CARDIOVASCULAR TISSUE ENGINEERING IN PRECISION AND REGENERATIVE MEDICINE

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Wyss translational center in Zurich

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erc European Research Council

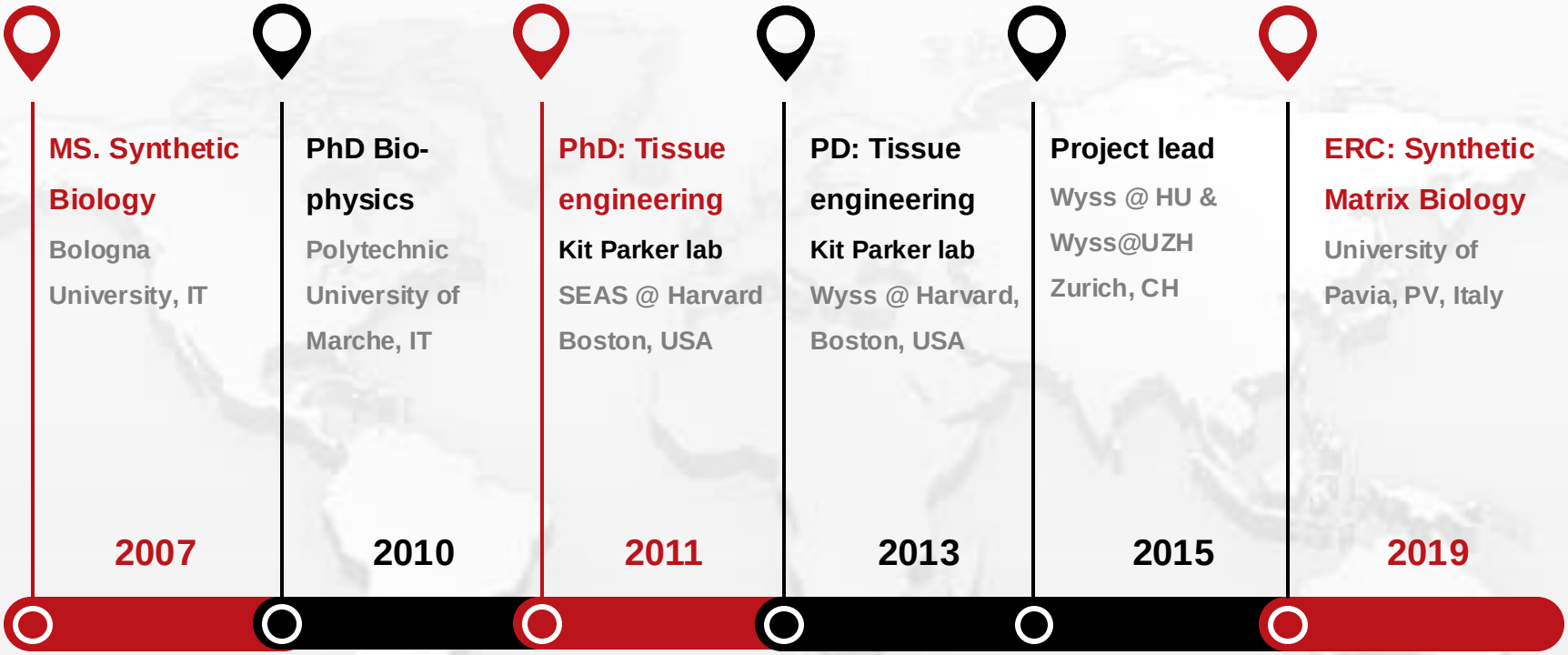
Finanziato dall'Unione europea NextGenerationEU

MUR

cabmm center for applied biotechnology and molecular medicine

Regione Lombardia

From synthetic biology, to tissue engineering, to synthetic matrix biology



Parker lab funding

DARPA DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

NIH National Center for Advancing Translational Sciences

Pasqualini lab funding

cabmm center for applied biotechnology and molecular medicine

erc European Research Council

Regione Lombardia

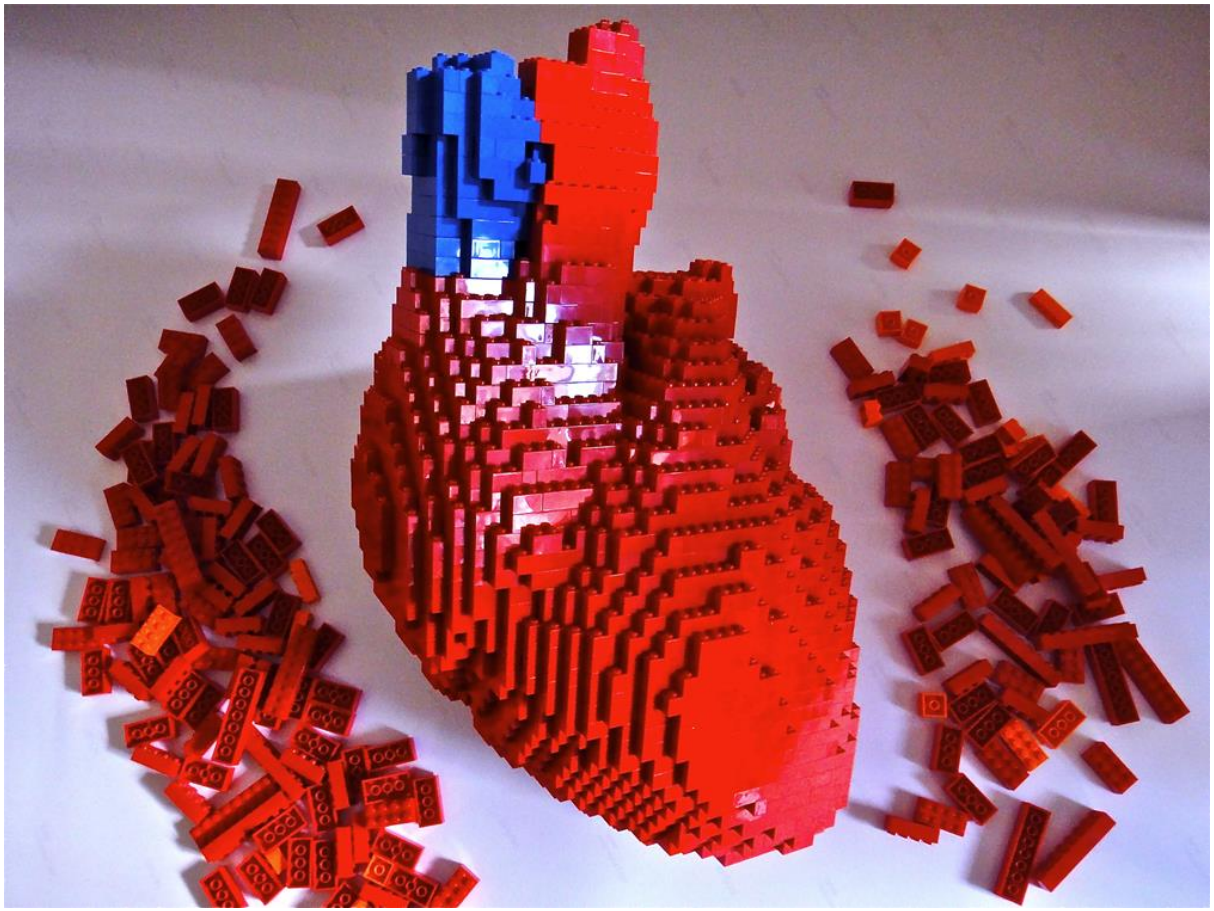
My original quest: What does it take to make cardiac tissue engineered products?

Roadmap (from 2010):

- **Organ replacement: 20 years, >20B cells, different cell types**
- **Regenerative medicines: 10 years, >100Ms cells,**
- **Drug discovery: 5 years, <1M cells, single/multiple cell types**

Roadblocks:

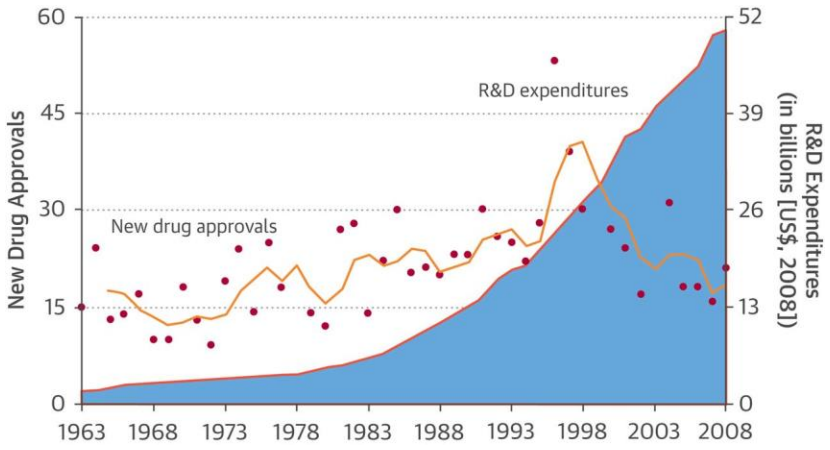
- **Cells as building blocks**
- **High-quantity**
- **High-quality**

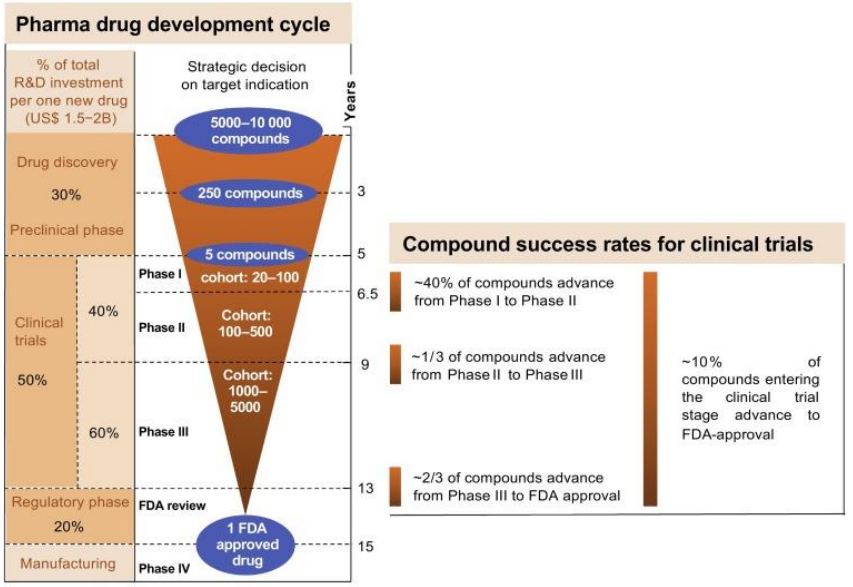


Agenda

1. **Background: Organ-chips in precision and regenerative medicine**
2. **My work with the heart-chip in Boston**
3. **My work with regenerative cardiovascular prostheses in Zurich**
4. **My work in Synthetic Matrix Biology in Pavia**
5. **Discussion**

Organs-on-chips (OOC) are fail-fast/cheap platforms for predictive pre-clinical investigations





Eroom law

- The cost of getting a drug to the market has doubled yearly over the last 30 yrs

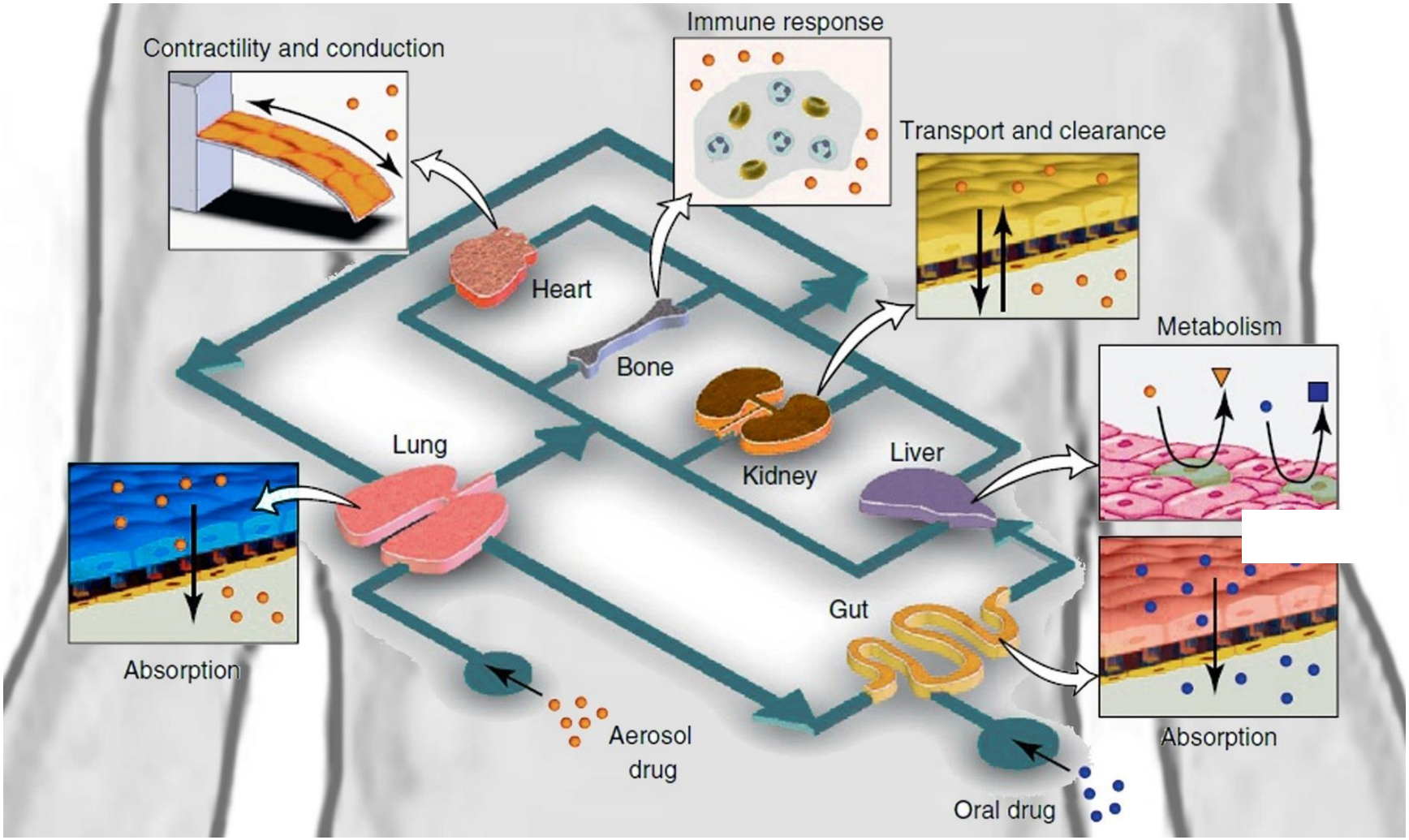
Drug discovery economics

- Failures in the clinical trial phase are ethical and economical catastrophes

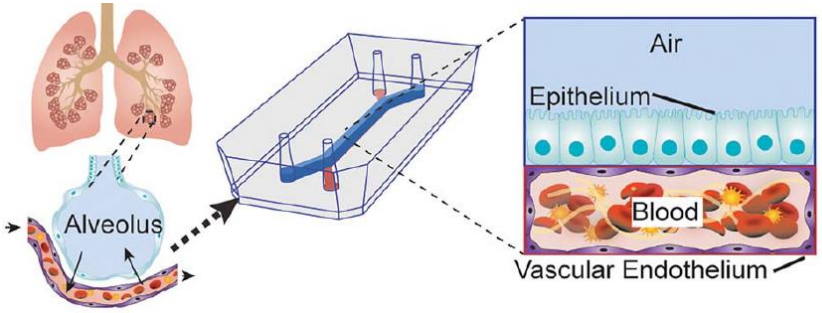
OOC are assays meant to fail candidate compounds pre-clinically

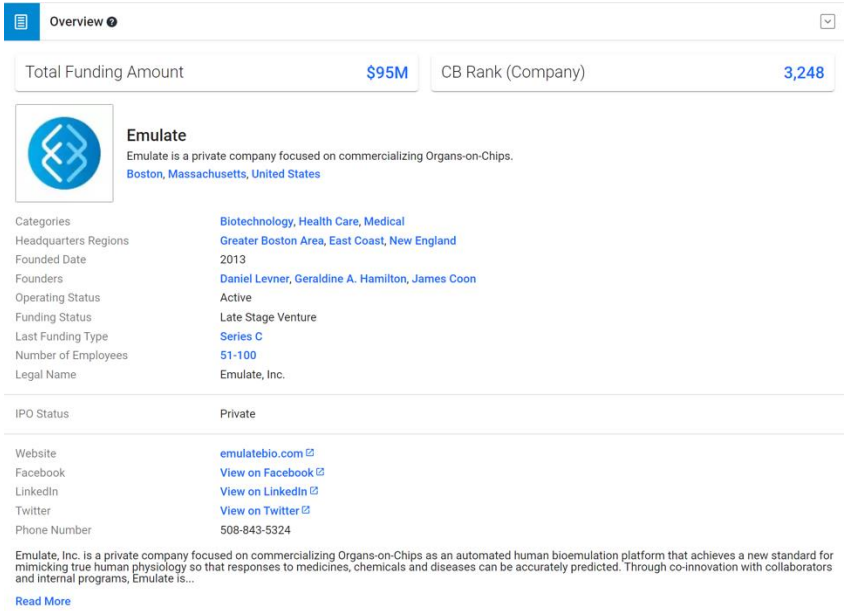
Fordyce et al, JACC 2015; Harrer et al, Trends Pharmacol Sci, 2019

Organs-on-chips (OOC) are fail-fast/cheap platforms for predictive pre-clinical investigations



The Wyss/Emulate approach: General-purpose OOCs for safety, efficacy, and everything in between





The Harvard Wyss Institute OOC

- Human cell types + biomimetic stretch
- ~50 M$ in DARPA/NIH funding

Emulate, Inc

- >100 M$ in private investments
- Co-development deals with Pharma

Emulate, the dominant player in this market, doesn't have a heart chip

https://raportuldegarda.ro/articol/lung-on-a-chip-cum-pot-fi-descoperite-noi-medicamente-pentru-fibroza-pulmonara-idiopatica-prin-intermediul-biotehnologiei/. Crunchbase

Heart-chips powered by human pluripotent stem cell-derived cardiomyocytes for disease modeling

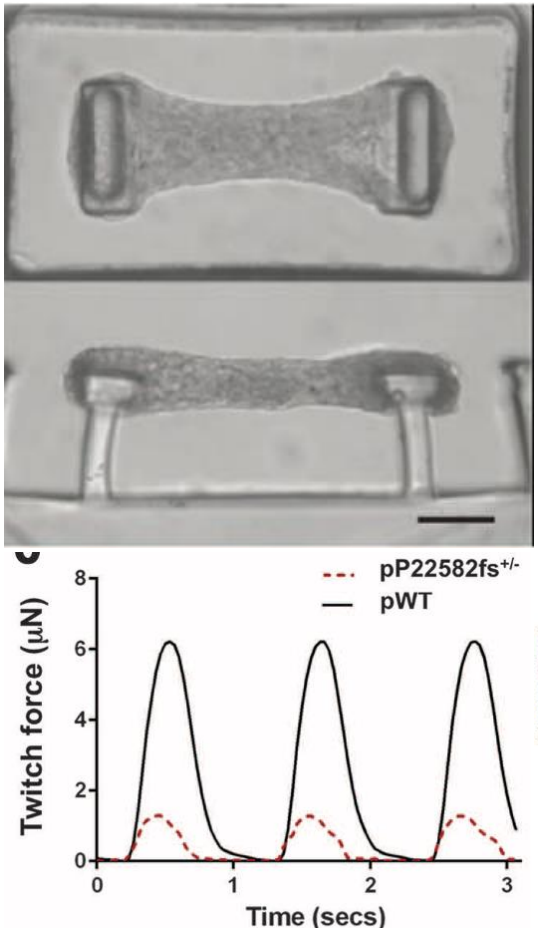
Cell sources

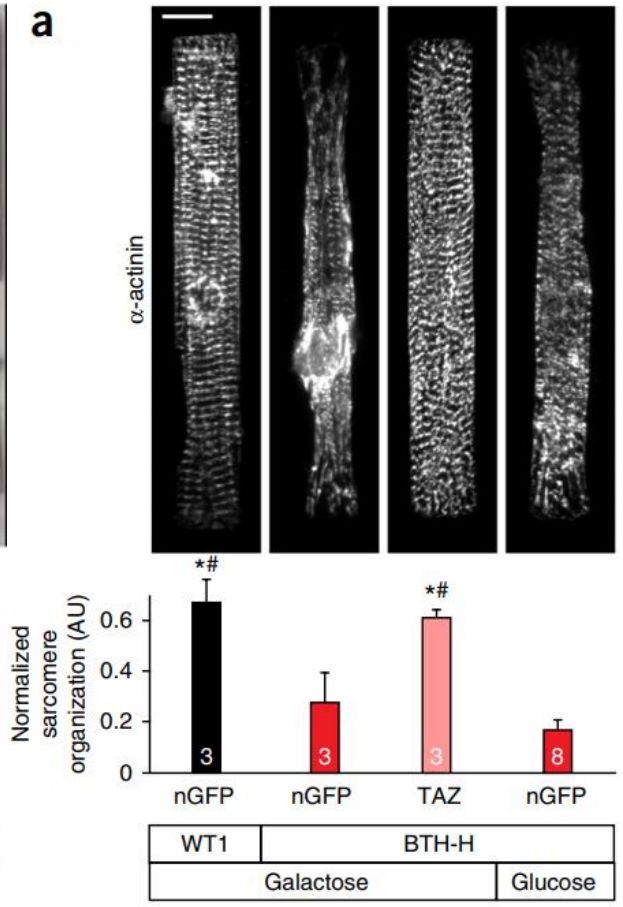
- Patient-specific
- Genome-edited

Type of diseases

- Genetic disorders
- Single mutations

Key application in this space is the ability to model diseases





Hinson et al, Science, 2015; Wang et al, Nat Medicine, 2014

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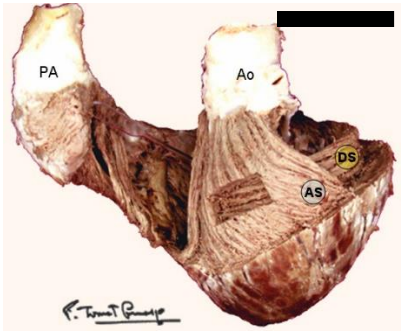
Microfabrication Techniques to Recapitulate Cardiac Cell and Tissue Structure

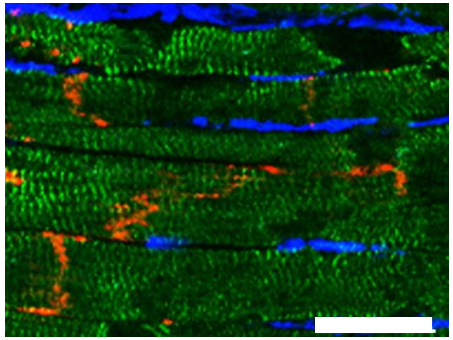
Contractile structure in the heart:

- Laminar tissues
- Aligned myofibrils
- Striated muscle cells

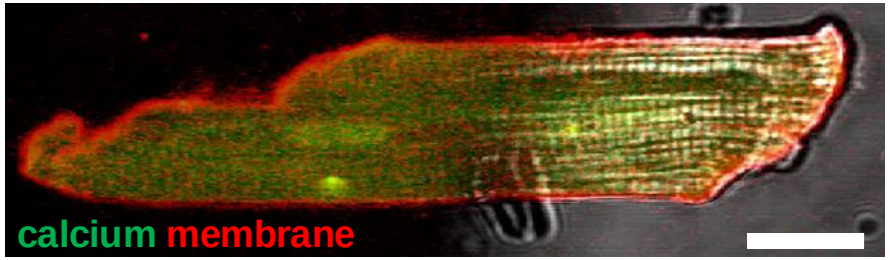


Scale: ~10 cm

α-actinin, β-catenin, fibro

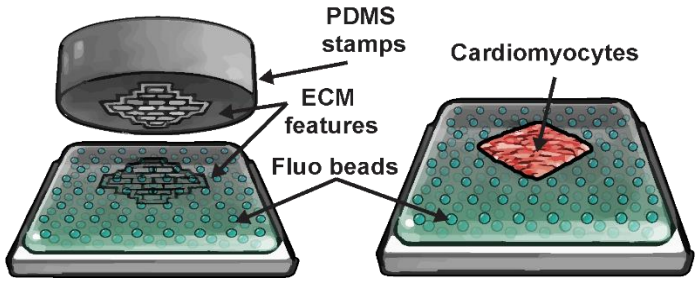


Scale: ~250 um

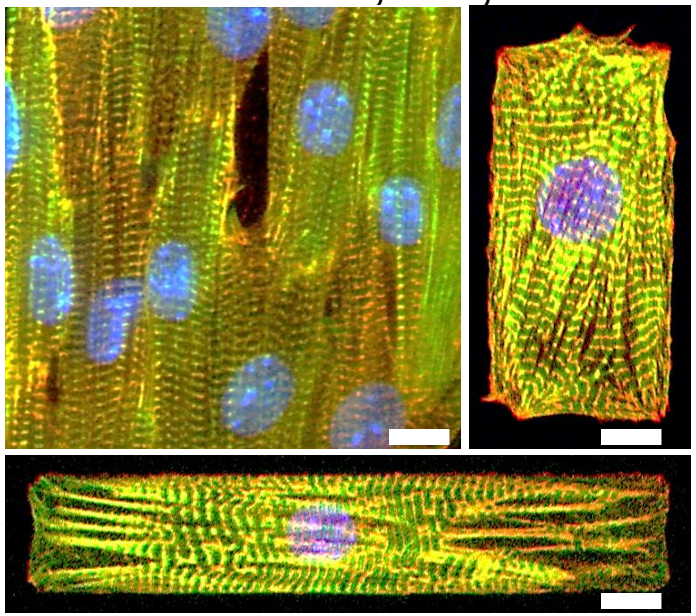


Scale: ~25 um

Microcontact printing



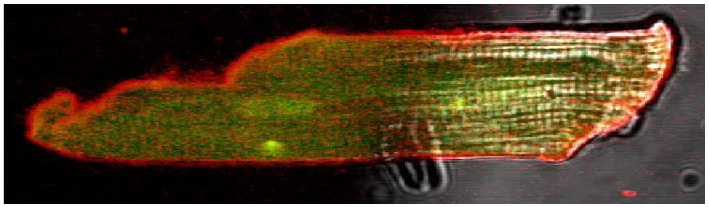
α-actinin, actin, chromatin



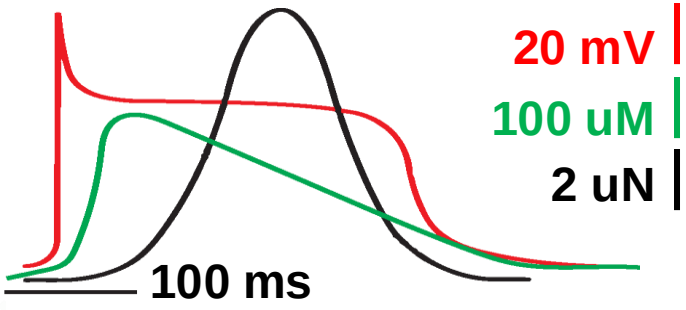
Scale: 10 um

Multiparametric assessment of cardiac tissue
structure and function in the Heart-chip

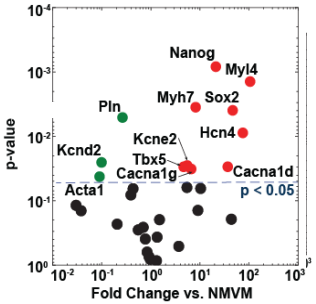
Rat cardiomyocyte



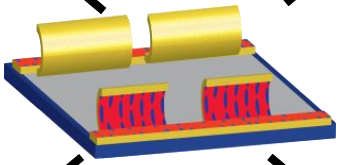
calcium membrane



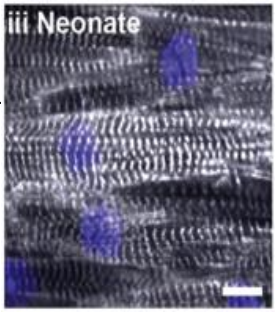
Gene Expression



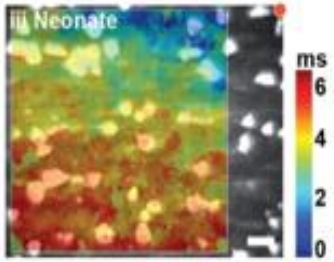
Heart-on-a-Chip



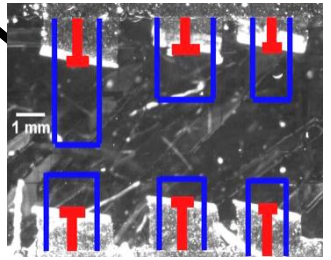
Structure



Electrical Activity



Contractility



Functional signals in the heart:

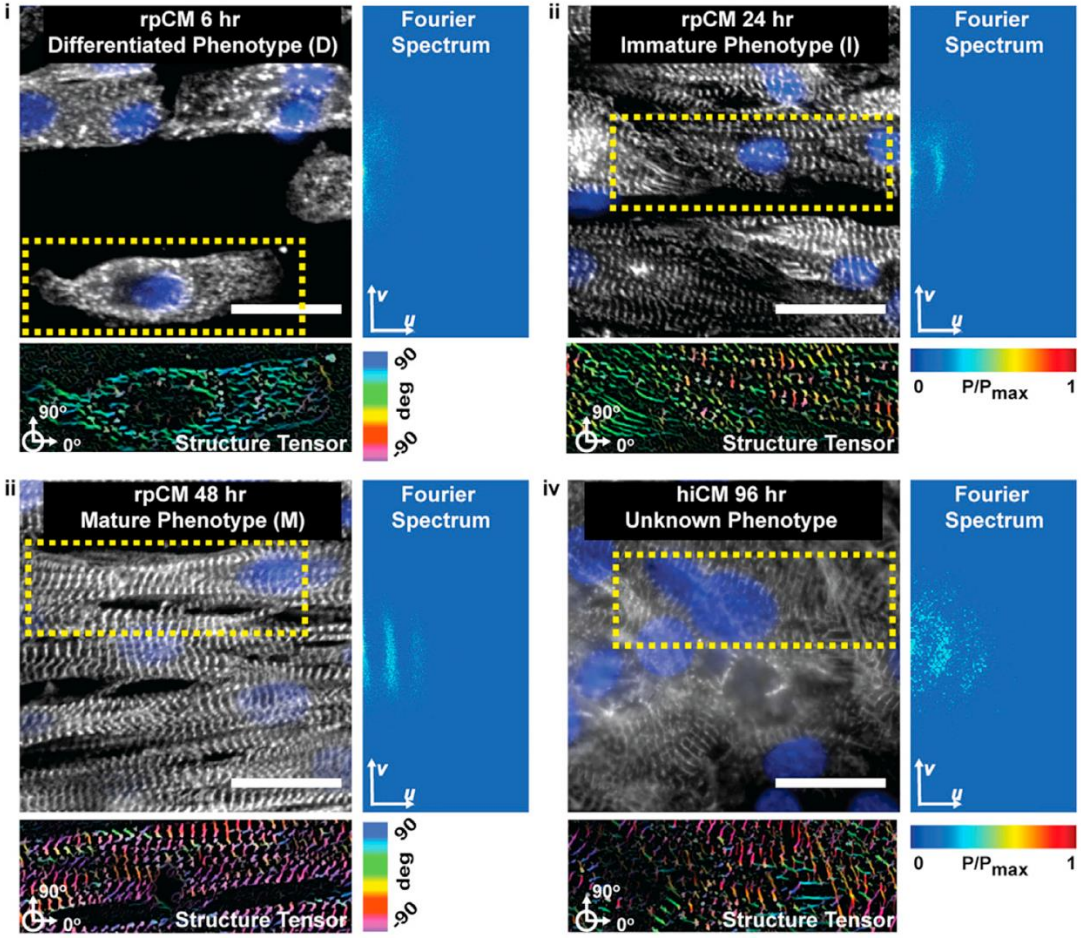
- Action potential
- Calcium transient
- Contractile force

Heart-Chip Assay:

- Gene expression
- Electrochemical coupling
- Contractility

Pasqualini, Stem Cell Reports, 2015

Quantifying the structural maturation of hiPSC-CMs using a heart-chip platform

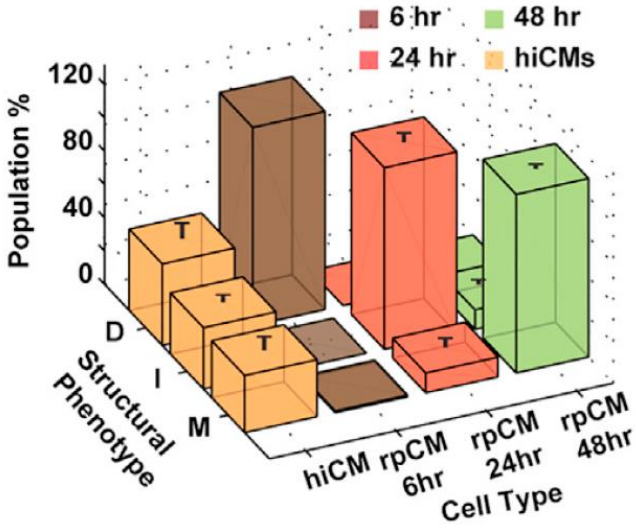


α-actinin chromatin

Scale: 25 um

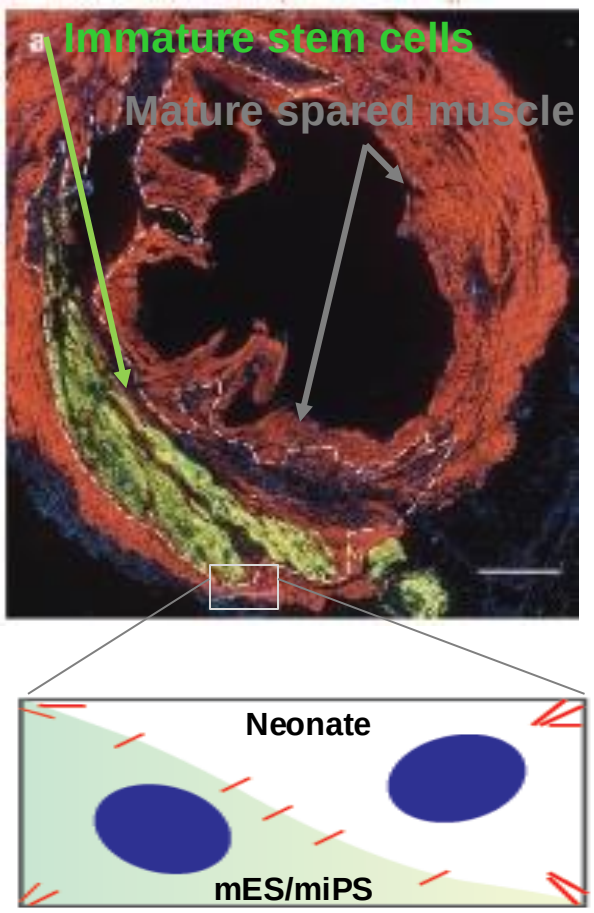
30% of hiPS-CM have mature contractile structure

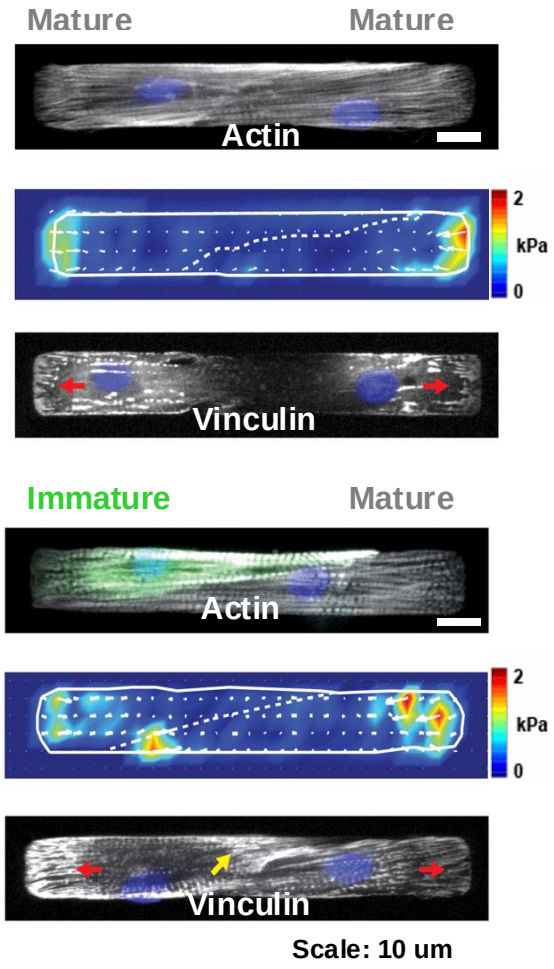
- *Training dataset: Primary engineered tissues*
- *Test set: hiPS-CM*
- *Machine learning: Three independent classifiers*

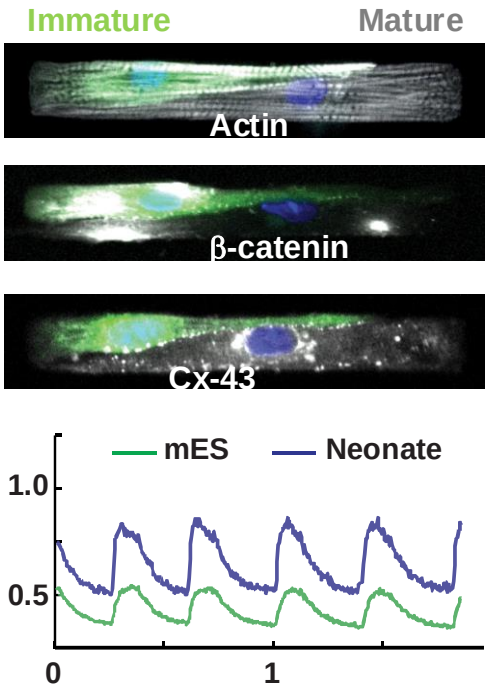


Pasqualini, Stem Cell Reports, 2015

A heart-chip potency assay for cardiac cell therapy



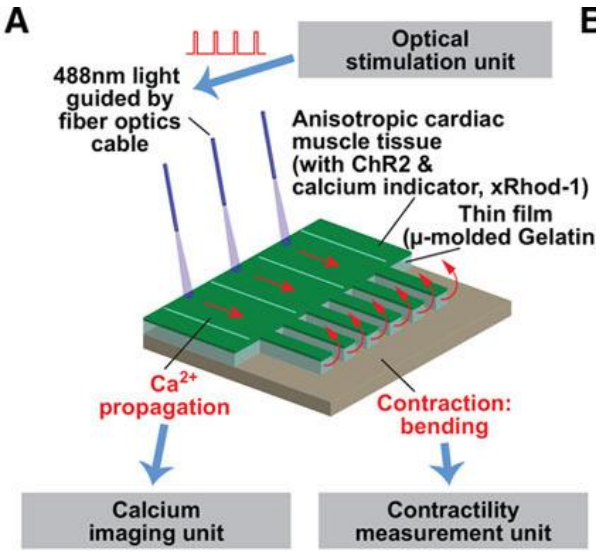


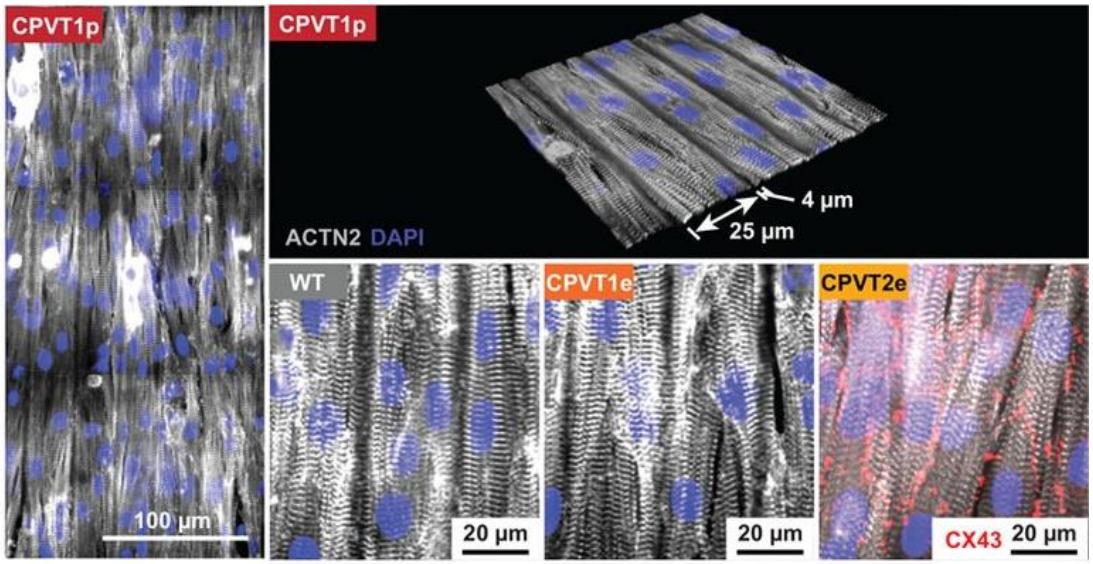


We modeled the (non-)therapeutic effect seen in large animal studies

Chong, Nature, 2014; Pasqualini, J. Cell Bio, 2016

Heart-chip based disease-modeling





Park et al, Circ, 2019

Heart chip

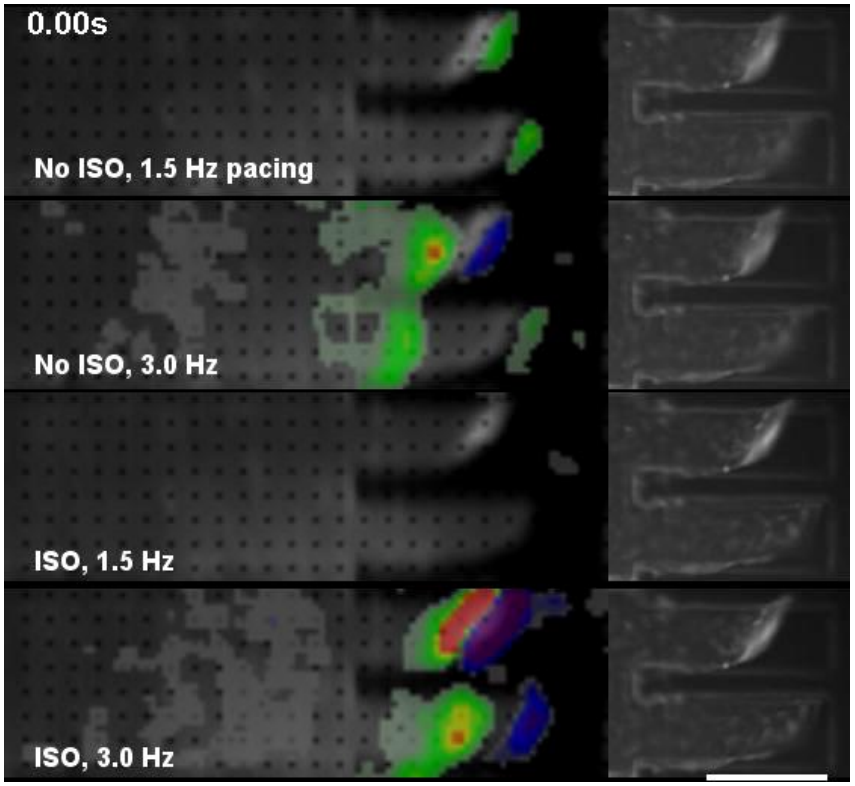
- Optogenetics
- Muscular thin film
- Custom-made to have larger areas to study calcium propagation

Engineered WT and CVPT tissues

- 2.5D platform
- Laminar tissue architecture
- Well-developed contractile apparatus
- Electrically-competent tissues

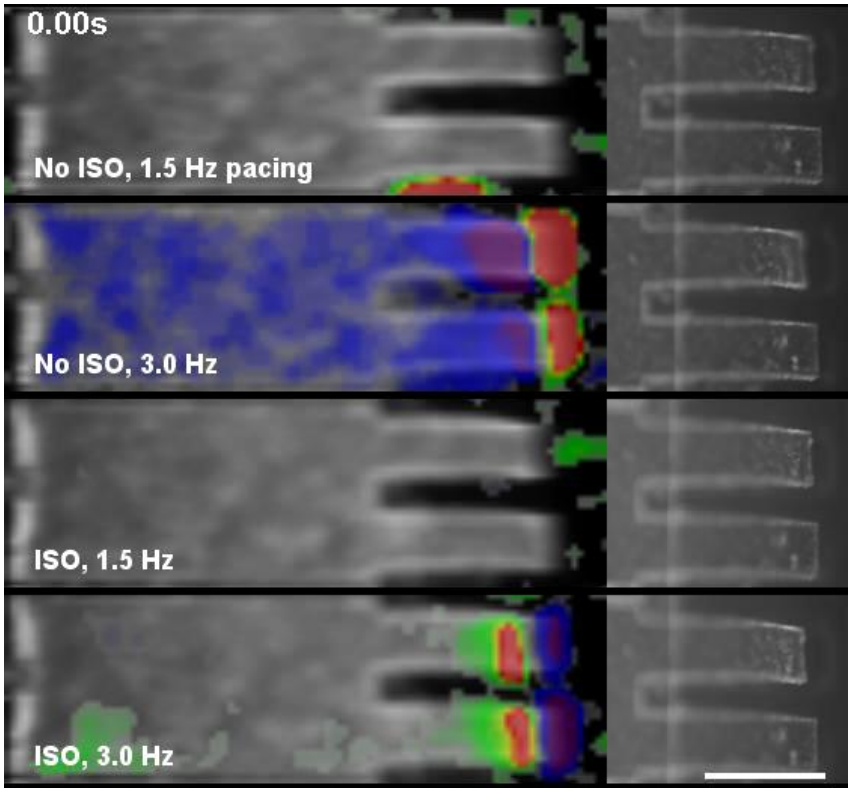
Park et al, Circ, 2019

Tissue models from CPVT patient-derived hiPSC-CMs formed rotors under an exercise-mimicking stimulation protocol



Healthy heart chip

- Regular calcium waves w/out ISO



CPVT heart chip

- Reentry with exercise-like stimulation

Park et al, Circ, 2019

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The extracellular matrix (ECM) in the heart

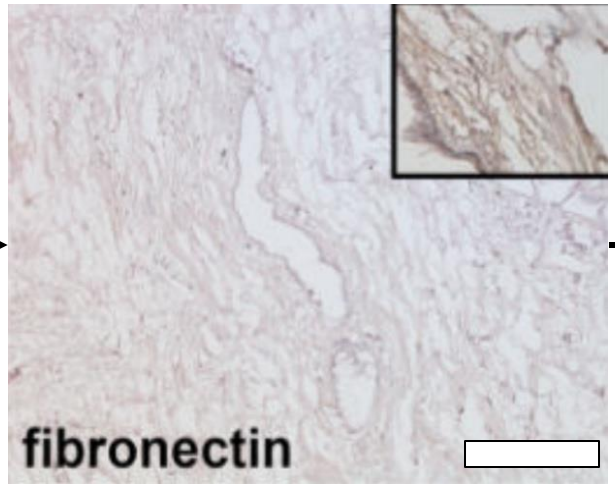
Whole heart

Ott, Taylor@UMn, 2008

Scale: ~2.5 cm

2D layer

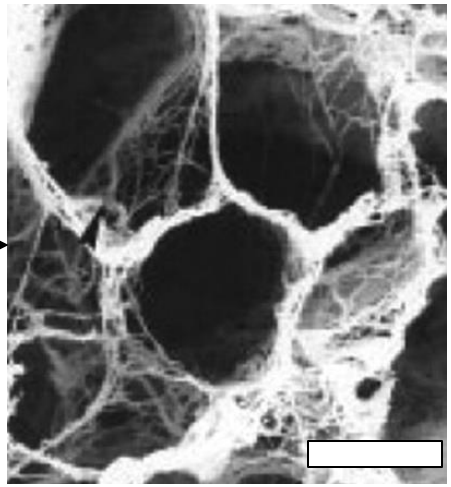
Guyette, Ott@MGH, Circ Res, 2016



Scale: ~250 um

Single cells

Spinale@MUSC, Phy Rev, 2007



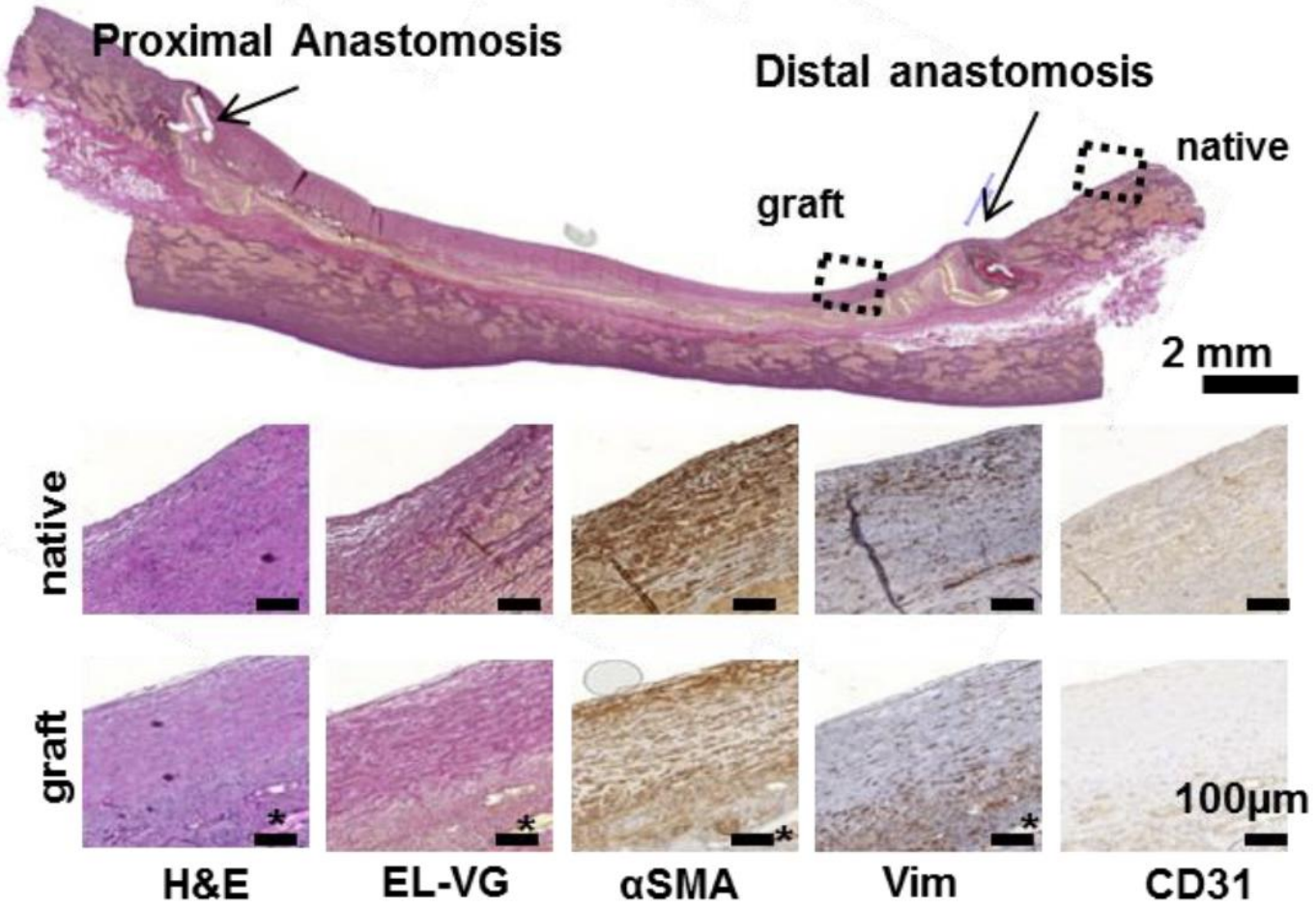
Scale: ~25 um

Multiscale organization of the extracellular matrix (ECM):

- Fibrous, compact ECM maintain multi-chambered organ structure
- Anisotropic distribution of ECM proteins sustain laminar tissues
- ECM boundaries that physically constrained single cells

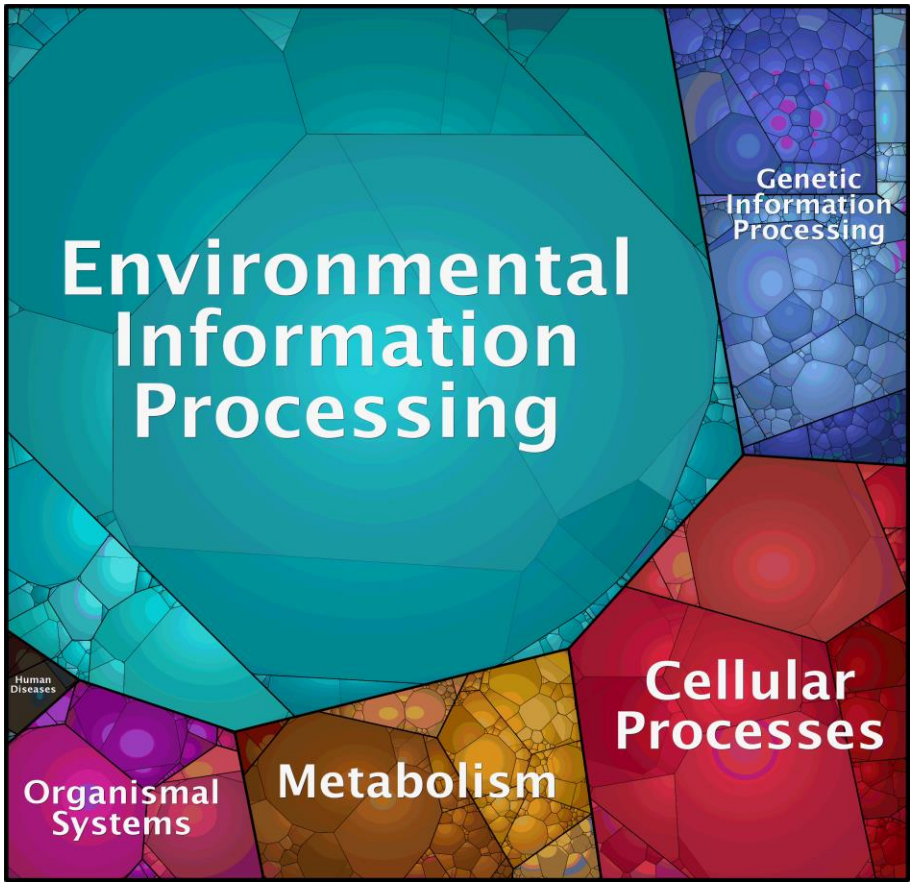
Biomimetic ECM cues to engineer native-like cardiac tissues

Decellularized ECM from tissue-engineered heart vessels is highly regenerative in a sheep model after one year.



Source: Simon Hoerstrup's LifeMatrix project (GMP fabrication and GLP analysis in preparation for a clinical trial)

The regenerative decellularized ECM is a *complex mix of structural and functional proteins* (and even more with sugars)



Regenerative decellularized human ECM:

- **~6000 proteins**

By gene ontology:

- **~60% of ECM proteins are structural.**
- **~40% of ECM proteins are functional.**

Source: Pasqualini et al, 2018 – Unpublished GLP proteomics by Biognosis, AG

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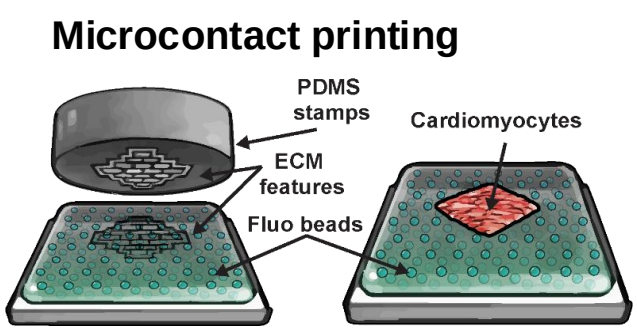
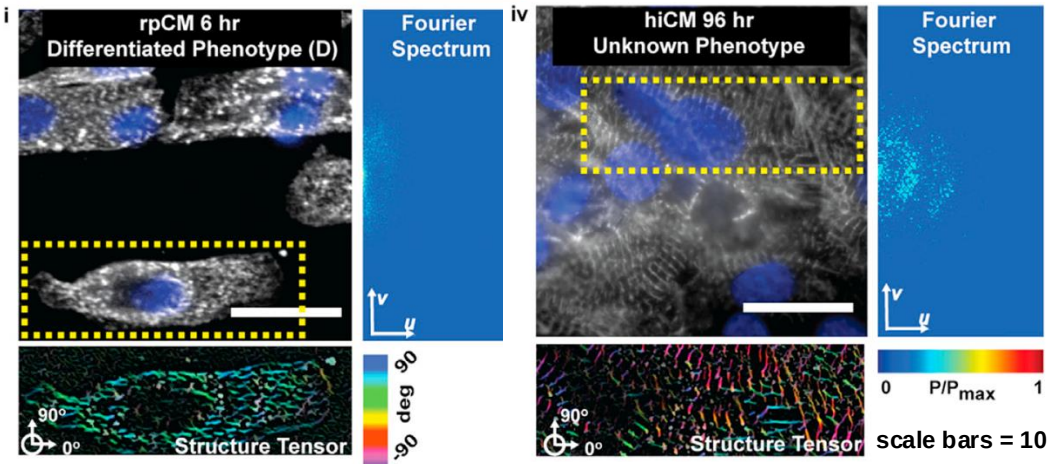
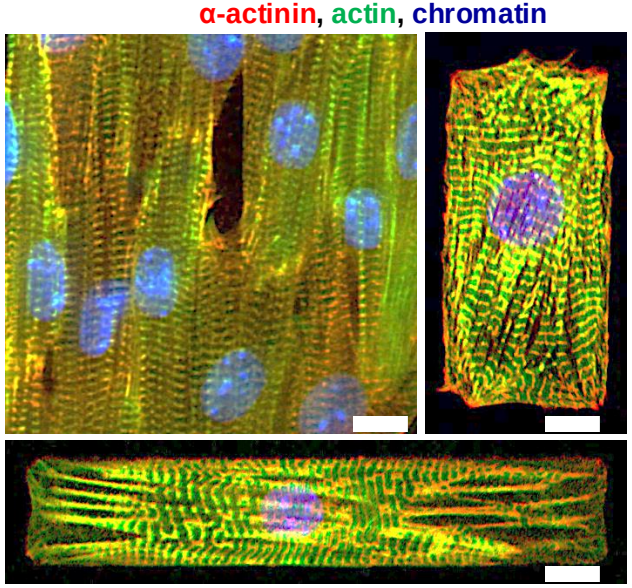
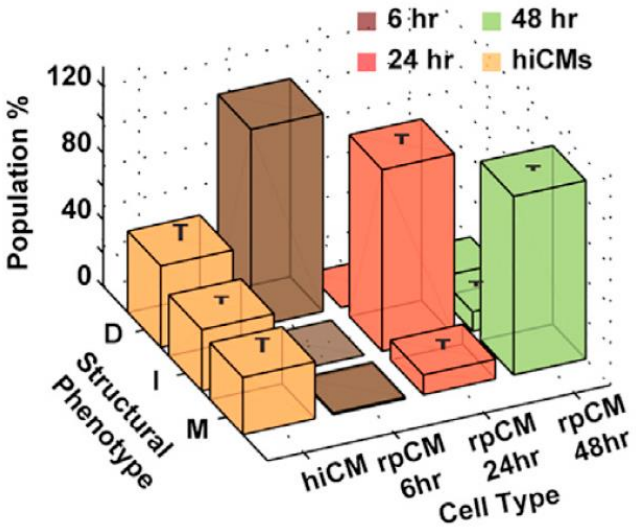
What I used to believe: We could build a heart by sticking together high-quality cells





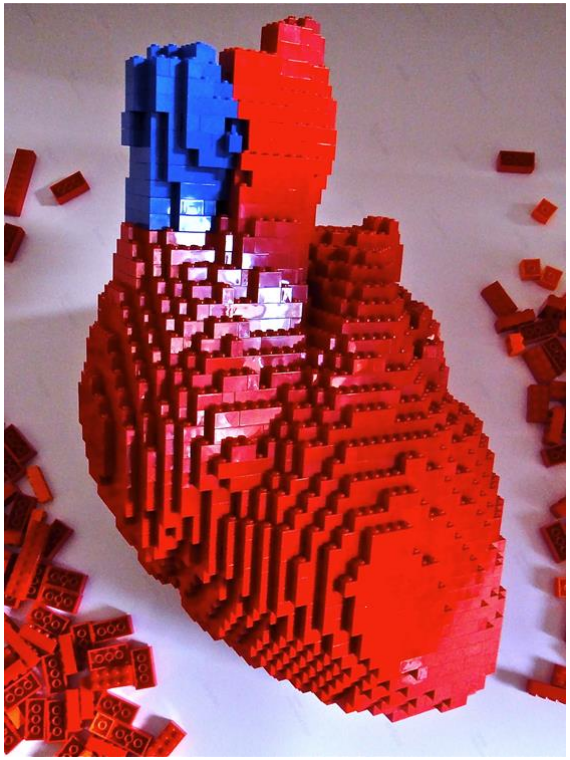




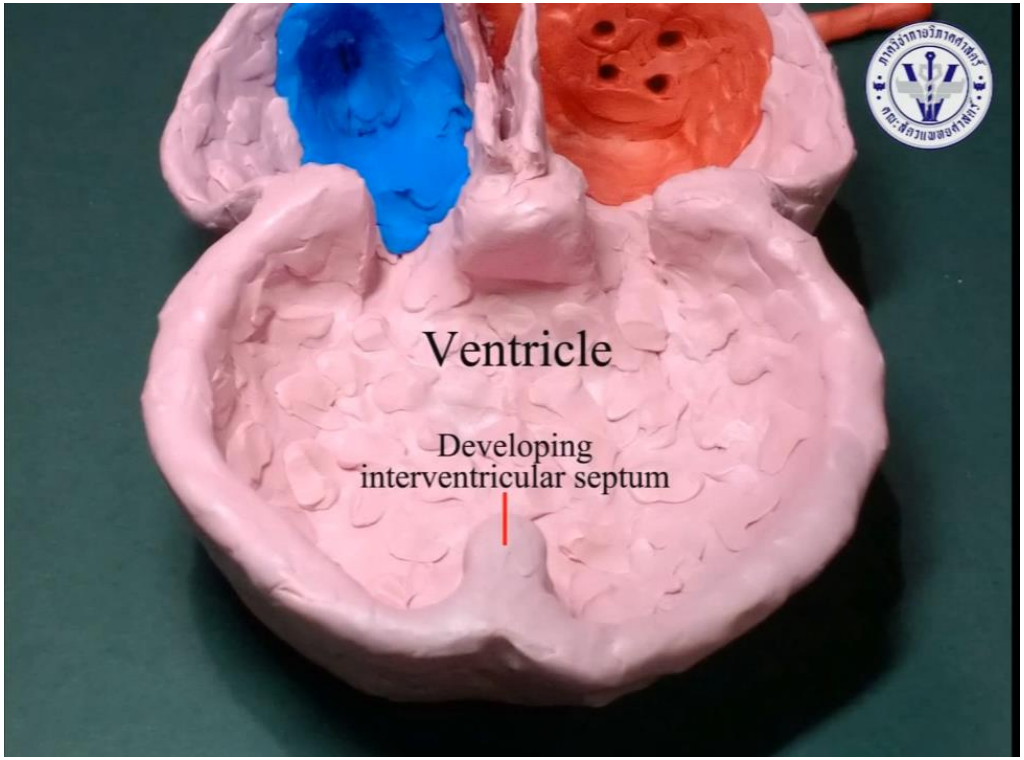
Pasqualini et al, 2014, 2015, 2016, 2018; US20160203262A1

I was wrong #1! Stem-cell-derived cardiac cells are fetal, so we should use them to grow hearts out, not build them.

Building a heart



Growing a heart



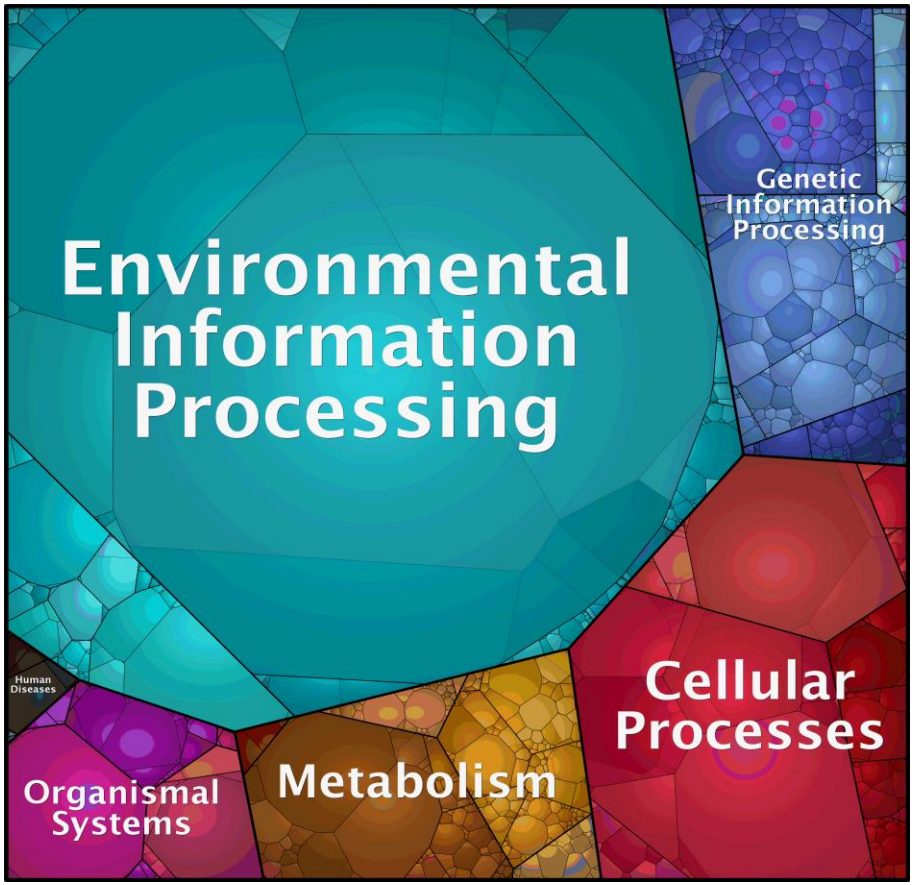
I was wrong #2! If the ECM is just the glue that keeps the cells together, *why is it so complex?!*

Cells: functional blocks

ECM: structural glue

Decell. human tissues proteomics:

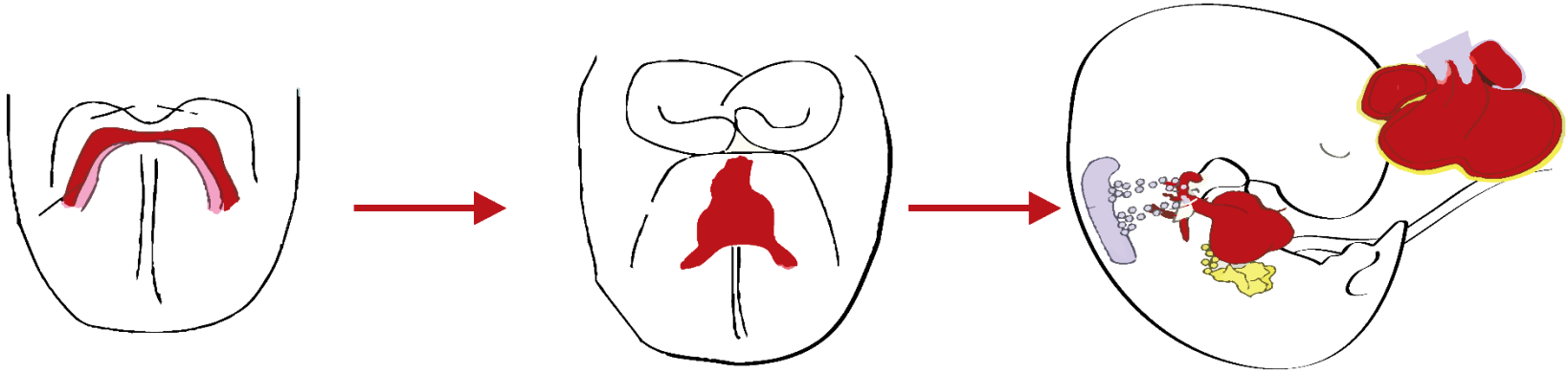
40% of ECM proteins are functional.



Source: Pasqualini et al, 2018 – Unpublished GLP proteomics by Biognosis, AG

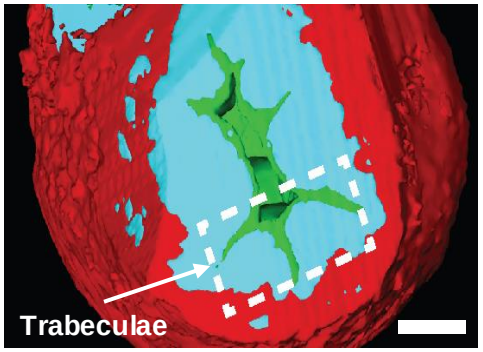
Synthetic Matrix Biology: Can use ECM parts to program tissues in the same way synthetic biologists use DNA parts to program cells?

Complexity



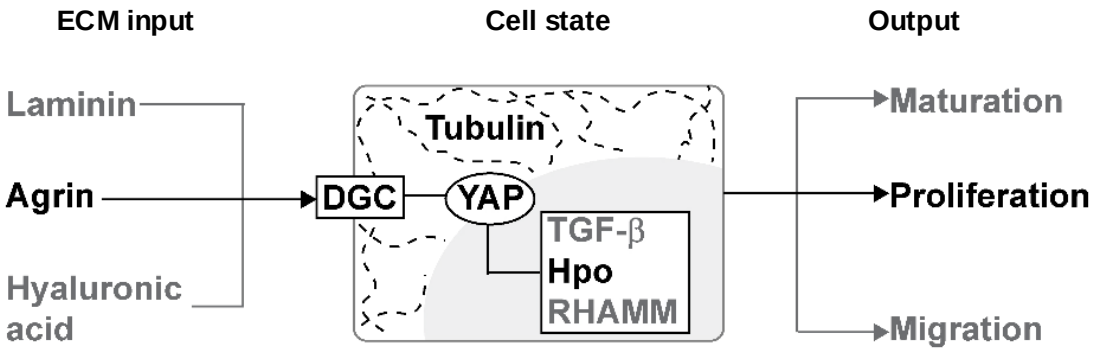
Heart development in mouse

Myocardial morphogenesis: Trabeculation



Del Monte-Nieto, 2018* Scale: 500 µm

Mouse heart tube: Myocytes on agrin, Endothelial cells on laminin, hyaluronic acid.



Team: A multidisciplinary, diverse group of people committed to understanding biology through engineering and *vice-versa*

Senior scientists

Moises di Sante

Molecular Biology

Alessandro Enrico

Materials science

Julius Zimmermann

Modeling and simulation / Image analysis

Post-doctoral fellows

Sandipan Chattaraj

Polymer physics

Saranya Vasudevan

Molecular dynamics

PhD students

Bohdana Horda

Materials and manufacturing for bioengineering

Eloisa Torchia

Mechanobiology in engineered cell culture platforms

Melissa Pezzotti

Advanced optical methods in tissue engineering

Agenda: New tools

1. New Cells
2. New Cells
3. ECM-Cell interactions

FUCCIplex: A multiplexable cell cycle sensor for imaging-based phenotyping

What do you need to make a cardiac tissue model?

Cardiac (muscle) cells and their extracellular matrix (ECM)

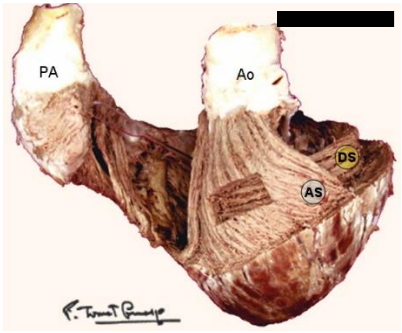
Contractile structure in the heart:

- Laminar tissues
- Aligned myofibrils
- Striated muscle cells

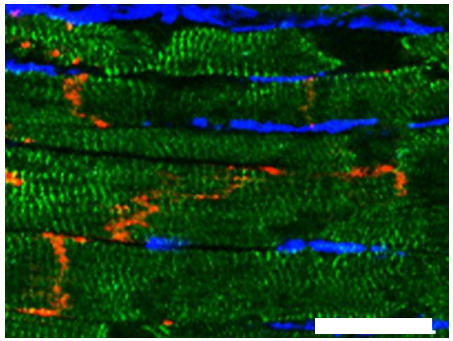
ECM Multiscale organization:

- Multi-chambered organ
- Anisotropic 2D distribution
- Single-cell constraints

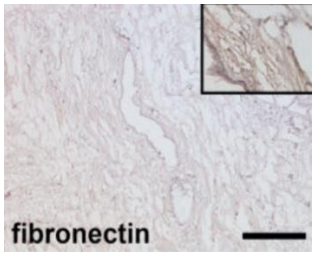
α-actinin, β-catenin, fibro

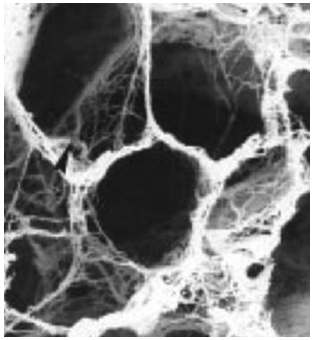


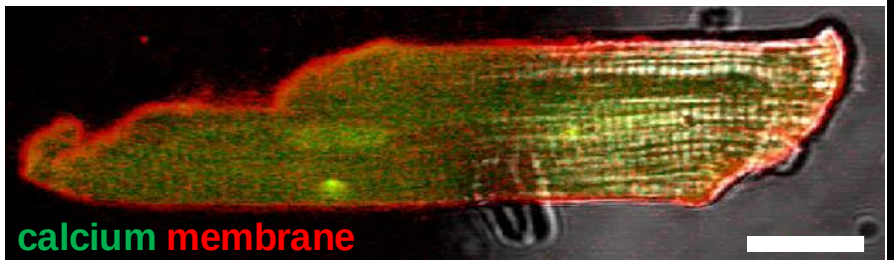
Scale: ~10 cm



Scale: ~250 um







Scale: ~25 um

Scale: 10 um

Sources: (Left) Camelliti, Card Res, 2005. Courtesy: Dr. Gosain and Brey; (Right) Ott, Taylor@UMn, 2008, Guyette, Ott@MGH, Circ Res, 2016, Spinale@MUSC, Phy Rev, 2007

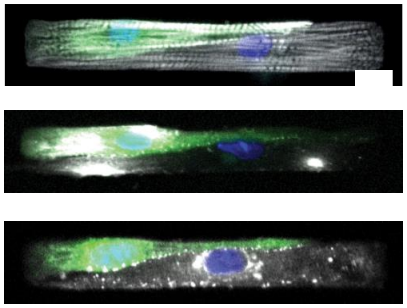
FUCCIplex: A cell cycle sensor that can be multiplexed with existing GFP/RFP-based sensors for cell structure/function

PROBLEM

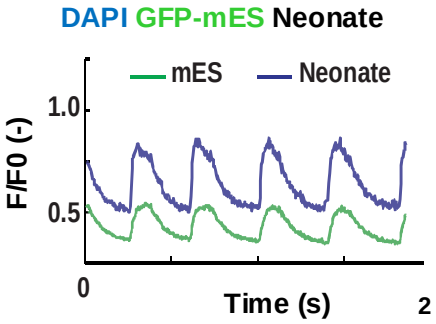
SOLUTION

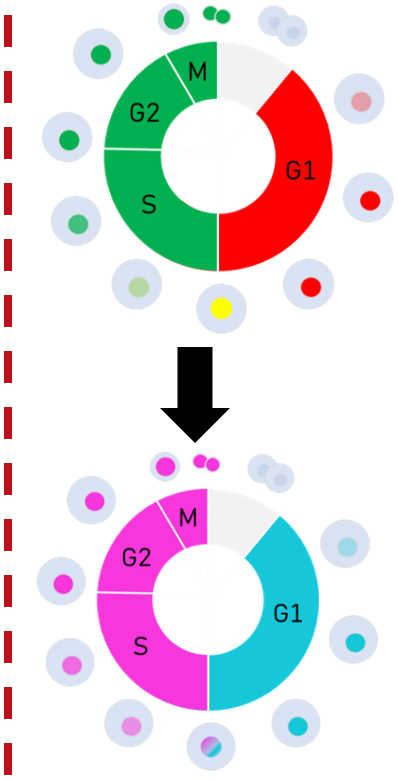
Physiological signals in neonate and mES-derived cardiomyocytes using

- **FURA-RED**
- **GFP**



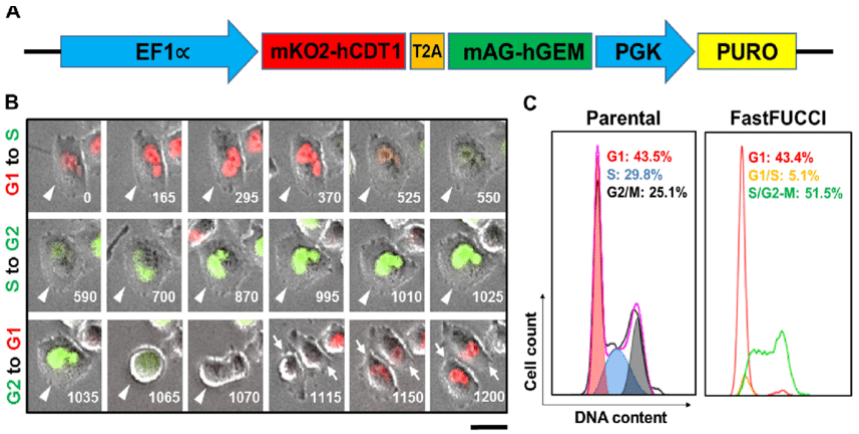
Scale: 10 um





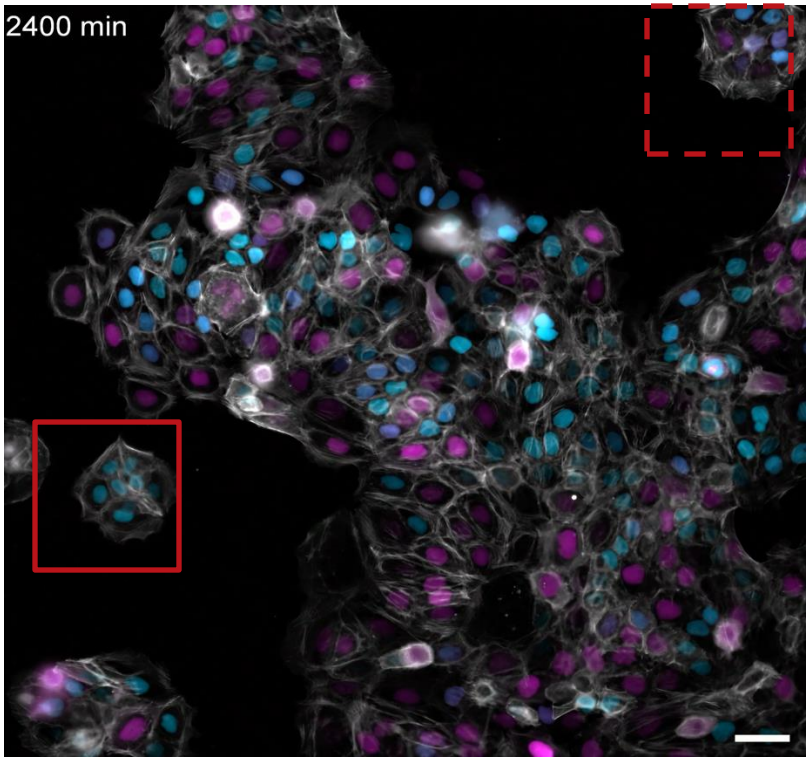
Tracking of cell cycle in pancreatic cancer cells using

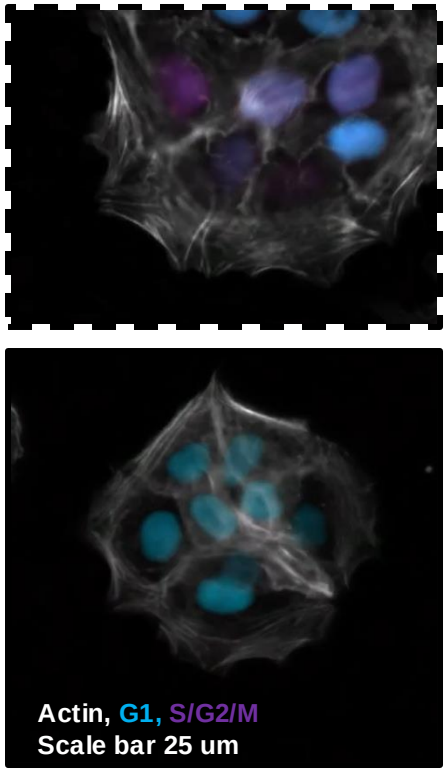
- **RFP-cdt1**
- **GFP-geminin**

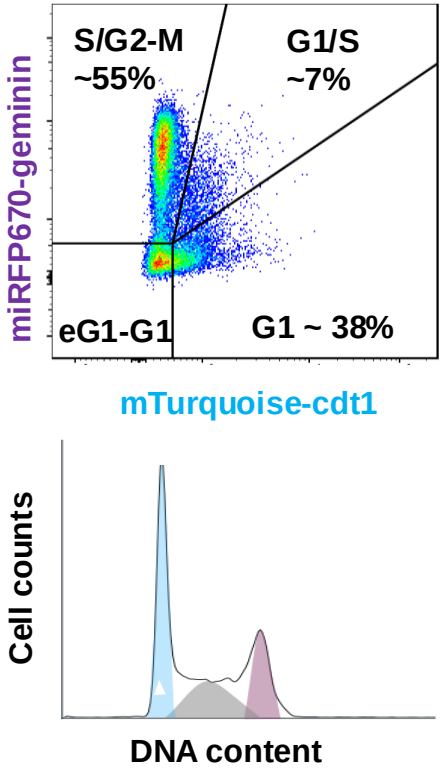


Sources: Pasqualini, JCB 2016 (top) and Koh et al, JCS 2017 (bottom)

FUCCIplex is a good cell cycle sensor in HaCaT cells also expressing RFP-LifeAct

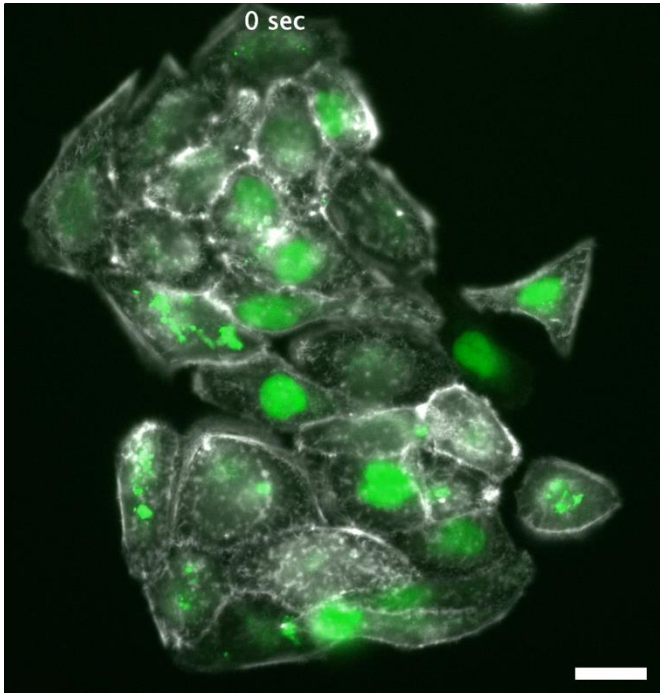


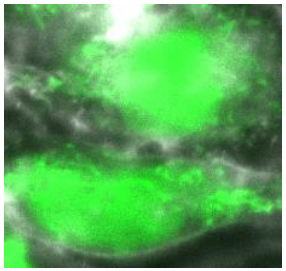


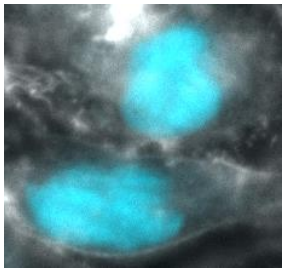


Further multiplexing with GFP-based calcium sensors (Fluo-4) suggests cell cycle-dependent ATP-response

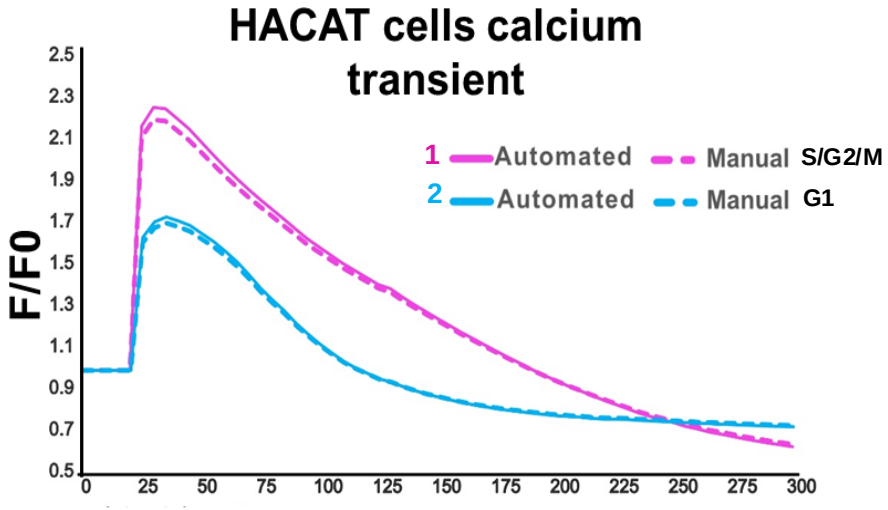
Raw data

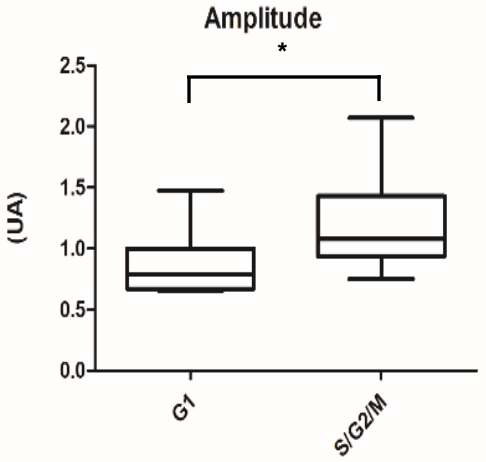




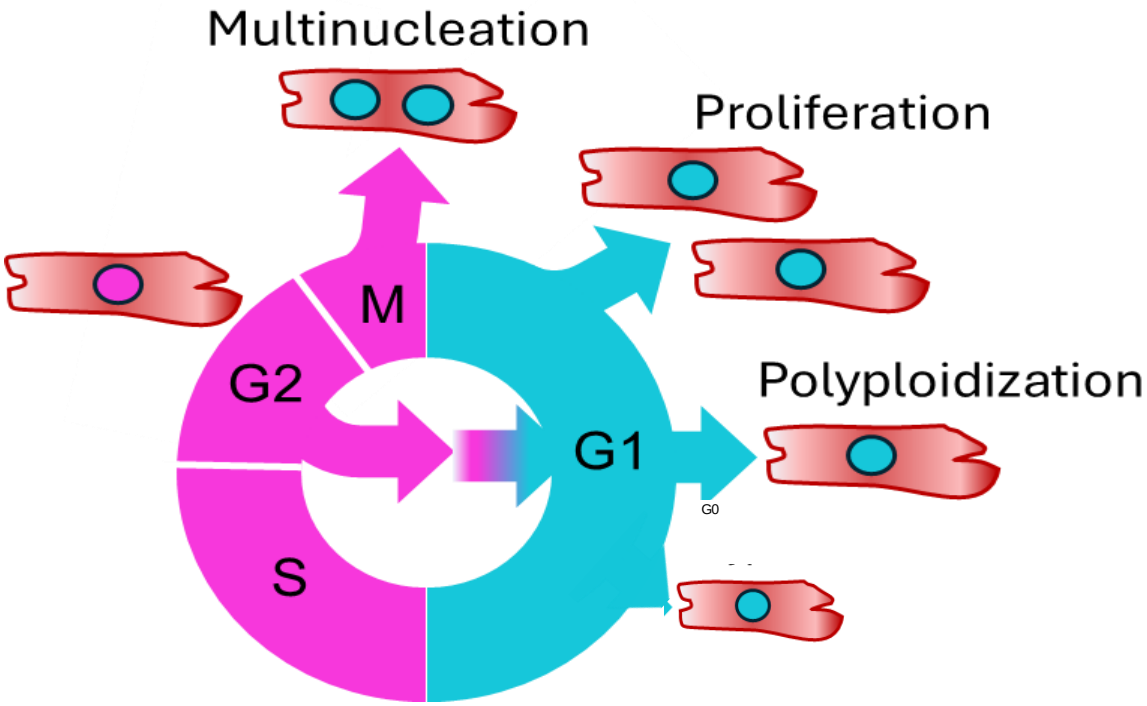


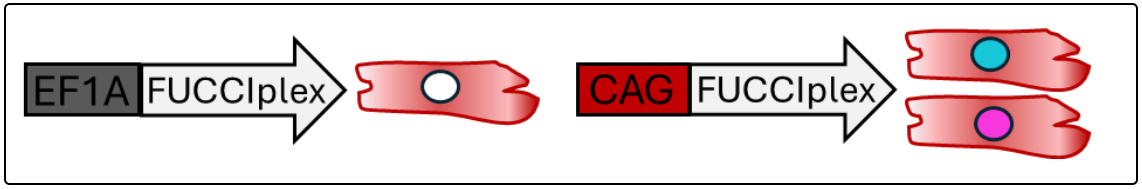
Fluo-4 calcium sensitive dye





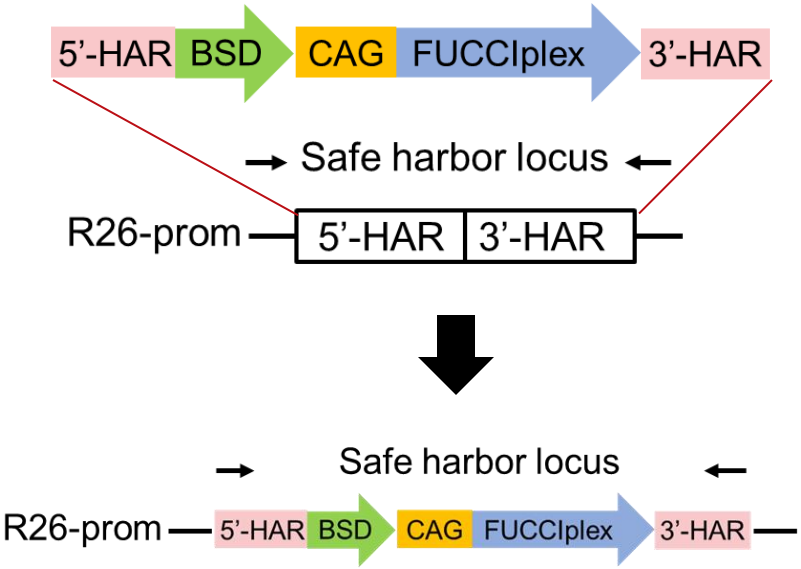
FUCCIplex in hiPSC and hiPSC-derived cardiomyocytes: strategies for drug testing or regenerative medicine

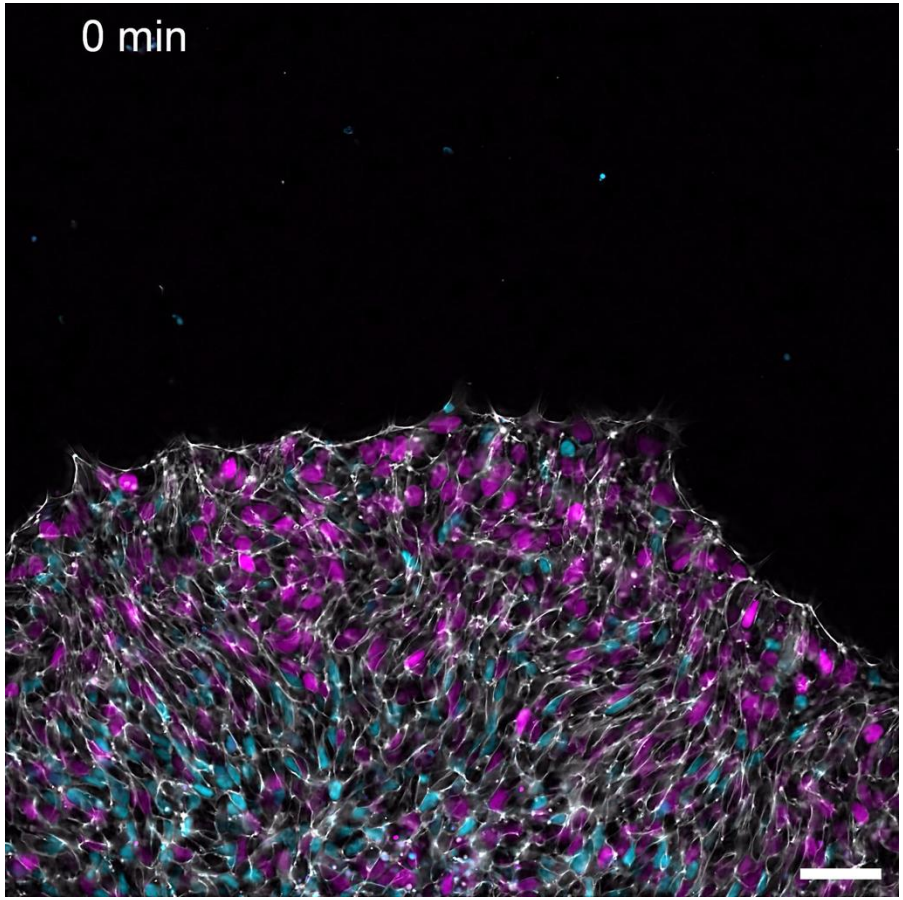




Source: [left https://link.springer.com/article/10.1007/s11886-022-01682-9

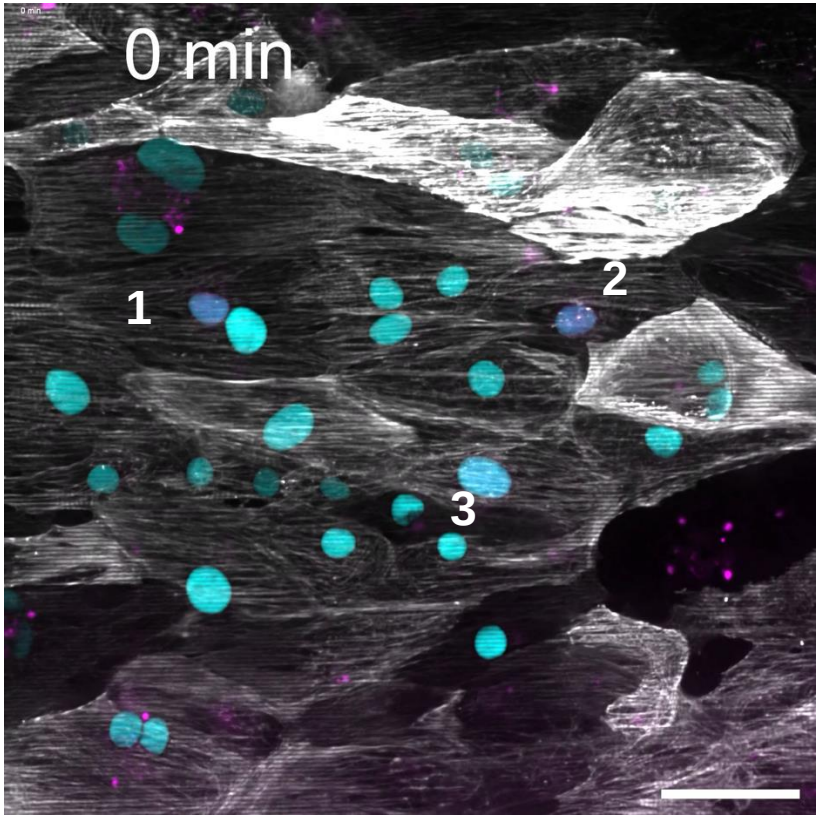
CAG-FUCCIplex expression from human Rosa26 to have a 4-color reporter line for all the tests

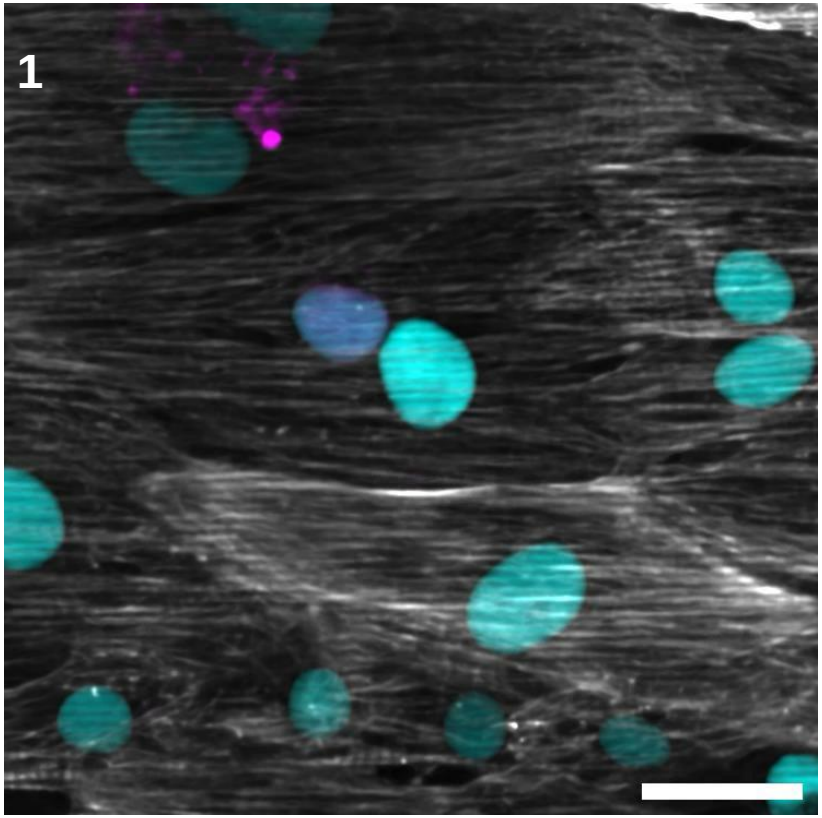




Actin, G1, S/G2/M Scale 25 um

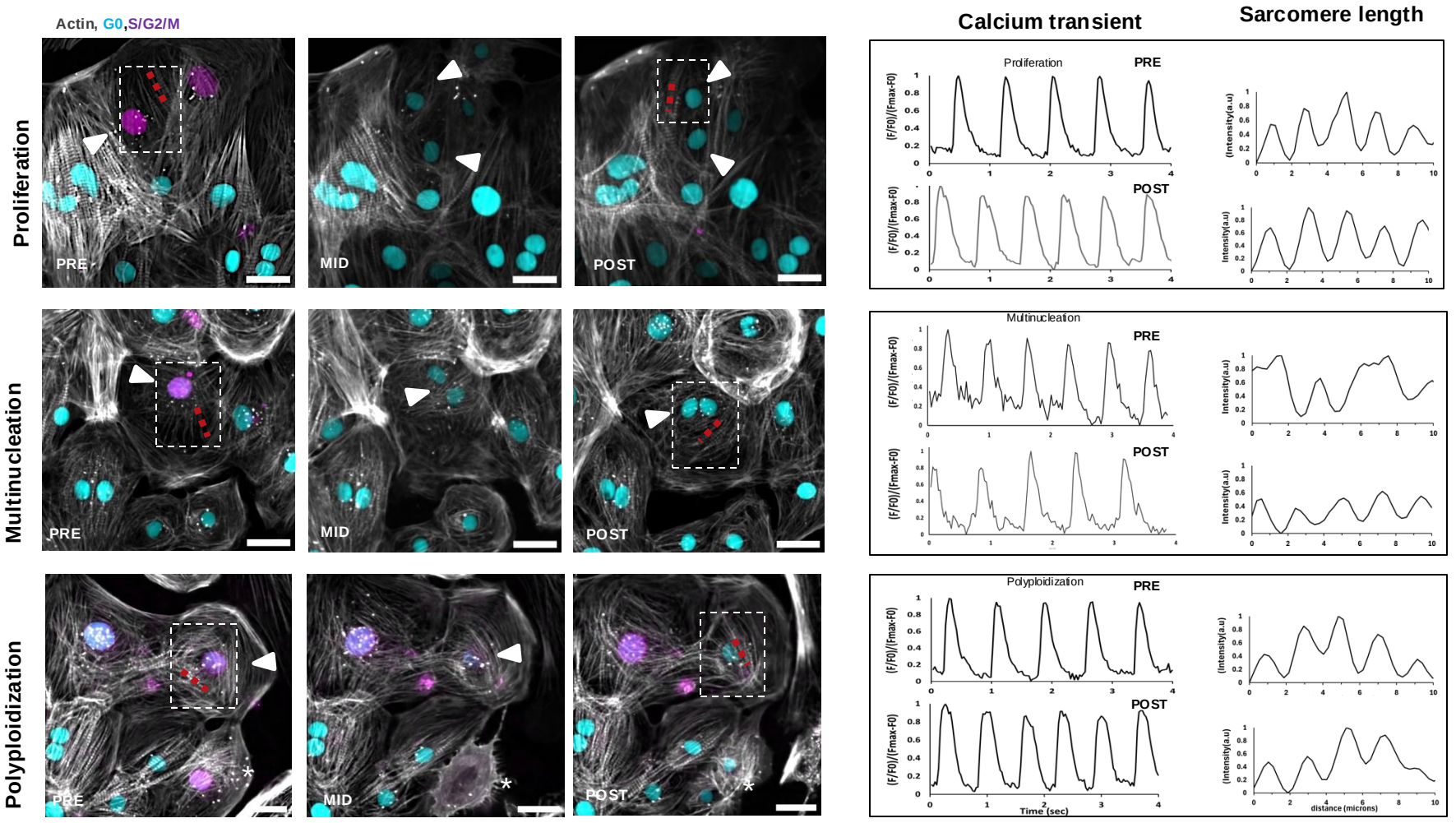
Regenerative medicine: hRosa26-CAG-FUCCIplex enables imaging of hiPSC-CM cell cycle re-entry with Agrin treatment



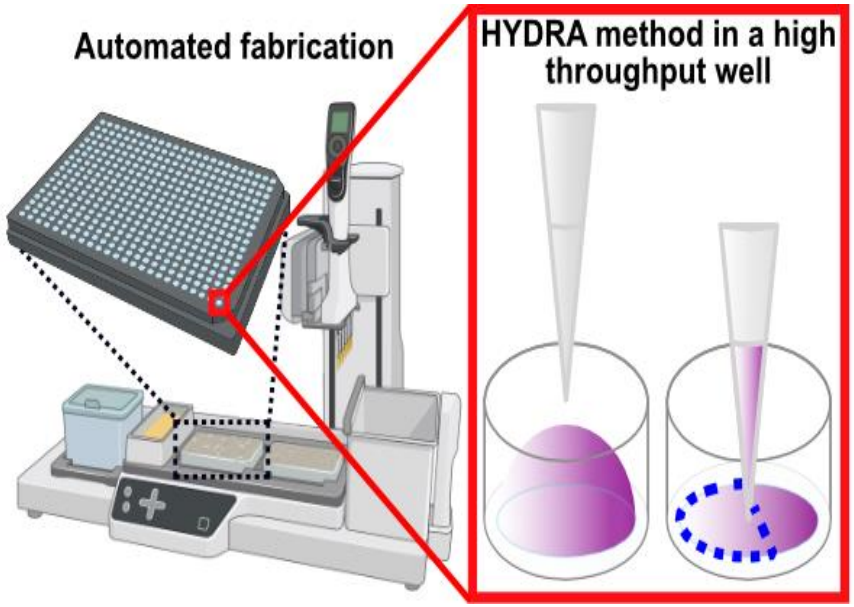


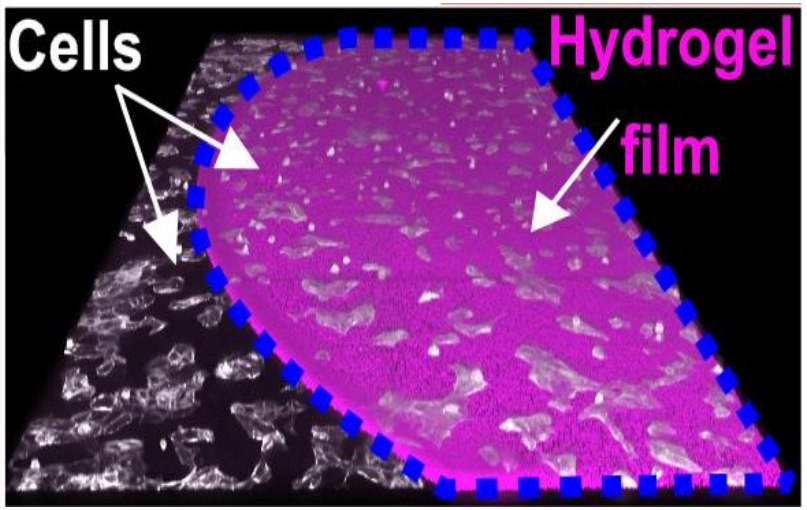
Actin, G1, S/G2/M Scale 100 and 25 um

Regenerative medicine: We can look at calcium transients and sarcomere structures before and after cell cycle events



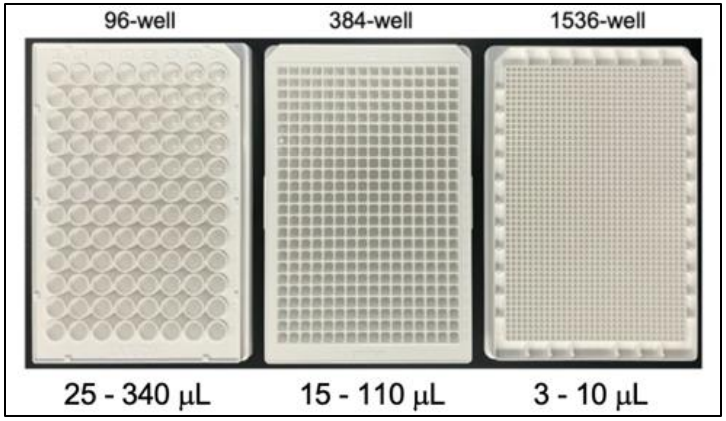
HYDRA: HYdrogel Dispensing with Robotic Automation



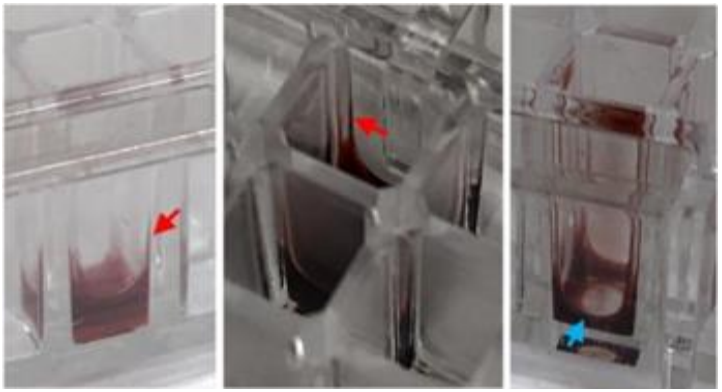


We develop HYDRA (HYdrogel Dispensing with Robotic Automation) to automate the fabrication of thin hydrogels in multi-well plates.

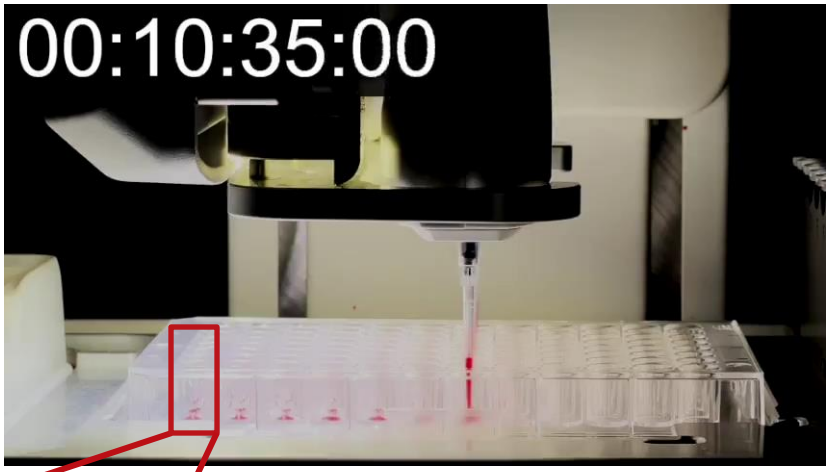
Working volumes in HT plates



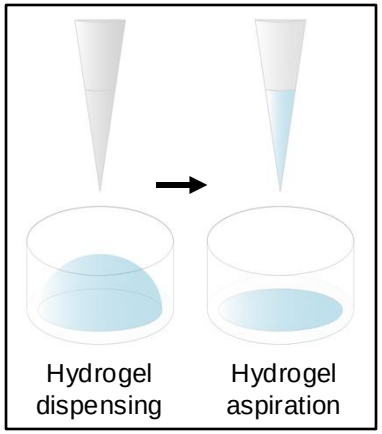
Meniscus effect in HT wells



HYDRA method in a TC 96-well plate

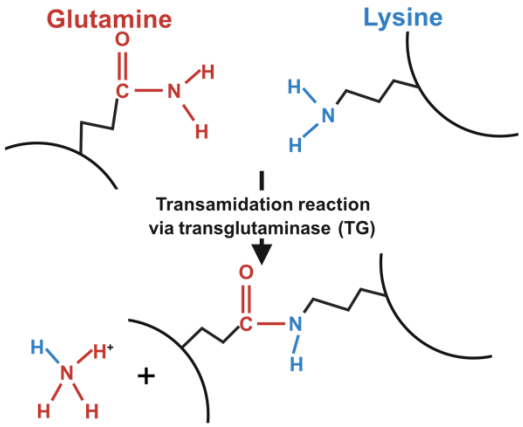


Zoom on a single well

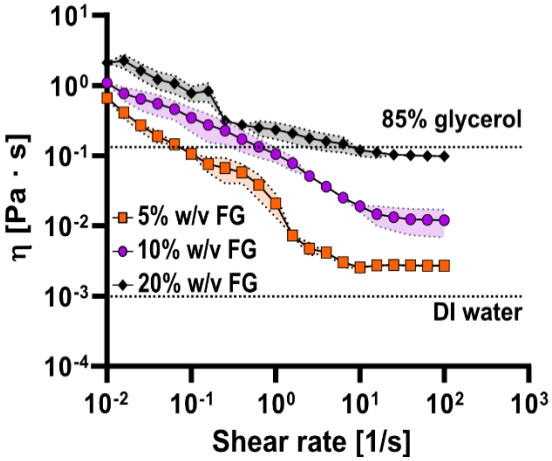


Feasibility – We characterized fish gelatin viscosity and stiffness by chemically crosslinking it with transglutaminase (TG)

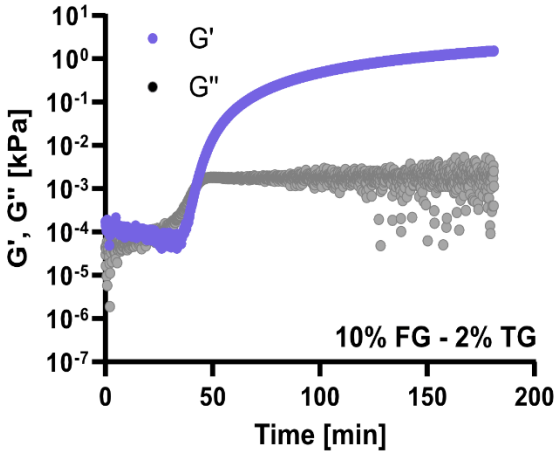
Chemical crosslinking schematic



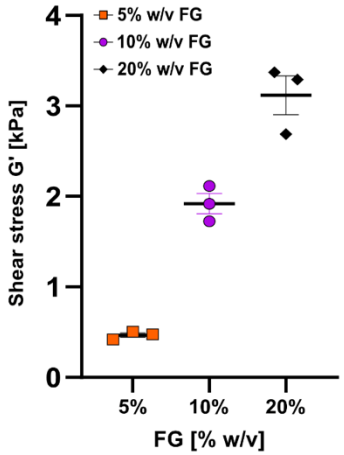
Fish gelatin viscosity

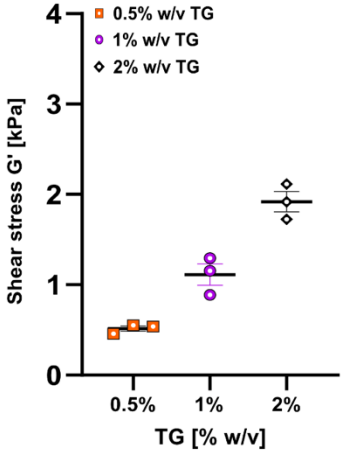


Crosslinking kinetic



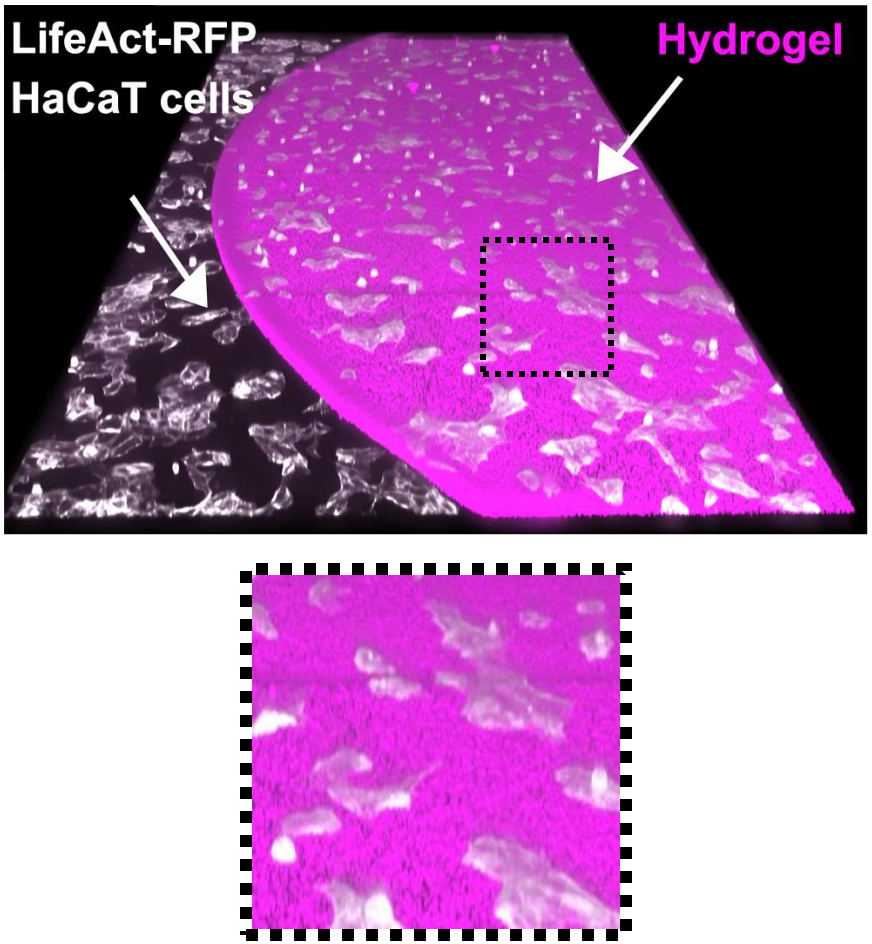
Fish gelatin stiffness by varying gelatin or TG concentration



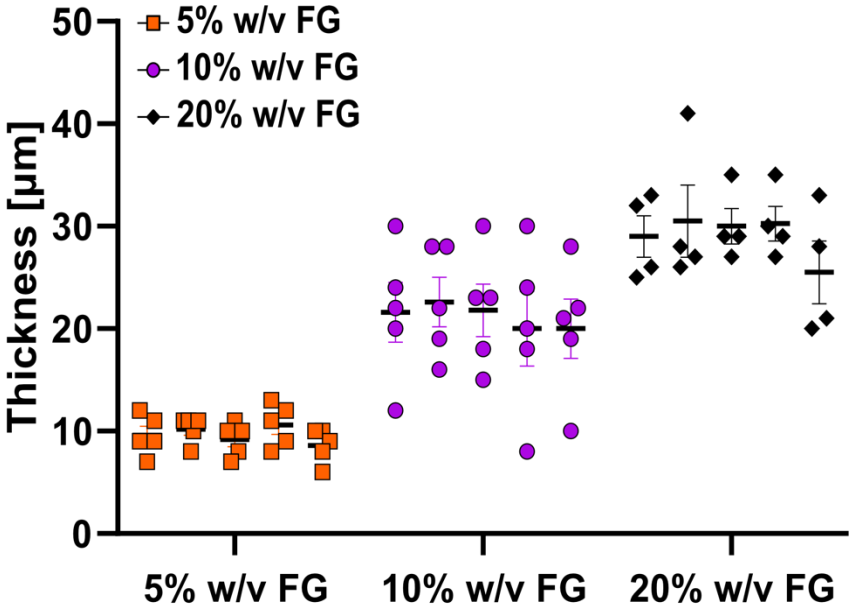


Feasibility – We characterized fish gelatin hydrogel thickness and flatness using confocal imaging.

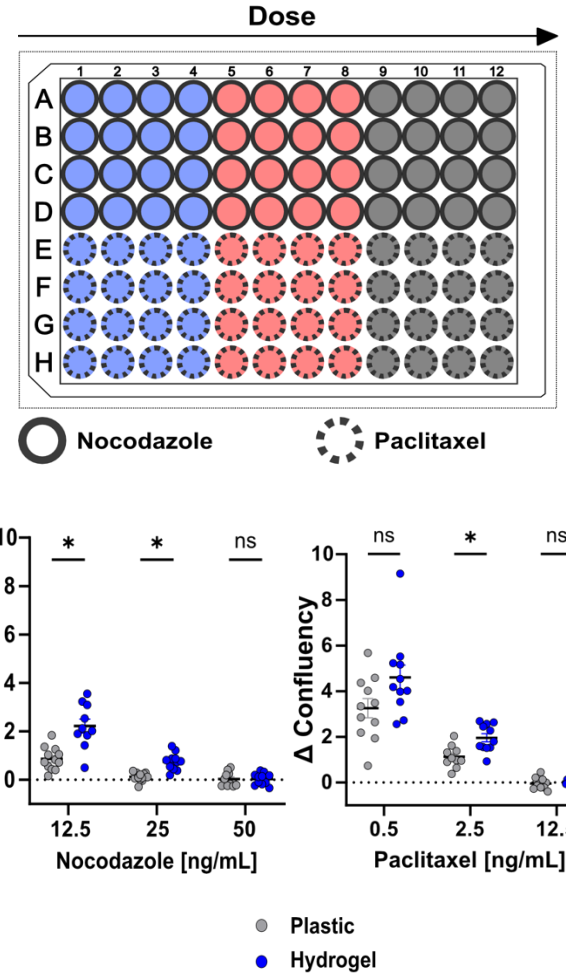
3D rendering of HYDRA-like hydrogels embedding fluorescent beads

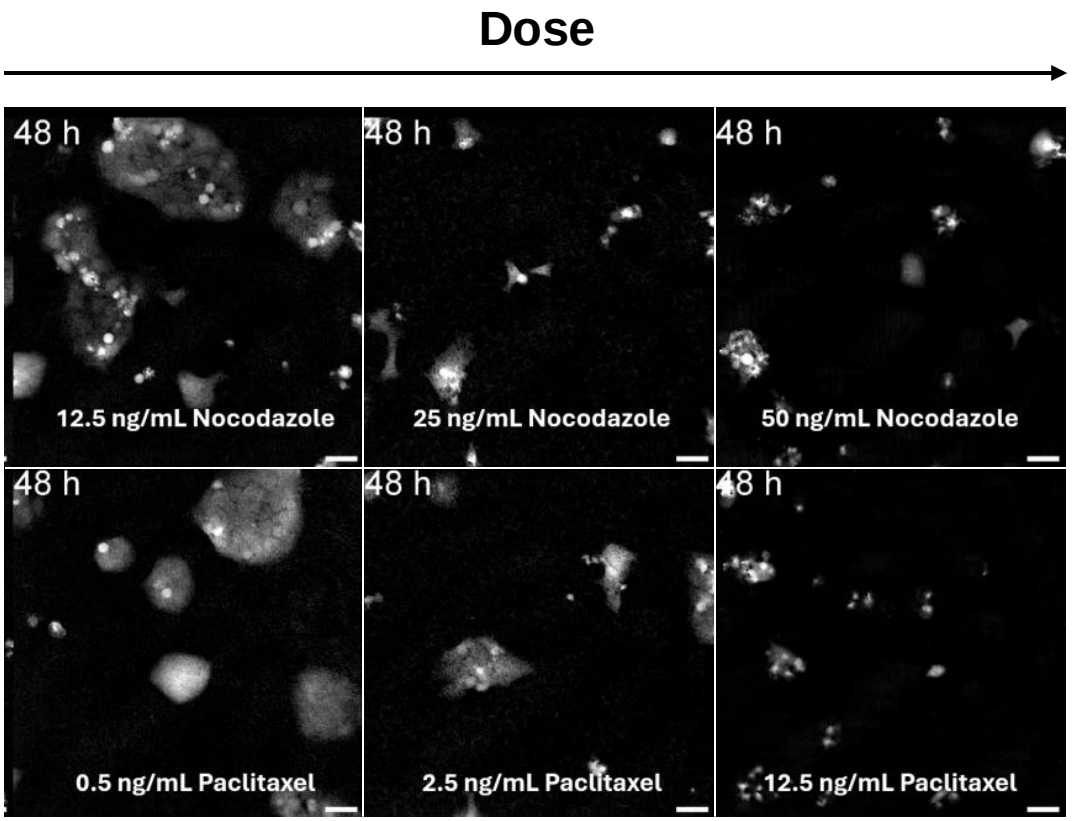


Thickness measurements by varying gelatin concentrations



Quantitative Phase Imaging compatibility – We demonstrate HYDRA HTS plate can be used for imaging-based screening (drugs).



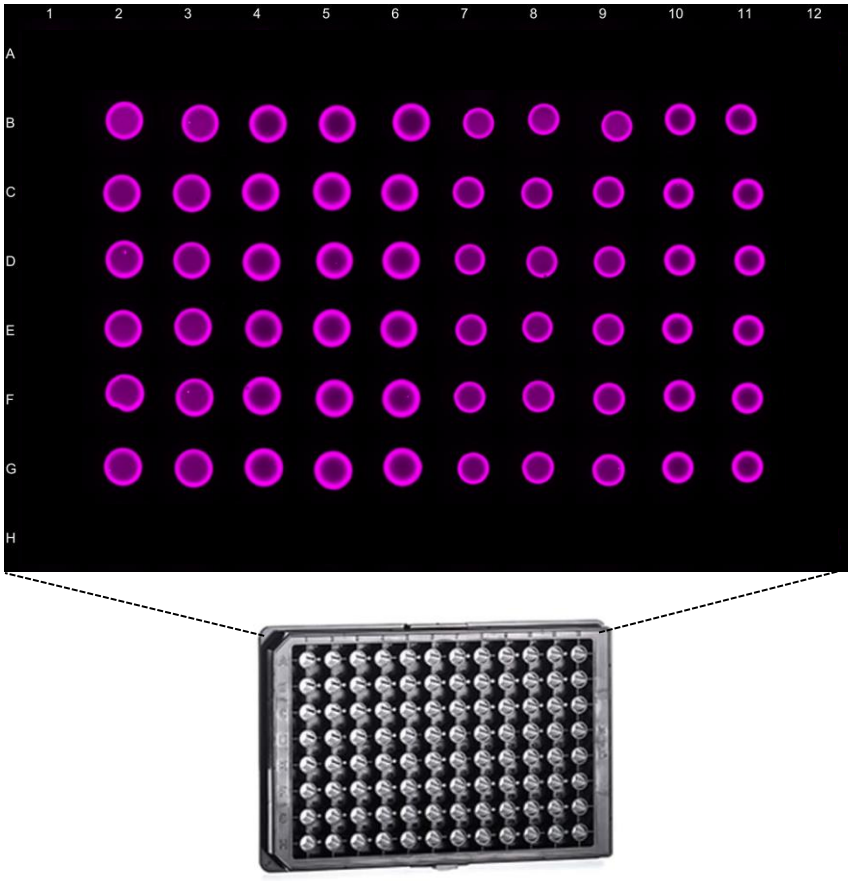


Holographic 48 hours imaging of HaCaT cells on hydrogel thin films

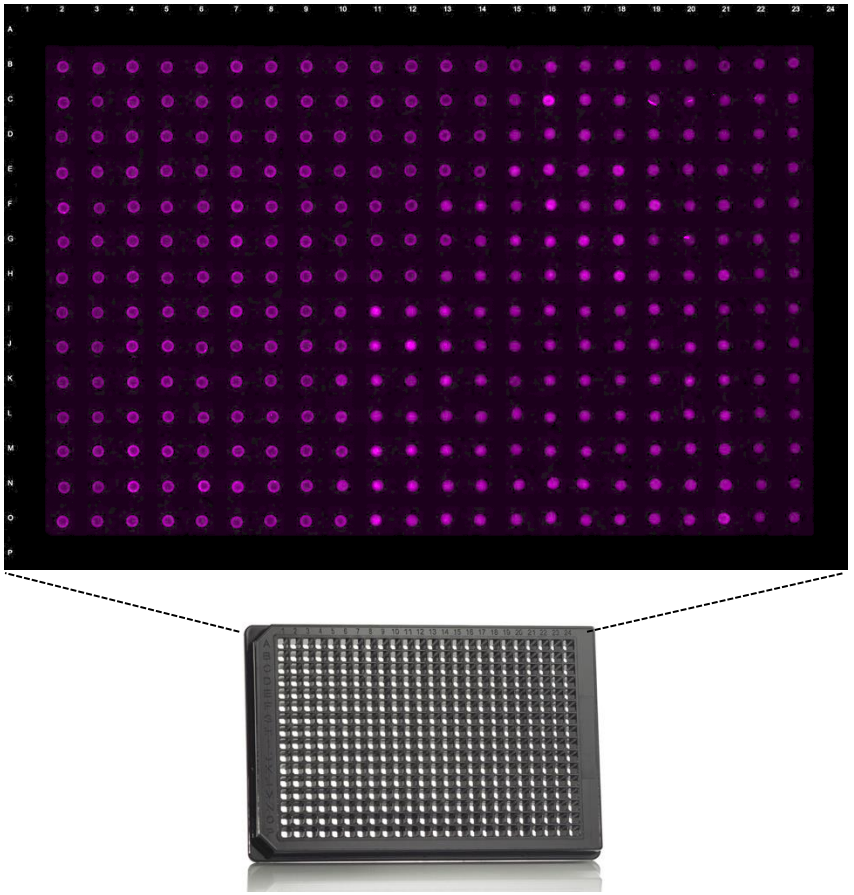
Scale bars: 50 µm

Scalability - We move from a 96- to a 384-well plate scaling hydrogel volumes.

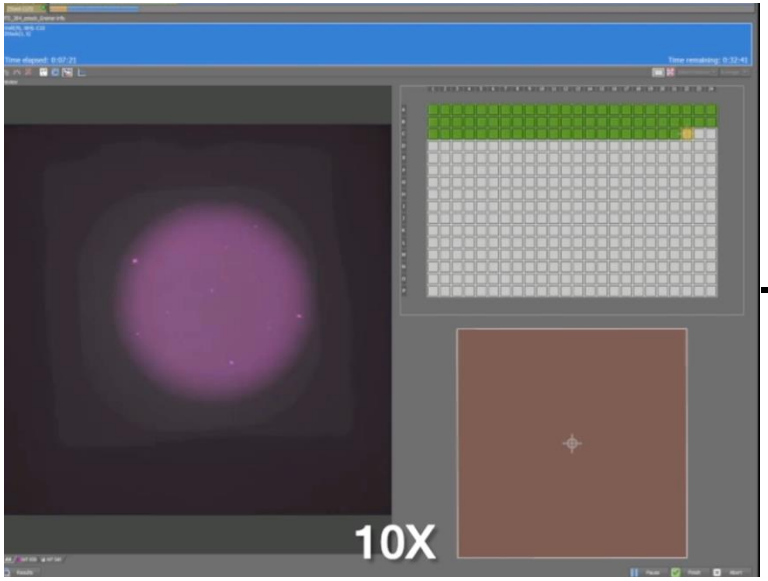
96-well plate (12 uL)



384-well plate (1 uL)

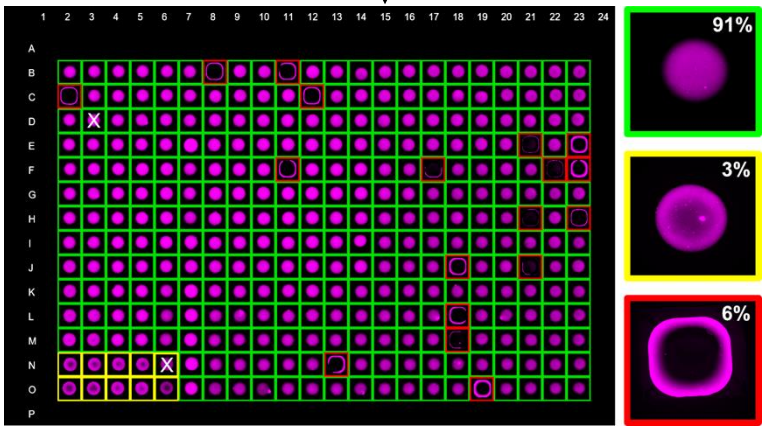


Scalability - We move from a 96- to a 384-well plate scaling hydrogel volumes.



Automated fluorescence imaging of hydrogel w/ beads

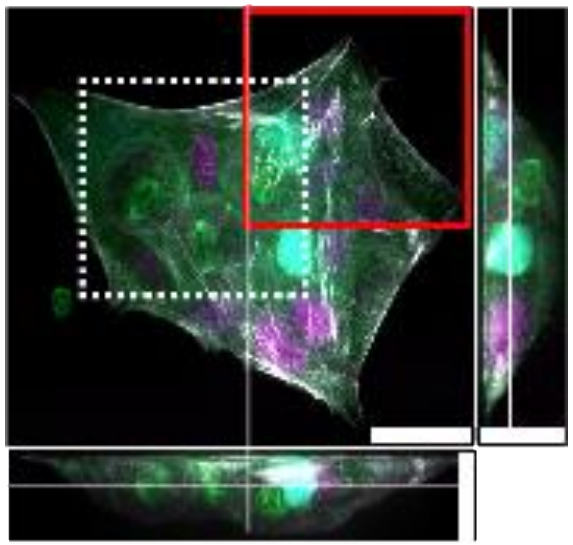
Quality control single-gel analysis

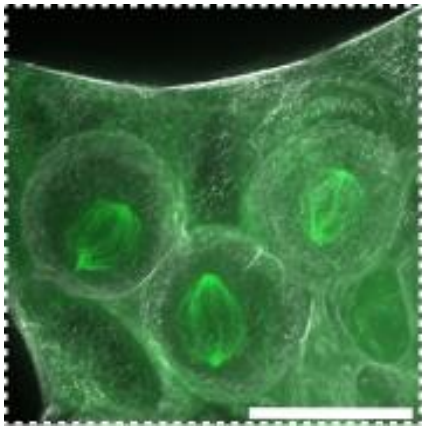


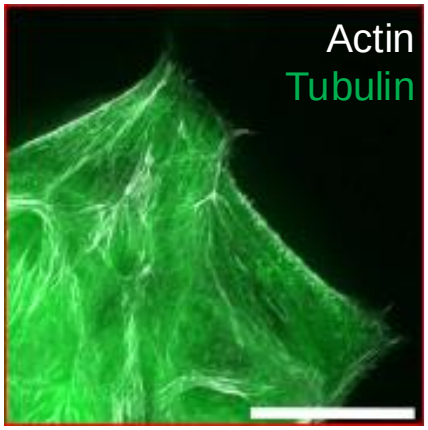
HYDRA 384-well plates can be used in advanced fluorescence microscopy applications.

18-hour HaCaT cell proliferation experiment

High-resolution confocal imaging







Scale bars: 25 µm

Summary

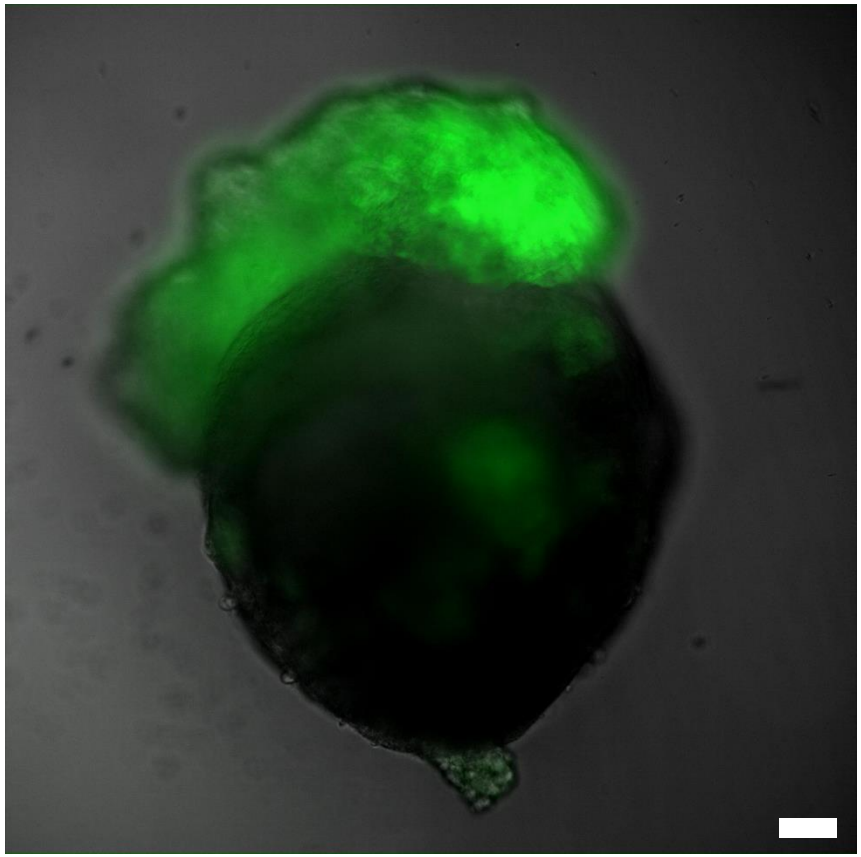
1. **HYDRA: High-throughput (really!!) engineered cell culture platforms to study ECM-cell interactions (pre-print out by the end of the month)**

2. **TEMPO: a suite of genetically encoded suite of fluorescent sensors for in hiPSCs (pre-print out by the end of the month)**

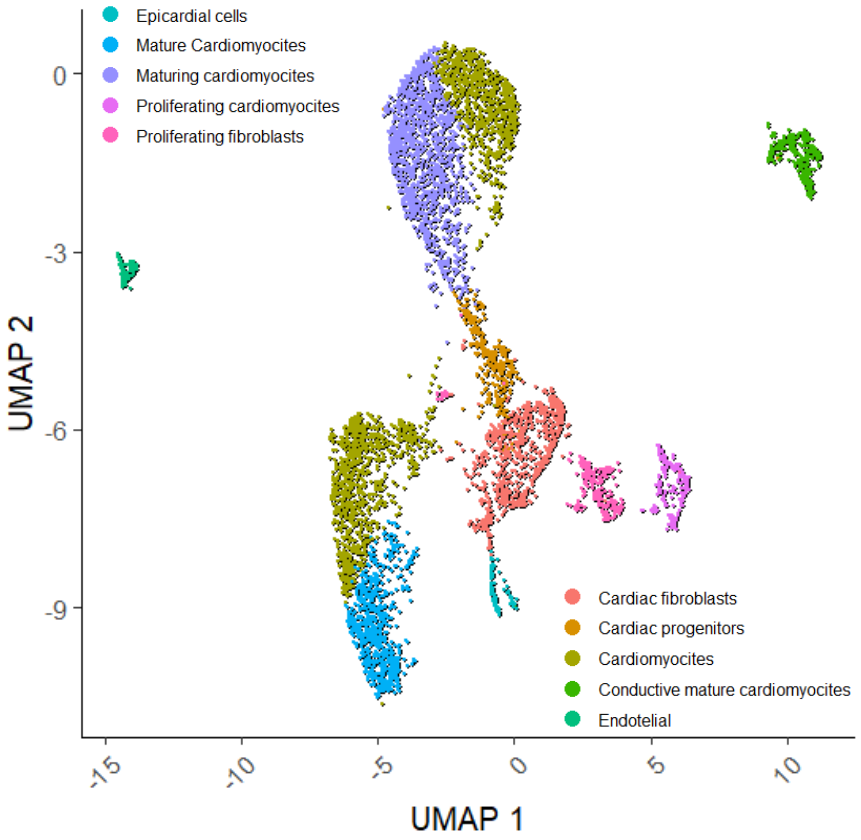
But what about cell-ECM interactions?!

We can use FUCCIplex hiPSC to produce cardiac organoids or cardioids: in-vitro models of cardiac morphogenesis

hiPSC-derived cardiac organoid (live)

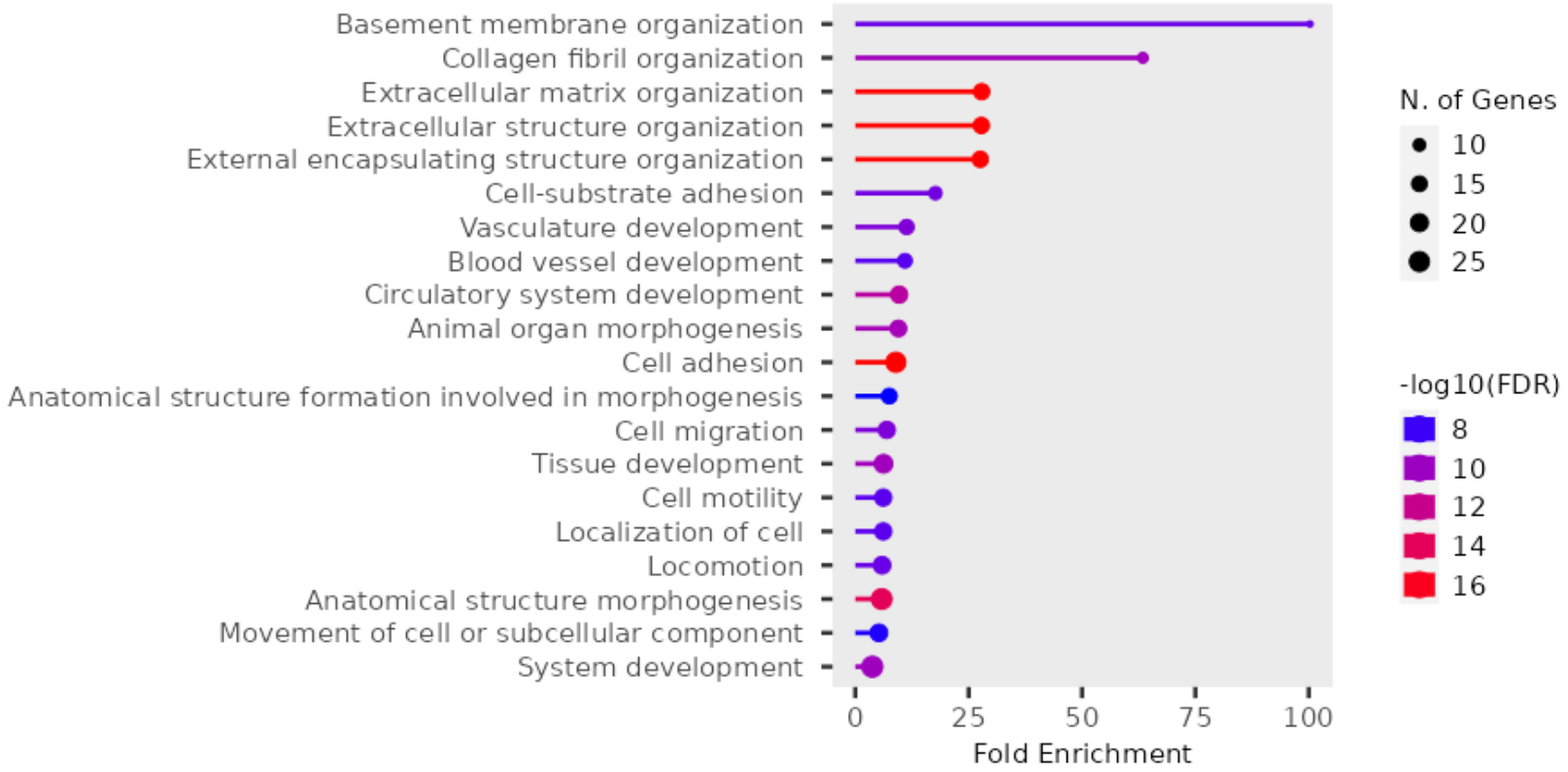


Scale: 100 um



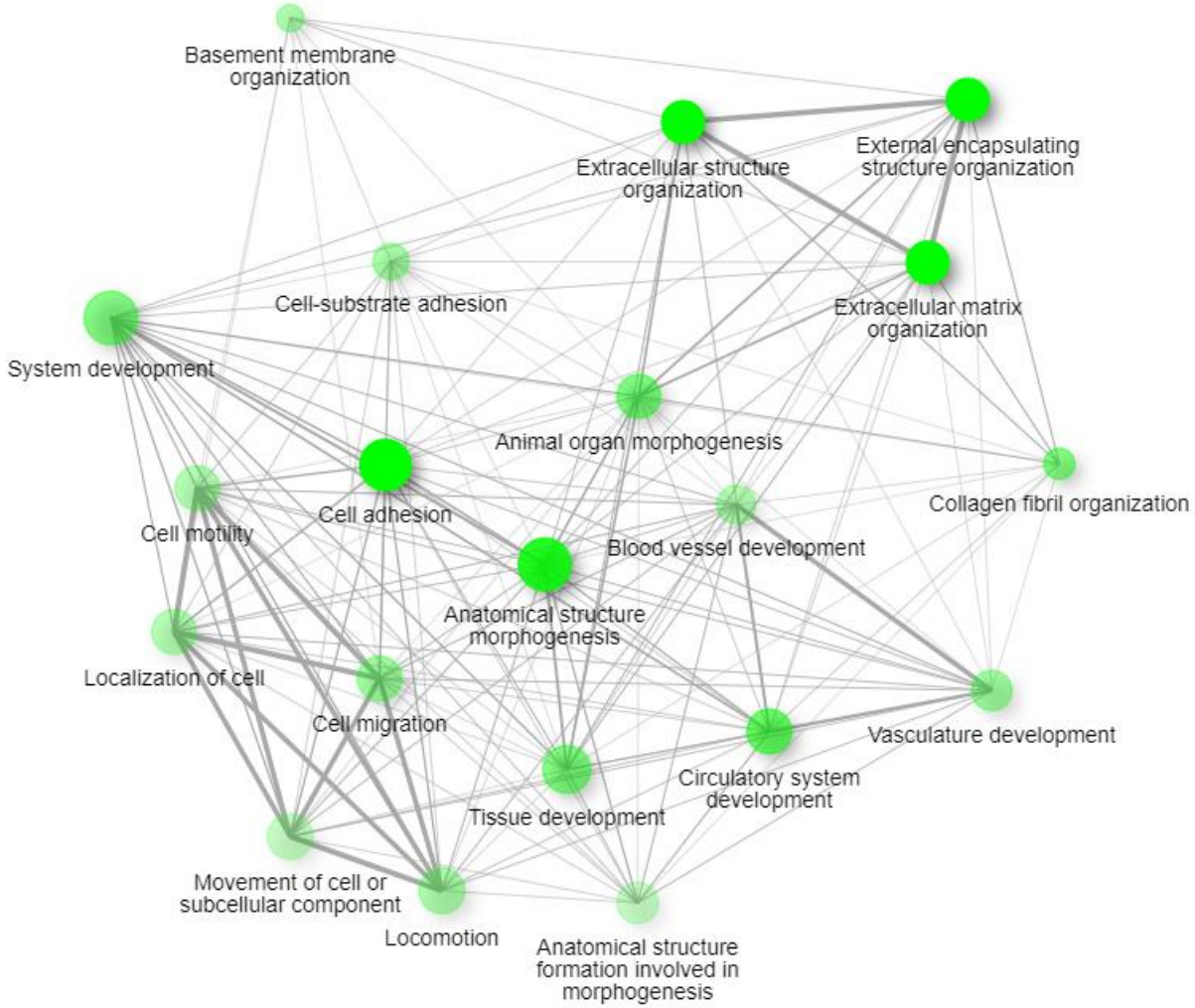
With Bertero's lab @ UniTO

We can further mine the dataset with the Matrisome database: by gene ontology ECM-associated genes participate in myocardial morphogenesis



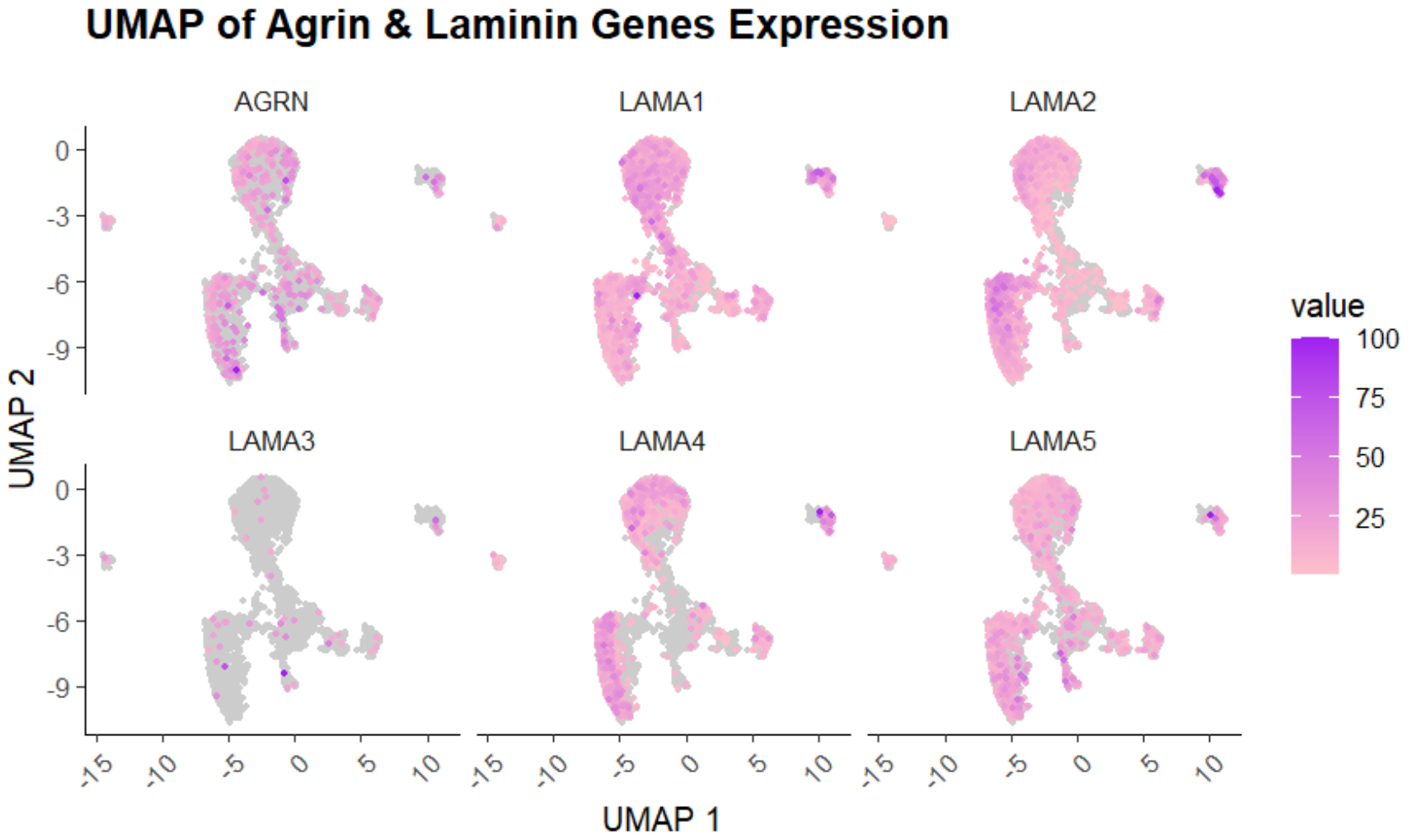
Analysis with shinyGO 0.8 (http://bioinformatics.sdstate.edu/go/)

Interaction network analysis further supports an integrative role of the ECM in the biological processes linked with cardiac development.

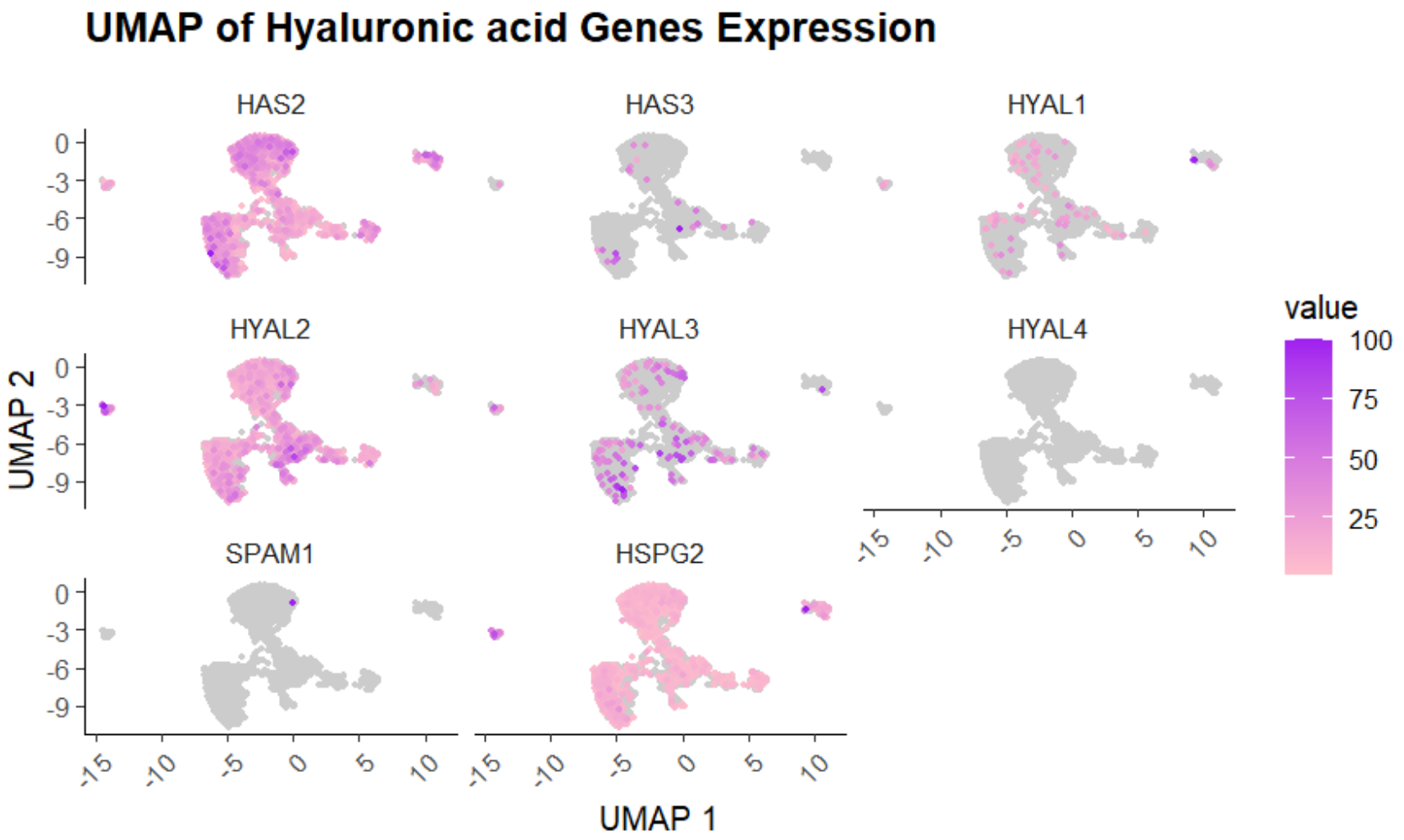


Analysis with shinyGO 0.8 (http://bioinformatics.sdstate.edu/go/)

Agrin and laminin genes are differentially expressed across cell types



Hyaluronic acid genes are differentially expressed across cell types



Thinking Outside the Cell: Let's make a Functionally Annotated ECM Atlas!