Notch Signaling Coordinates with Cell Contractility to Drive Biliary Differentiation of Liver Progenitor Cells[1,2]

Premise:
cell type: BMEL 9A1 (bipotential mouse embryonic progenitor cells)
platform: glass slide with polyacrylamide (PA) on top, micropatterned with varying ECM composition and gel stiffness to test for cell differentiation. ECM conjugated to PA *via* sulfo-SANPAH (NHS-based convalent linking).

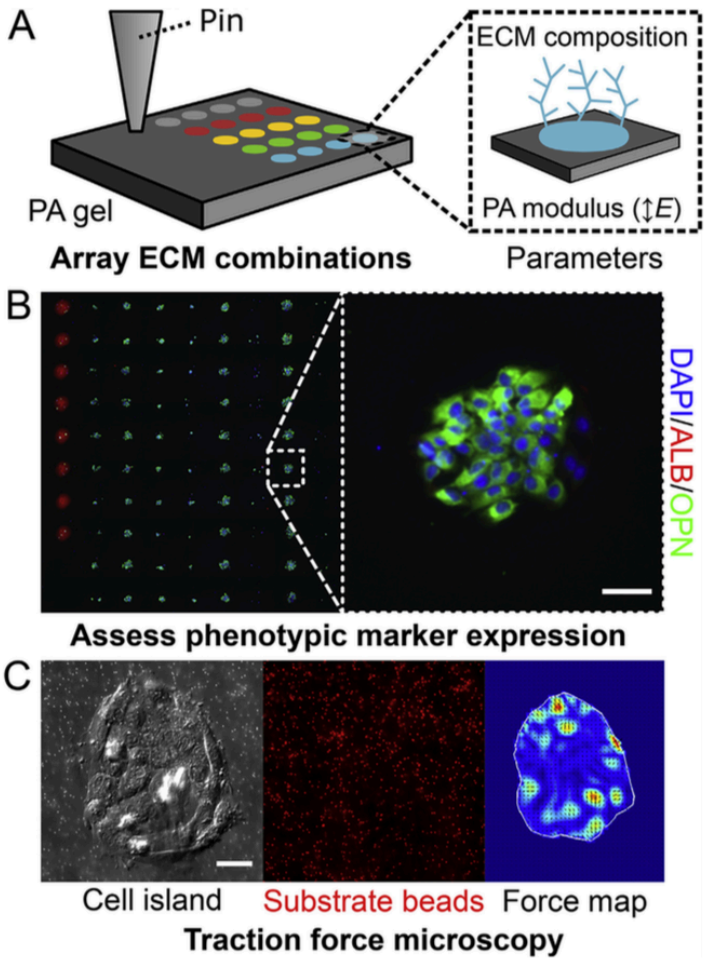
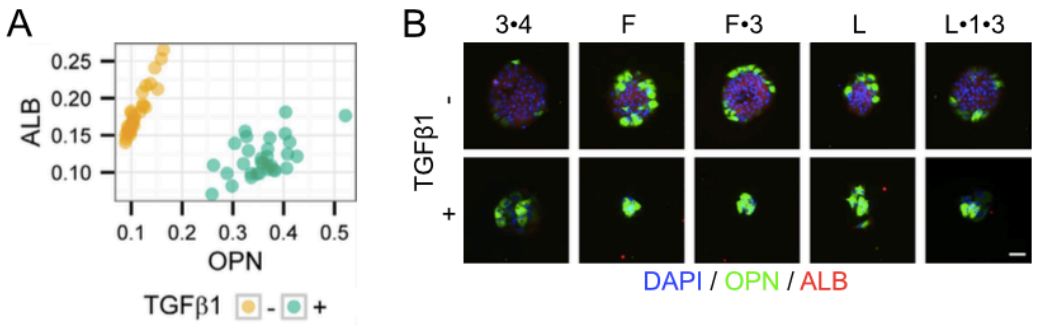


Fig. 1. An integrated platform for correlating fate processes with both biochemical and biophysical parameters. (A) ECM combinations were arrayed on a PA hydrogel substrate, enabling simultaneous control of ECM composition and PA modulus. (B) Cells cultured on ECM arrays were assessed for phenotypic marker expression. (C) Cells on PA substrates doped with fluorescent beads, enabling measurement of cell-generated forces on ECM arrays. Scale bars are 50 μm.

Method:
1. Seed the undifferentiated cells on the platform.
2. Assess marker expression (pattern can be seen after 24 h).
3. Data: Fluorescence images of different markers. Scatterplots much like those in flow cytometry can be obtained.
ALB = albumin, OPN = osteopontin.
ALB ↑ and OPN ↓ = hepatocyte differentiation.
ALB ↓ and OPN ↑ = cholangiocyte/biliary differentiation.



Interesting Find:

1. On fibronectin (FN), cells showed lower differentiation toward the cholangiocyte/biliary lineage when the substrate stiffness lowered from 30 kPa to 4 kPa. ON collagen, cell differentiation is independent of stiffness.
2. Disease Model: Alagille syndrome. Children with Alagille syndrome usually suffer a progressive loss of the bile ducts within the liver over the first year of life and narrowing of bile ducts outside the liver. This leads to a buildup of bile in the liver, causing damage to liver cells. Scarring may occur and lead to cirrhosis in about 30 to 50% of affected children.
3. ECM ± Notch ligands/receptors. Inhibition of E-cadherin and reduction of Notch signaling increased podocyte differentiation. (E-cadherin and Notch receptors are both involved in cell-cell interaction to favor MET transition).

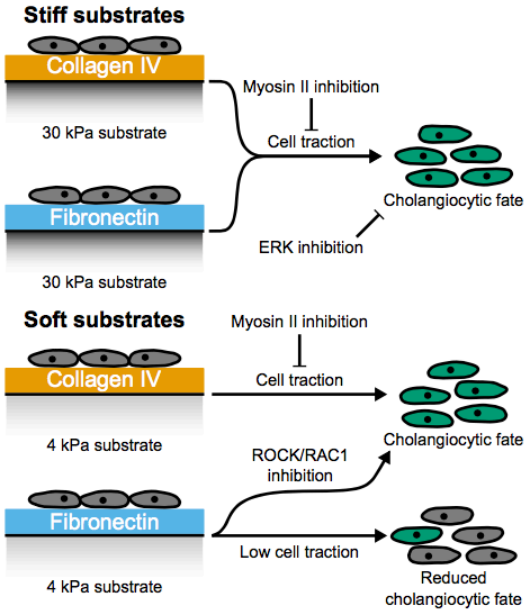


Fig. 8. Schematic summary of overall findings. Both C4 and FN support cholangiocyte differentiation of liver progenitors on stiff substrates while only C4 does so on soft substrates. Differentiation on stiff C4 and FN substrates is dependent on myosin II contractility and ERK signaling and is further associated with high traction stresses. In contrast, soft FN substrates are associated with low traction stress and result in reduced cholangiocyte differentiation. Inhibition of either ROCK or RAC1 increases cholangiocyte fate specification on soft FN substrates.

Audience Q&A:

Q: Will you include shear stress next?

Stem Cell Mechanotransduction, Differentiation and Migration on Linear Stiffness Gradient Hydrogels[3]

Premise:
cell type: human adipose-derived stem cells (hASCs or hADSC).
platform: Two wedges of PA gel with different stiffness, combined to form a rectangular composite with a stiffness gradient.

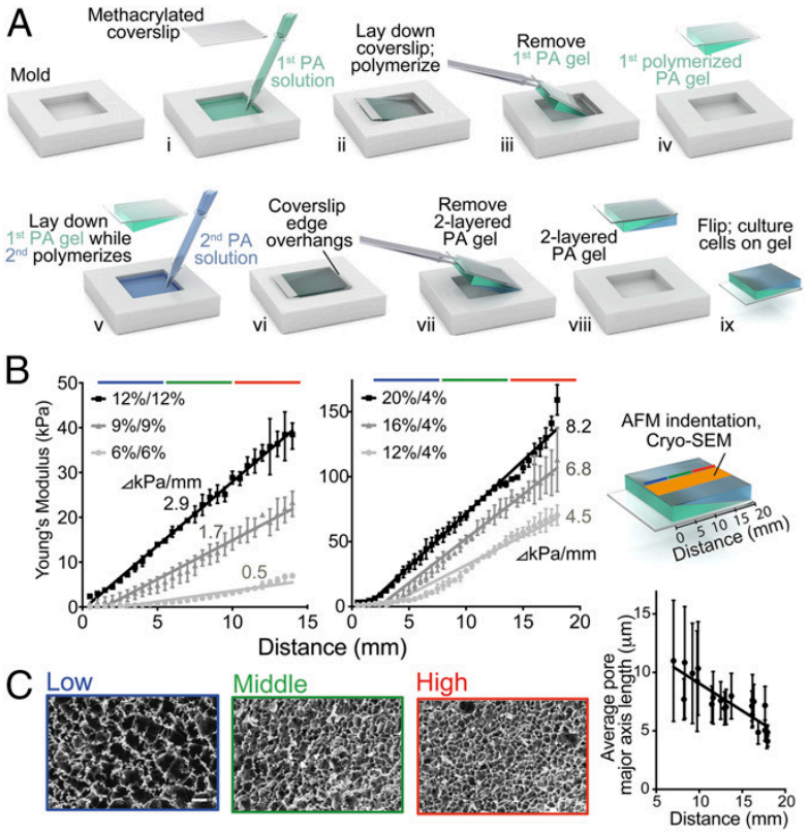
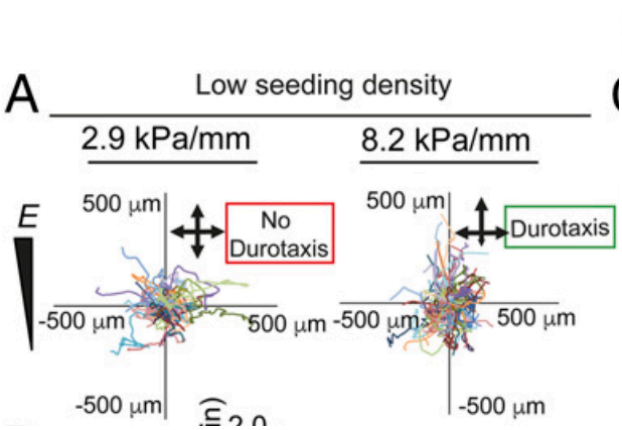
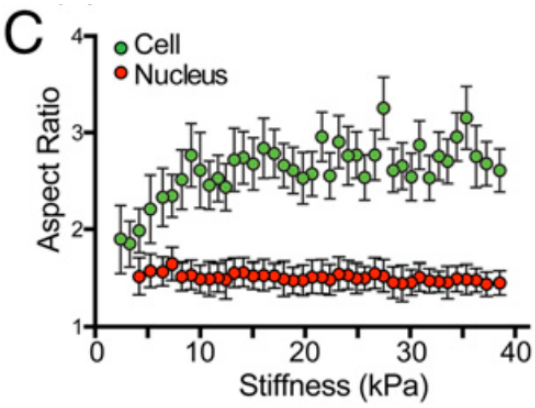


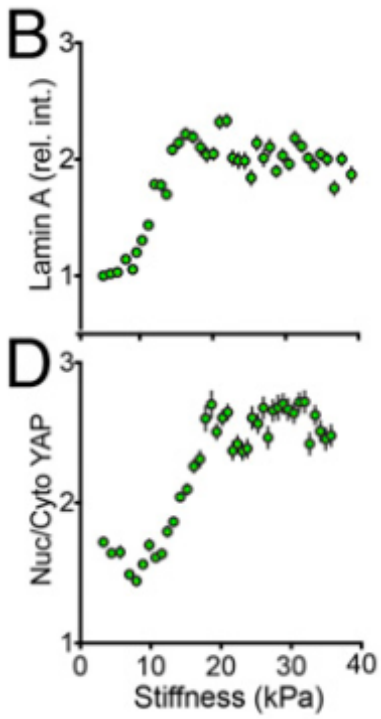
Fig. 1. Fabrication and characterization of stiffness gradient PA hydrogels. (*A*) A 24- × 20-mm rectangular mold is used to fabricate hydrogels in a double polymerization process creating a two-layered PA hydrogel. (*B*) AFM was used to probe the surface Young's modulus, *E*, for hydrogels composed of 12/12% = 2.9 kPa/mm (R^2 = 0.9965) (black squares); 9/9% = 1.7 kPa/mm (R^2 = 0.9764) (dark gray triangles); or 6/6% = 0.5 kPa/mm (R^2 = 0.9041) (light gray circles) (*Left*) or 20/4% = 8.2 kPa/mm (R^2 = 0.9783) (black squares); 16/4% = 6.8 kPa/mm (R^2 = 0.9532) (dark gray triangles); or 12/4% = 4.5 kPa/mm (R^2 = 0.9415) (light gray circles) (*Right*). (*C*, *Left*) Cryo-SEM images of a 12/12% PA hydrogel surface at low (blue), middle (green), and high (red) stiffness ranges. (Scale bar: 25 μm.) (*Right*) The corresponding graph shows pore size is inversely proportional to stiffness where the slope = −0.4676 μm/mm (R^2 = 0.7312).

Method:
1. Seed the cells on the platform.
2. Assess YAP/Taz ratio and cell migration
3. Data: Fluorescence images of different markers. Scatterplots much like those in flow cytometry can be obtained.

Interesting Find:
1. With higher substrate stiffness, nuclear aspect ratio is maintained while the cell aspect ratio did not.
2. Higher substrate stiffness results in higher laminin A expression.
3, Not unexpected, higher substrate stiffness results in higher Nuc/Cyto YAP.
4. Durotaxis seen on substrate with a steep stiffness gradient (8.2 kPa/mm).







Audience Q&A:
Q: Did you try out the system with mesenchymal stem cell (MSC)?
A: No.

Optical Control of Gene Expression for Neuronal Fate Specification

Premise:
cell type: ???
platform: use light to switch genes on and off. This approach has the advantages over chemical approach in terms of spatial and temporal resolution in regulation.

Interesting Find:
1. Used blue light to control without affecting the cell much (2W and 1.1 W).

Audience Q&A:
Q: Did you not fried the cells with blue light?
A: No.

Engineering Neural Tissue Using the Novel Functionalized Transcription Factor IASCL1

Premise:
cell type: Fibroblast
platform: Lentiviral transfection of ASCL-1 (achaete scute homolog-1) into fibroblast to create neutron cells.

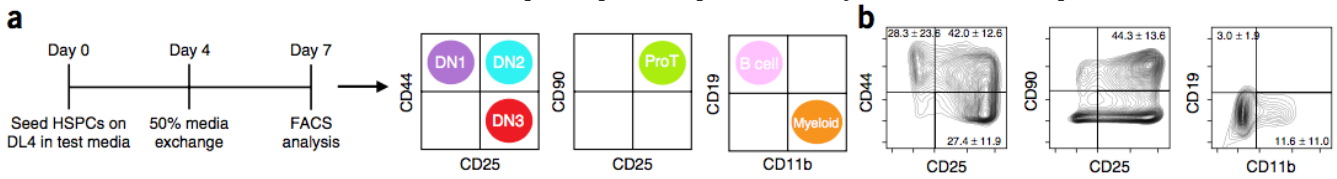
Interesting Find:
1. Maintenance of differentiation up to 21 days.
2. iProgen IPDT (intracellular protein delivery technology) is used to sneaks theASCL-1-IPDT protein sneaks into cells.
3. Protease are everywhere, but the ASCL-1 is stable in media up to 3 days.
3. Grant reviewers comment: Why not reprogram using astrocyte?

Audience Q&A:
Q: Did you not fried the cells with blue light?
A: No.

Progenitor T-cell Differentiation from Stem Cells Using Niche Engineering[4]

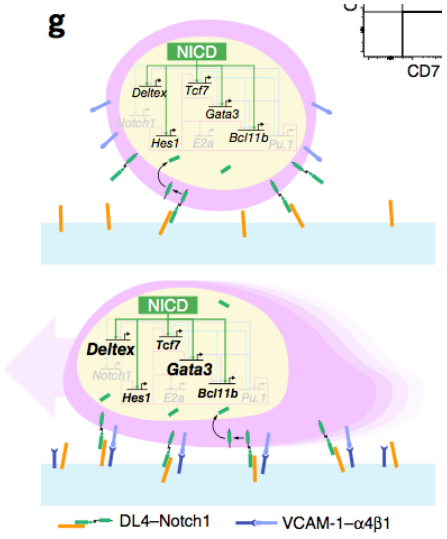
Premise:
cell type: hematopoietic stem and progenitor cells (HSPCs).
platform: Differentiates HSPCs into T cells using a "thymus niche" (with serum-free media). Essentially, a 96 well plate coated with the notch ligand delta-like 4 (DL-4) and vascular cell adhesion molecule 1 (VCAM-1).

Method:
Cell differentiation assessed based on the principal component analysis of marker expression:



Interesting Find:
Increased pulling on Notch promotes maturation into CD4 and CD8 cells. CD4 cells, also called T4 cells, are "helper" cells. They lead the attack against infections. CD8 cells, (T8 cells), are "suppressor" cells that complete the immune response. CD8+ cells can also be "killer" cells that kill cancer cells and other cells that are infected by a virus.

Proposed Mechanism:



mechanotransduction

Work in Progress:
1. Modeling of differentiation using Boolean logic.
2. Creating a hydrogel-based, injectable thymus for *in vivo* culture as opposed to the ex vivo culture commonly done right now. Currently, the intradermal route of administration seems feasible.

Development of Human-on-a-Chip Systems for Understanding Disease and for Preclinical Drug Discovery

Premise:
cell types: Various human cells.
platform: different organ-on-a-chip combined into one, developed by the Hespseros Inc. (founded by Mike Shuler). All cells are cultured in serum-free media. The most frequently mentioned platform contains cantilevers made using silicon for the monitoring cardiomyocytes. Capable of measuring the QT interval.

Showcase:
1. Heart-Liver system with Aztrazeneca. Monitoring of function in real time without loss in cell viability.
2. Heart-Liver-Cancer with Rosche. Recapitulate data where the drug killed cancer but no the drug-resistant cancer.
3. Look at dose response to Botox
4. ALS motor neuron.
5. Demonstrated 2D liver culture for over 28 days
6. In presence of other cells the liver cells are more stable (indicated by a more stable secretion of albumin).
7. Looked at the effects of chronic low dose doxorubicin.
8. Notice breaks in some cardiomyocytes after 9 days (however, still beating).
9. Try to deal with protein/drug adsorption to the microfluidic housing.
10. Skin with L'Oréal. Recapitulated
11. Recapitulation of BBB value *in vivo*.
12. Meet with FDA to look at rare disease.

Audience Q&A:
Q: Concerned at all with phenotypic variation from hiPSC?
A: For each project, we only used cells from the same lot. The variation should not be a concern. In fact, they should be welcomed. We will win by number to account for variation between individuals (i.e. n = 3-000 per study).

Q: Concerned with the reversal in flow direction?
A: Currently did not pose a problem.

Rapid and Facile Fabrication of Organs-on-Chips: Toward a Patient Derived Intestine-On-A-Chip

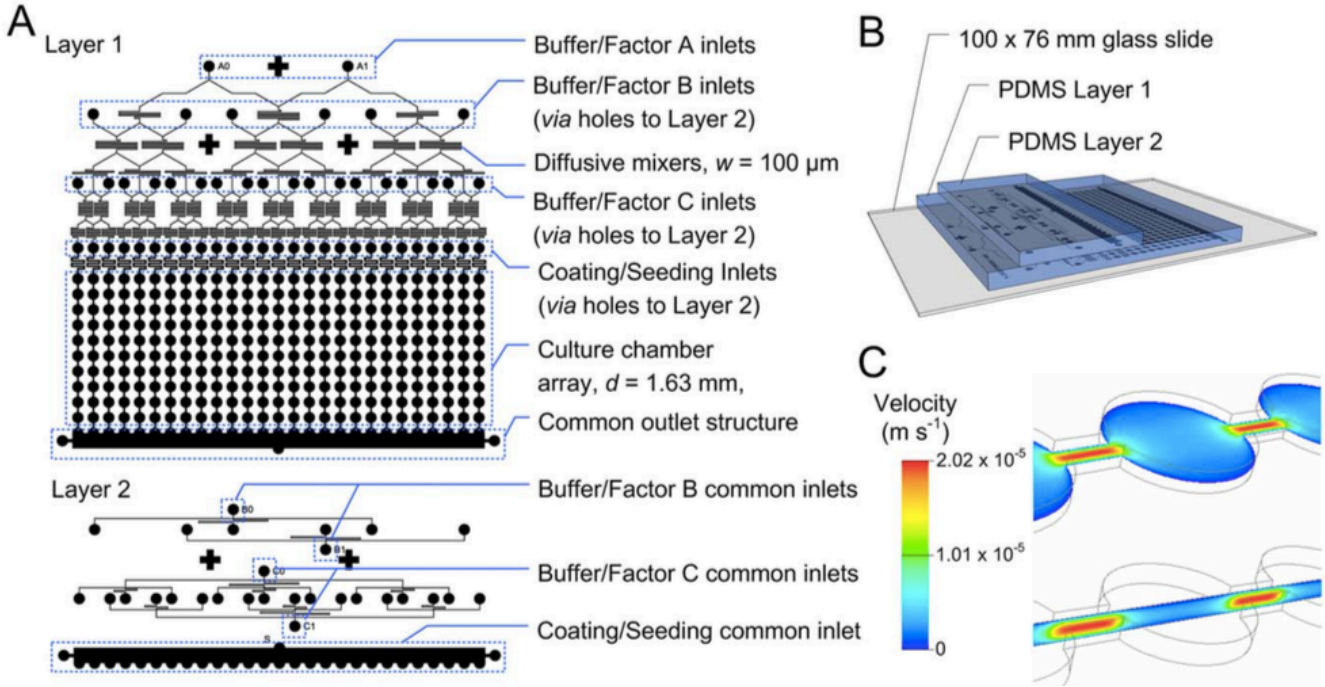
Premise:
cell type: ihPSC-derived enterocyte and Caco2 (enterocyte cell line, accounting for more than 90% of cells in the intestine).
platform: thermoplastic-based microfluidic device with 1.0 μm PC membrane. Claim: Thermoplastic can ehlp control oxygen tension better. Interesting fabrication approach: acrylic adhesive tapes used as the channel layer (patterned using a laser cutter).

Method:
1. Mature Caco2 for 3-4 weeks. Looking at alkaline phosphatase as the marker for maturation. Note: actually mature faster than Caco2 on the Transwell control.
2. Grow ihPSC in tem cell medias, then switch to media with 20% FBS later after 2 days. Some ihPSCs would die during this transition.

Interesting Facts:
1. The ihPSC attained the differentiated phenotype faster than the Caco2.
2. Maturaion is 4 times faster with flow.

High Throughput Optimisation of Differentiation from Human Pluripotent Cells To Kidney Lineages Using Microbioreactors Arrays

Premise:
cell type: ihPSC.
platform: PDMS-based device with multiple channels. Each channel contains a set of wells to facilitate the growth of ihPSC colonies.



Method:
1. Seed hiPSC into the device, with each array subjected to different soluble factors, i.e. WNT agaonist in order to get nephrons s and proximal tubules.

2. Differentiation toward nephrons assessed via marker expression, i.e. WNT, BMP2.

Long-term Micro uidic Human Liver Co-cultures For Drug Development[5]

Premise:
Hepatocyte tend lose phenotypic expression very fast.
Magic bullet: Co-culture of hepatocyte with other cell types tend to help maintain the hepatocyte phenotypes.
Cell type: Primary human hepatocytes (PHH) or induced pluripotent stem cell-derived hepatocyte-like cells (iPSC-HH).
Platform: triple culture of hepatocyte, 3T3-J2 fibroblast, and HUVEC, in either co-planar (random or patterned) or in 3D-bioprinted form (hosted on Transwell).

random **patterned *via* collagen filled in microfabricated pits**

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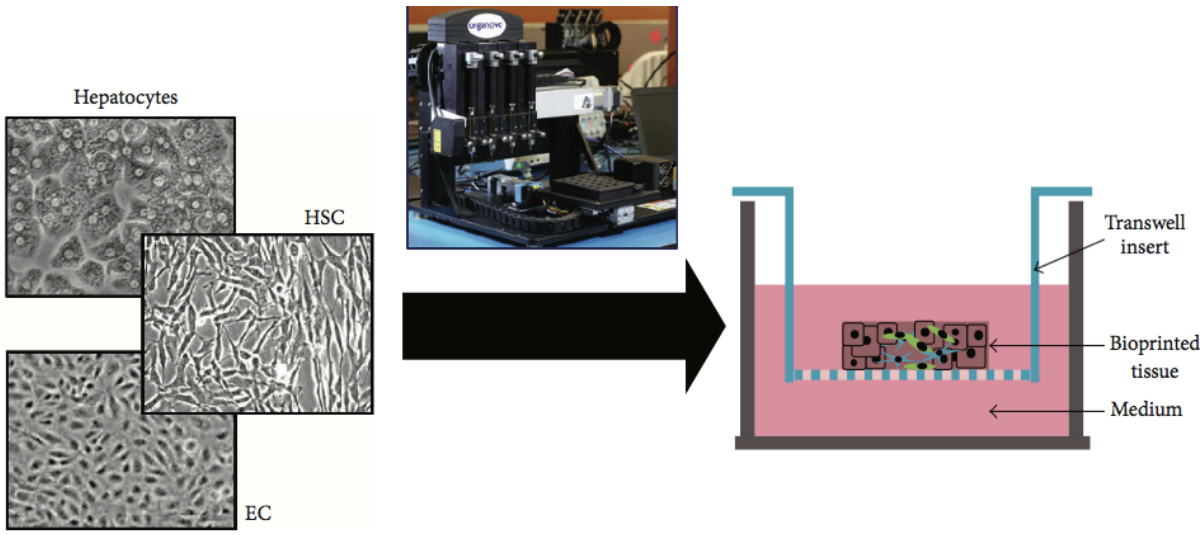


3D-bioprinted on Transwell

Interesting Fact:
1. Fluid flow did not help maintain the differentiated phenotype in this case. In fcat, the secretion of albumin decreased to about 75%. Generally, the higher the albumin secretion the better for hepatocytes.
2. Exosomes from the 3T3 J2 alone did not help maintain the differentiated phenotypes.

Shear-induced Fibrillogenesis of Fibronectin Nanobers for Enhanced Cutaneous Wound Healing

Premise:
Wound healing in embryo is different from in adult. The FN arrangement is more random, whereas in adult the FN is more aligned…. Hence scarring. For adult the collagen I deposition is much faster.

Method:
1. Spin FN or Fibrinogen by rotary spinning at high centrifugal force (essentially a cotton candy machine).
2. Use the resulting nanofibers (much like a piece of cotton candy) to help close wound.

Interesting Fact:
1. One can unfold FN in a concentrated FN solution by pulling with a pipette.
2. Can even help hair follicle regrowth with the FN nanofiber.
3. The fibers are aligned rihght after fabrication, but the alignment is disrupted during handling. This helped reduce scarring.

The Role of Cell Geometry and Extracellular Matrix Ligands on Vinculin Loading and Actin Architectures

Premise:
Platform: Molecular engineering a FRET sensor on vinculin (a focal adhesion protein) to measure cell traction forces. In this case, high FRET signal means low force. The same group (PI: Breton Hoffman) also added FRET to NE-Cadheren.

Finding:
1. Higher stress at cell periphery (no surprise).
2. Also looked at micropatterned FN or serum. Compression only seen in cells on FN (at the cell center).
Spin FN or Fibrinogen by rotary spinning at high centrifugal force (essentially a cotton candy machine).

Interesting Known Fact:
Loss of vinculin lead to loss of traction force.

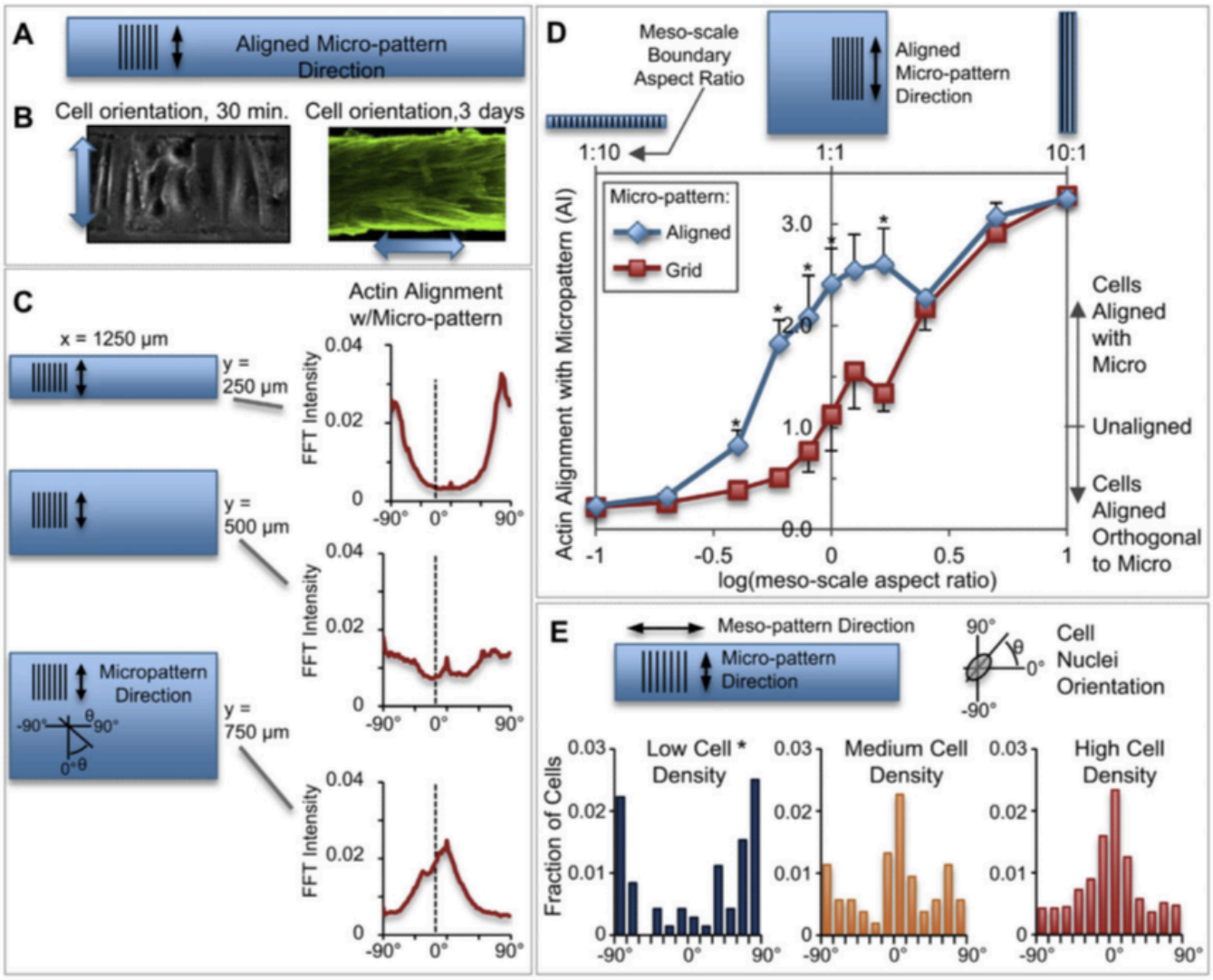
Vinculin-mediated Increase in Cardiac Function Confers Systemic Metabolic Efficiency and Extends Healthspan and Lifespan

Motivation:
From the Engler group: Vinculin overexpression 2Xed the life span of Drosophila (fruit fly). This benefit may be due to changed in sarcomere crystallinity downstream.

Methods:
Let the fly drink ^{13}C-labeled glucose and measure within 1 hr the metabolic activity *via* the TCA cycle. The vinculin ovexpression leads to higher heart muscle efficiency.

Directed Mesenchymal Stem Cell Migration During Aligned Neo-Tissue Formation

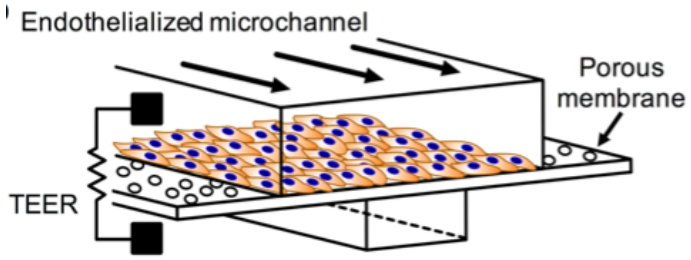
Premise:
Study stem cell migrations on ECM (FN or collagen) patterned in grids or arrays of lines or
Platform: A thin non-fouling hydrogel layer that resists cell adhesion and protein adsorption is created.
Patterns were made using photo-ablation, followed by ECM adsorption onto the abled patterns.



Take Home:
1. micro-pattern directs cells early on, but over time the macro-pattern can takes over time as cell number increases.

Detecting the Endothelial Conditional Response to Oscillatory Shear Stress[6]

Premise:
cell type: **HAECs**, iMAECs (Immortalized Mouse Aortic Endothelial Cells), and HUVECs.
platform: Laminar shear stress (LSS) and oscillatory shear stress (OSS) applied to a cultured endothelium in a microfluidic device:



Interesting Find:
1. Upregulation of ICAM-1 observed for cells subjected to OSS at the center of the channel. Note: β-catenin is a dual function protein, involved in regulation and coordination of cell–cell adhesion and gene transcription (Wnt signaling).

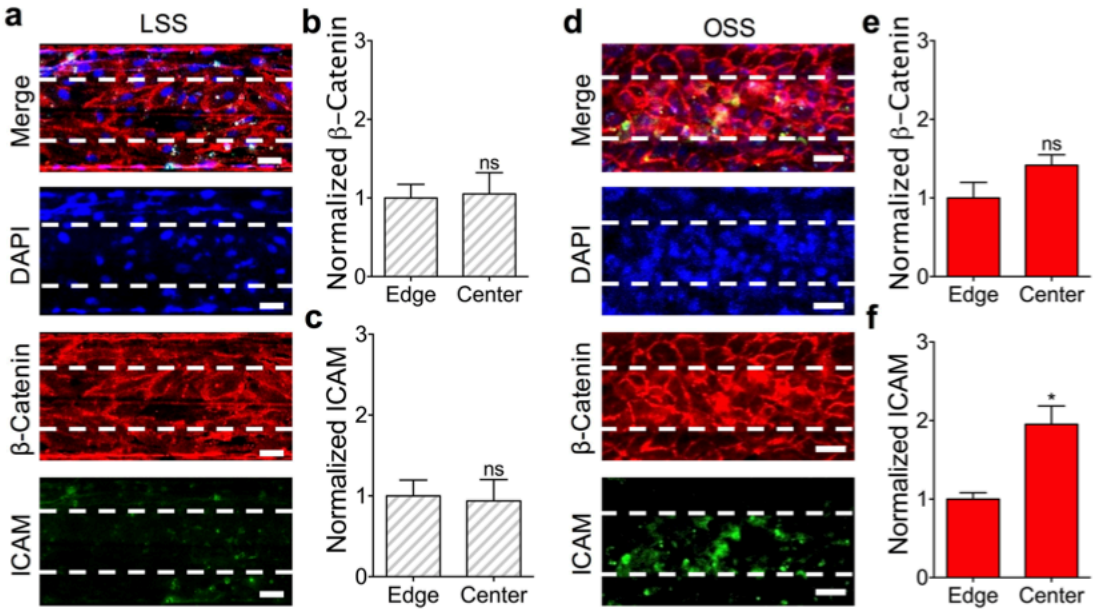


Figure 2. Microfluidic transcellular monitor design. (**a**) Confocal microscopy images were taken through the underside of the porous membrane of an HAEC monolayer cultured in the upper microchannel under LSS at 10 dyne/cm^2 that was stained for β-catenin and ICAM (lower microchannel outlined with dotted white lines). Scale bar is 40 μm. (**b**) β-catenin channel intensity measurements and (**c**) ICAM channel intensity measurements for the LSS case comparing the center and edge regions normalized to the edge region (N = 3). (**d**) Confocal microscopy images were taken through the underside of the porous membrane of an HAEC monolayer cultured in the upper microchannel under OSS at 1 Hz that was stained for β-catenin and ICAM (lower microchannel outlined with dotted white lines). Scale bar is 40 μm. (**e**) β-catenin channel intensity measurements and (**f**) ICAM channel intensity measurements for the OSS case comparing the center and edge regions normalized to the edge region (N = 3). Plotted as mean ± SEM where * is for $p < 0.05$.

2. For LSS, No differences in TEER and the expression of β-catenin and ICAM-1.

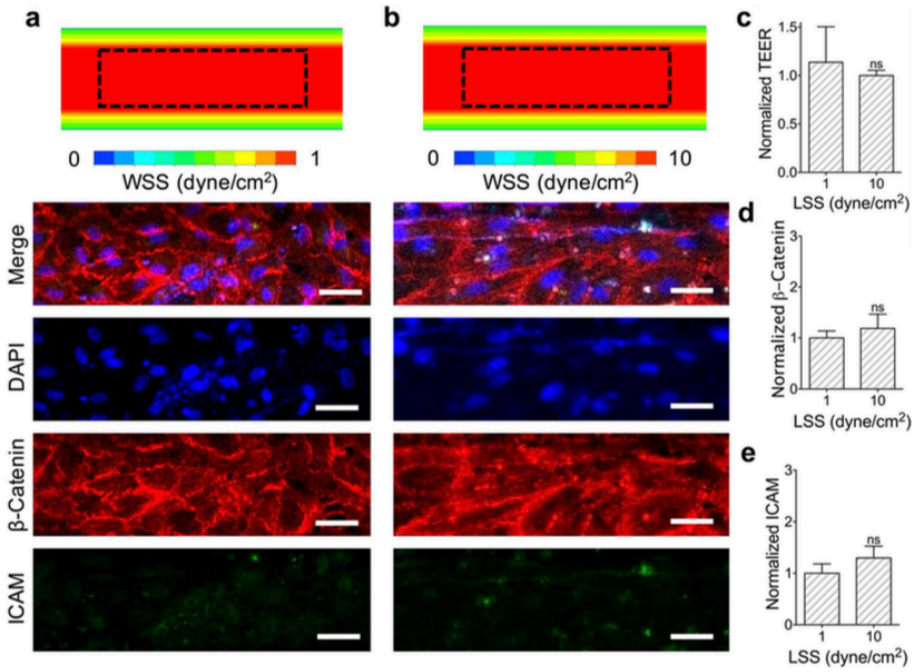


Figure 3. Comparing LSS cases for +1 and +10 dyne/cm². (**a**) Upper microchannel WSS distribution and confocal microscopy images of HAEC monolayers cultured under +1 dyne/cm² and (**b**) +10 dyne/cm² as viewed through the underside of the porous membrane. Confocal images used for comparison were taken from a region in the center of the upper microchannel (outlined). Scale bar is 40 μm. (**c**) Normalized TEER of HAEC monolayer cultured under +1 or +10 dyne/cm². (**d**) β-catenin channel intensity measurements and (**e**) ICAM channel intensity measurements for the LSS cases comparing +1 and +10 dyne/cm² normalized to 1 dyne/cm² case (N = 3). Plotted as mean ± SEM where * is for $p < 0.05$.

2. For OSS, differences in TEER and the expression of ICAM-1

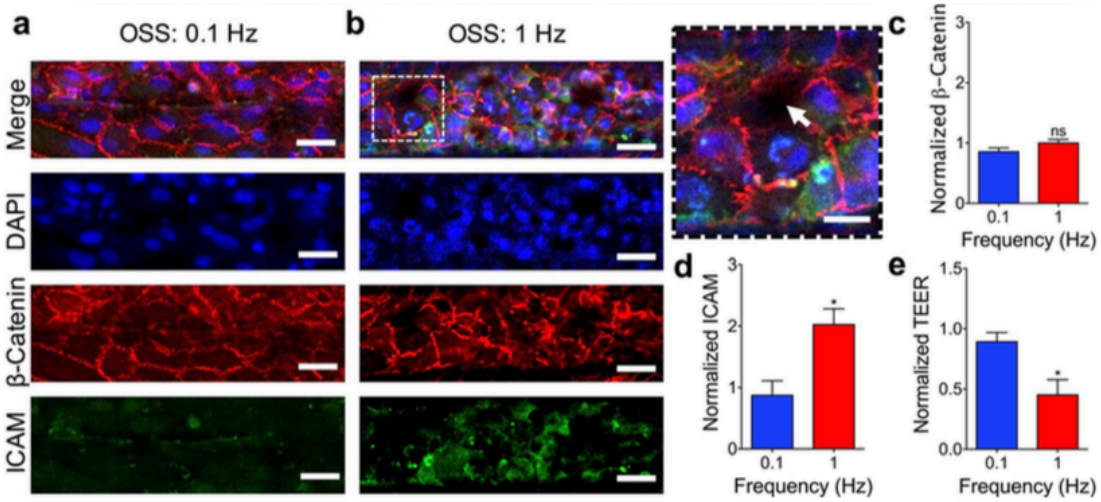


Figure 4. Monitoring of endothelial frequency response. (**a**) Top view confocal images of HAEC monolayer in the center region cultured under 0.1 Hz. (**b**) Top view confocal images of HAEC monolayer in the center region cultured under 1 Hz with disrupted endothelial junction (white arrow). Scale bar is 40 μm and 20 μm for enlarged image. (**c**) β-catenin intensity channel measurements for the OSS cases comparing 0.1 and 1 Hz normalized to 10 dyne/cm² case (N = 3). (**d**) ICAM intensity channel measurements for the OSS cases comparing 0.1 and 1 Hz normalized to 10 dyne/cm² LSS case (N = 3). (**e**) Normalized TEER of HAEC monolayer after 36 hours of culture under 12 hours of 0.1 or 1 Hz normalized to the 10 dyne/cm² LSS case. Plotted as mean ± SEM where * is for $p < 0.05$.

Tissue-Specific Endothelial Cell Response to Laminar Shear Stress

Motivation:
Endothelium exhibit structural and functional heterogeneity,

Methods:
Harvest cells from different endothelial bed and culture under flow of 4 dyn/cm^2 for 12hr on different ECM coating. The catch is, do these cell retain their *in vivo* phenotype *in vitro*. RNA is also isolated from the sample for additional assessment.

Lung EC and ESC-EC: perpendicular to flow first then align,
Cardiac EC and thymus EC: don't align much.
Brain, kidney, and liver EC don't want to align at all.

Also study the effect of shear stress on cell stiffness (measured *via* AFM).
For cardiac and thymus EC, the stiffness reduces when the shear stress increases.

Work in Progress:
1. Transcriptome profiling.
2.Grow the ECs using decellularized vessels.

Audience Q&A:
Q: Did you look at longer the longer time points.
A: Continue the observation up to 3-4 day. The pattern seens at the 12h time point is maintained.

Bioprinting Exosome Microenvironments

Methods:
1. Print cxosomes (with 10 μg/mL of protein content) in 60 μL of ink onto collagen.
2. Collagen will bind and retain exosomes for up to 3 weeks.
3. Can use reagents to up and down regulate the cellular uptake of the immobilized exosomes (i.e. heparin, β-cyclodextrin).
01. Exosomes obtained from J774a.1 mouse macrophage stimulated with 100 ng/mL LPS
02. Load BMP2 into the exosomes *via* sonication.
03. Purification of the BMP2-loaded exosomes *via* SEC.
04. Assess the expression of alkaline phosphatase, indicative of the recipient cells toward the osteogenic lineage.

Finding:
Indeed the recipient cells showed increased alkaline phosphatase expression.

The Twist:
Q: Audience asked what if you printed BMP2 directly, and forget the exosomes.
A: Same increase expression of alkaline phosphatase expression is seen.

Microvesicle Induction by the Metabolically-Regulated Glycocalyx

Motivation:
Larger glycocalyx are expressed in aggressive circulating tumor cells (CTC).
Question 1: What is the mechanism of glycocalyx shedding.
Question 2: Microvesicle (MV) biogenesis not well-defined.
Glycocalyx consists of many different glycoproteins. So narrowed the focus to specifically examine the effect of mucins (Muc) and hyaluronan (HA) deletion and truncation.

Interesting Find:
1. Glycocalyx truncation reduced the production of MVs.
2. Average production of 300 MVs/cell/hr.

Future Work:
1. Glycocalyx may play a rule in MV induction.
2. Glycocalyx size may be related to MV shedding.

Questions: Did you ID the MV cargo.
Ans: Did not.

Tom Questions: Are these MV's really different from exosomes?
Ans: Hard to say, but exosome expression also increased in number.

Question after talk: How did this discovery happened?
Ans: PI is a glycocalyx guy. When examine the glycocalyx of CTC *via* SEM we came across these mudding MVs.

Exosomes from Diabetic SMCs Induce Adhesion of Monocytes to Vascular Endothelium

Summary:

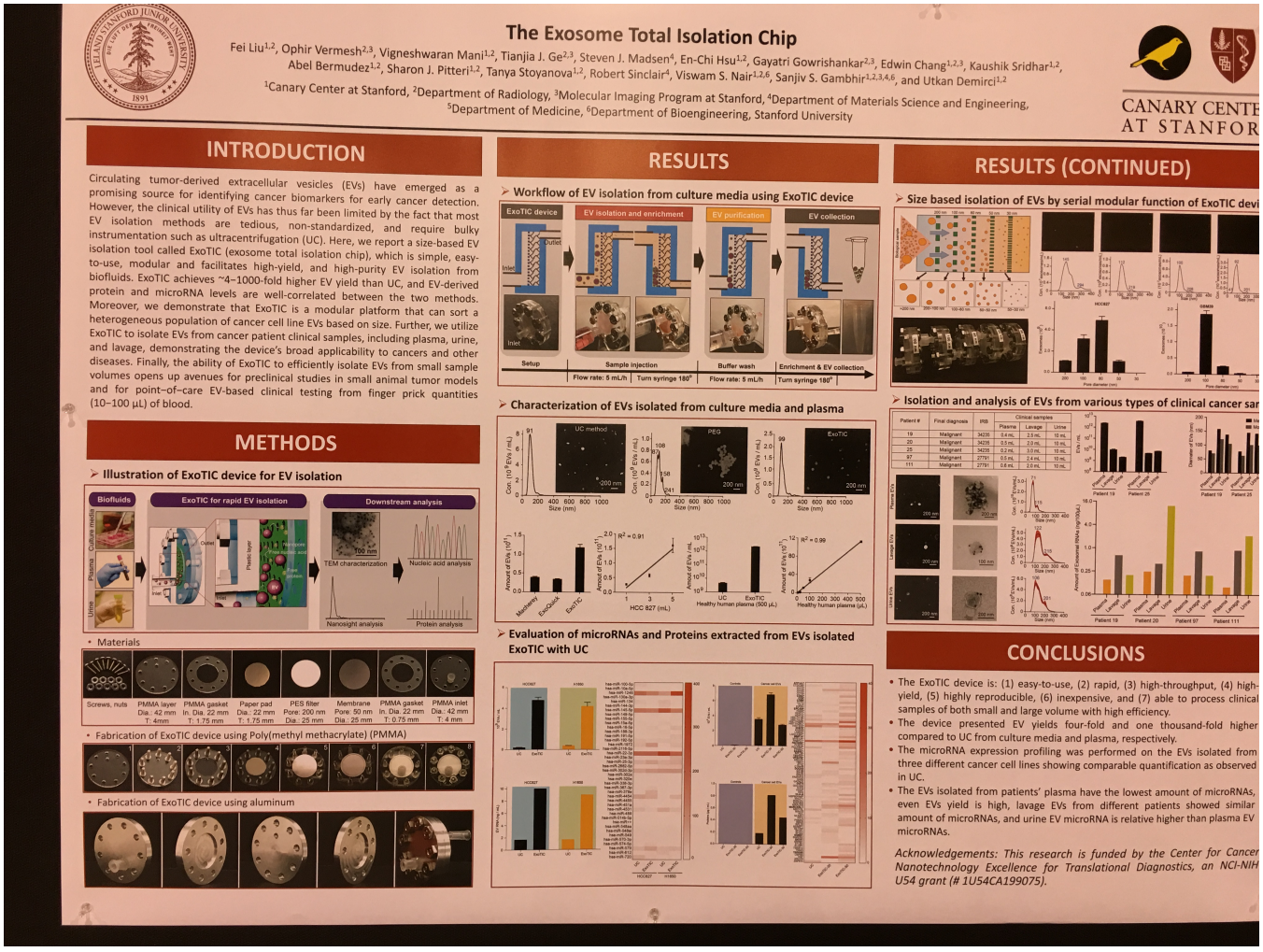
Exosomes from diabetic smooth muscle cells induce monocyte attachment to the endothelium (HCAEC/ Human Coronary and Iliac Artery Endothelial Cells or HUVEC). Upregulation of ICAM-1 is likely the mechanism for this increase in attachment. Control: TNF-α.

The Exosome Total Isolation Chip (ExoTIC) Device

Summary:

Exosomes capture using two different filters (PES membrane: 200 nm pore and "membrane": 50 nm pore) through the application of flow. The first filter removed debris and junks, then 2nd filter captures exosomes. The flow is normal at entrance, and tangential further down stream.

Patent Publication Number: WO2017136430 A1



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