without having to use an *in vivo* model - Alendronate used for osteoporosis

Create atherosclerosis condition

- Introduce **LDL** through shear stress when seeding endothelial cells
- Increase HR

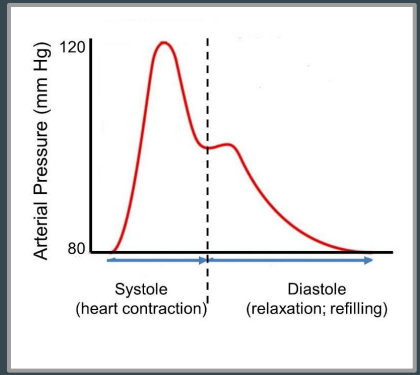
Implement drug treatment and observe

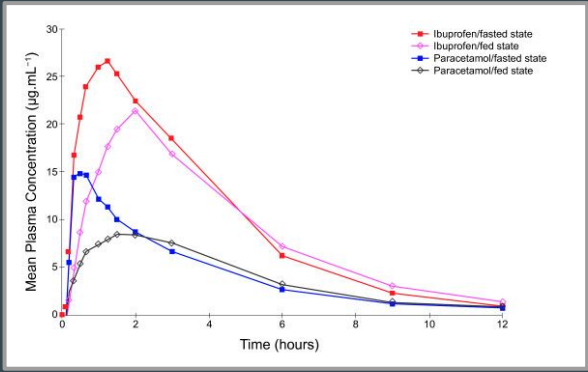
- Pump in **alendronate** in desired dosage and see if the amount of plaque reduces or if the rate of plaque buildup decreases



Limitations of Proposed OOC Technology for Coronary Arteries

1. **Simplification of Pumping Mechanism**
    a. Pressure gradient along the coronary arteries is variable
    b. Varied pressure gradient (and velocity) along the arteries can contribute to vascular insult
2. **Variable Composition of Fluid Over Time**
    a. Multi-organ interactions result in varied composition of molecules and cells within blood as a function of time
    b. Certain molecules can increase or decrease likelihood of vascular insult
        i. Over or under estimation of plaque build-up may result
3. **3D Bioprinting is a Relatively Novel Technology**
    a. Poses accessibility/scalability risk over current PDMS REM methods that are currently more utilized





Questions?

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