Human-on-a-Chip combined with PBPK modeling for *in vitro/in vivo* PK/PD extrapolation
Part II: PBPK model

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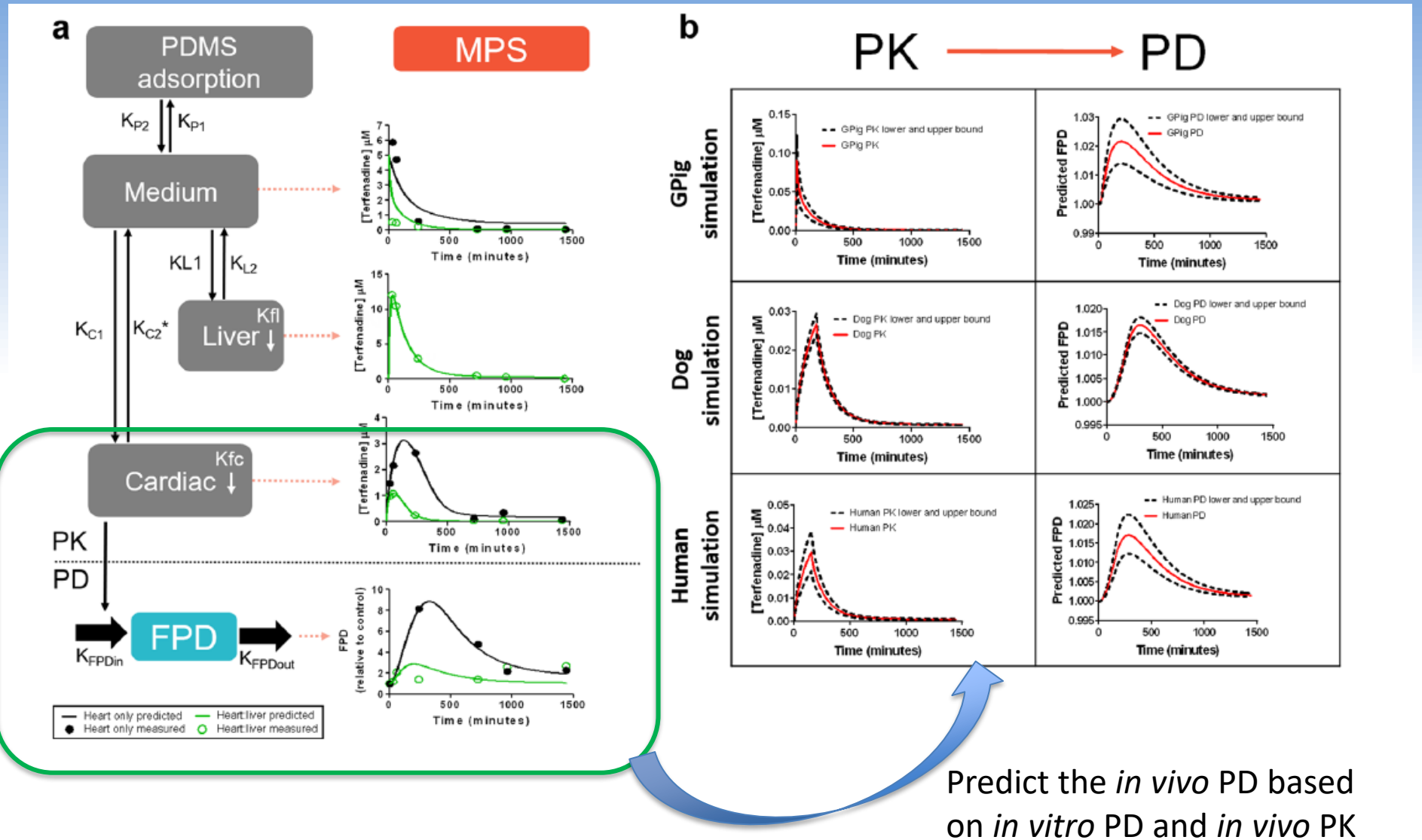
Compartmental HoaC Model



Predict the *in vivo* PD based on *in vitro* PD and *in vivo* PK

McAleer et al – Scientific Reports 2019, 9:9619

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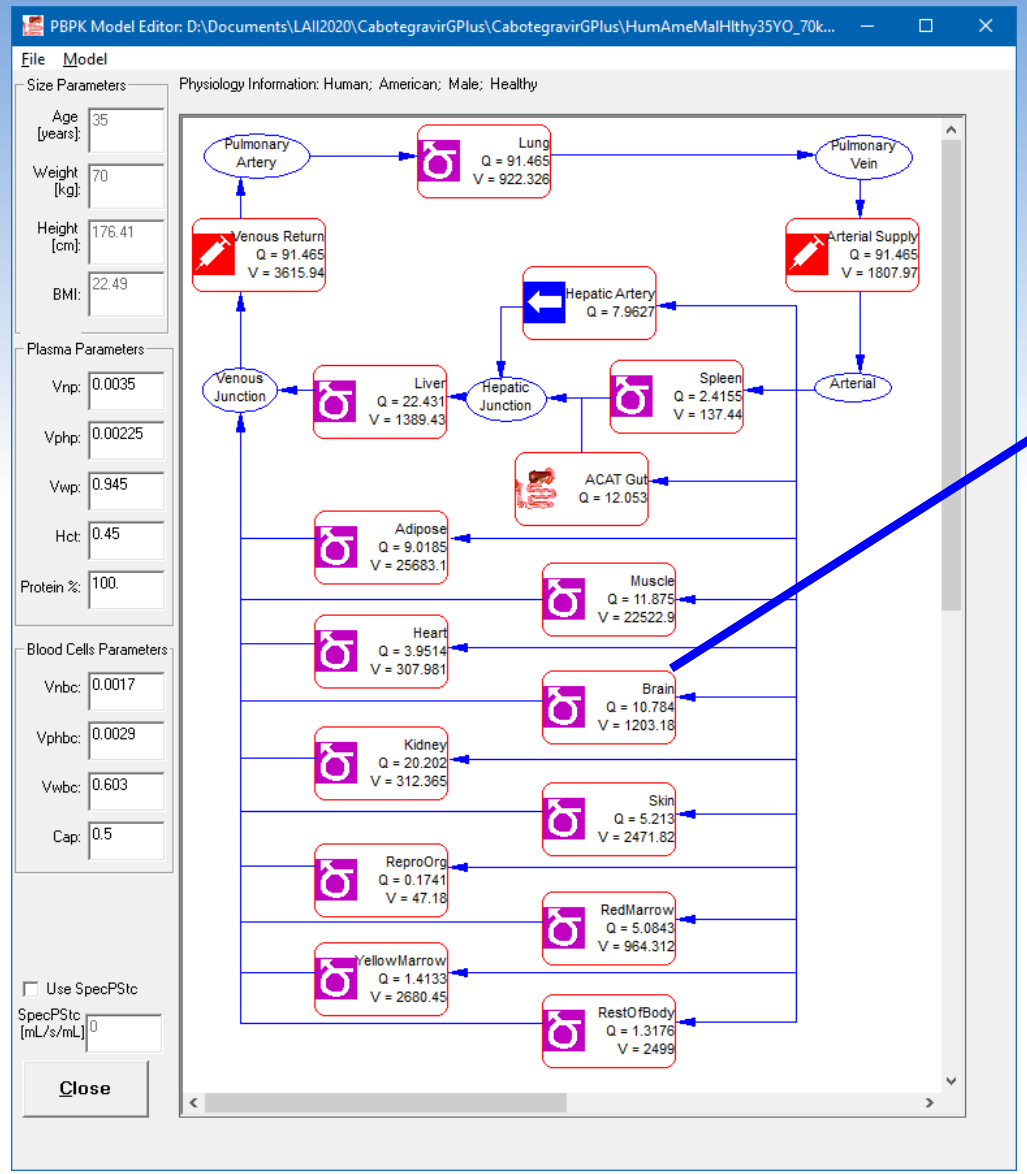
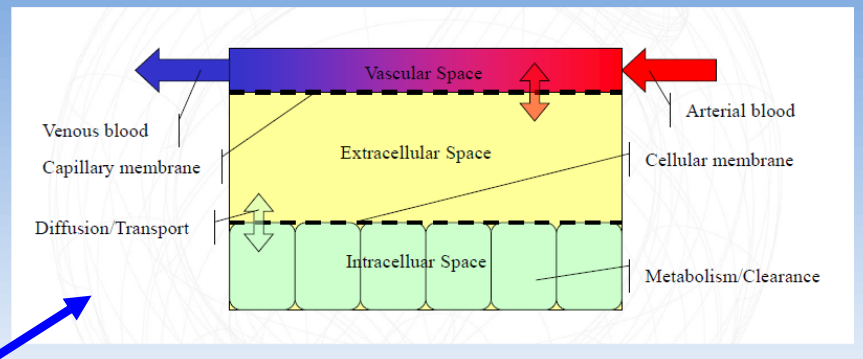
What is defined in a PBPK Model?





$$Vt\frac{dCt}{dt} = \left(Q \times Cbi - \frac{Q \times Ct \times R_{bp}}{Kp} - CL_{int,u}\left(\frac{Ct \times fu_p}{Kp}\right)\right)$$

- Each compartment represents a tissue:
  - Specific volume(s) *
  - Blood perfusion rate *
  - Enzyme/transporter expression levels *
  - Volume fractions of lipids & proteins *
  - Tissue: plasma partition coefficient (Kp)
    - Estimated from drug properties:
      - logD vs. pH
      - pKa(s)
      - Plasma protein binding
      - Blood: plasma concentration ratio

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Perfusion-Limited Tissues

$$V_T \frac{dC_T}{dt} = Q_T\left(Cb_i - \frac{C_T R_{bp}}{K_p}\right) - CL_{\text{int},u}\frac{C_T f_{up}}{K_p} - v_{metab}(C_{Tu})$$

V_T : Tissue volume

C_T : Drug concentration in tissue

Q_T : Tissue blood perfusion rate

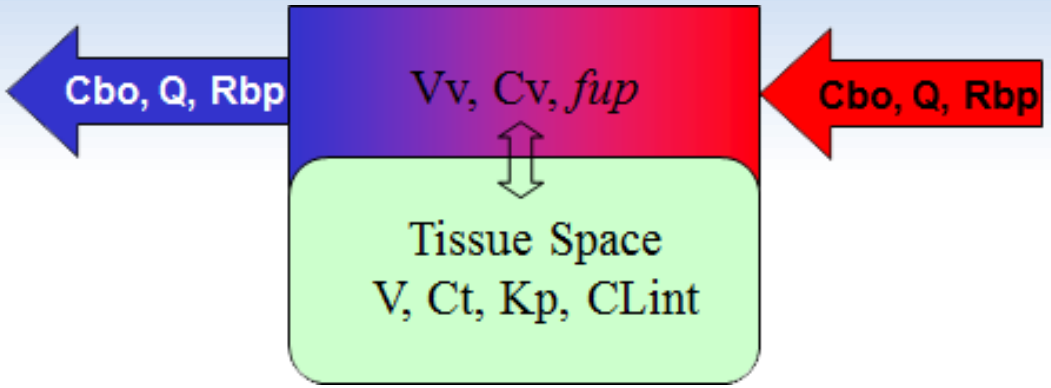
R_{bp} : Blood to plasma concentration ratio

K_p : Tissue to plasma partition coefficient

$CL_{\text{int},u}$: Unbound intrinsic clearance in tissue

v_{metab} : Metabolism rate in tissue - based on unbound intracell conc

f_{up} : Fraction unbound in plasma



Permeability-Limited Tissues

$$\left(V_{ect} + V_{vasc}\frac{R_{bp}}{K_p}\right)\frac{dC_{ect}}{dt} = Q_T\left(Cb_i - \frac{C_{ect}R_{bp}}{K_p}\right) - perm$$

$$perm = PS_{TC}(C_{ect,u} - C_{ict,u}) + v_{\text{influx}}(C_{ect,u}) - v_{\text{efflux}}(C_{ict,u})$$

$$V_{ict}\frac{dC_{ict}}{dt} = perm - v_{metab}(C_{ict,u}) - CL_{\text{int},u}C_{ict,u}$$

V_T : Tissue Volume

C_{ect} : Drug Concentration in extracellular space

C_{ict} : Drug Concentration in intracellular space

Cb_i : Drug Concentration in entering blood

Q_T : Tissue Perfusion

R_{bp} : Blood to Plasma Ratio

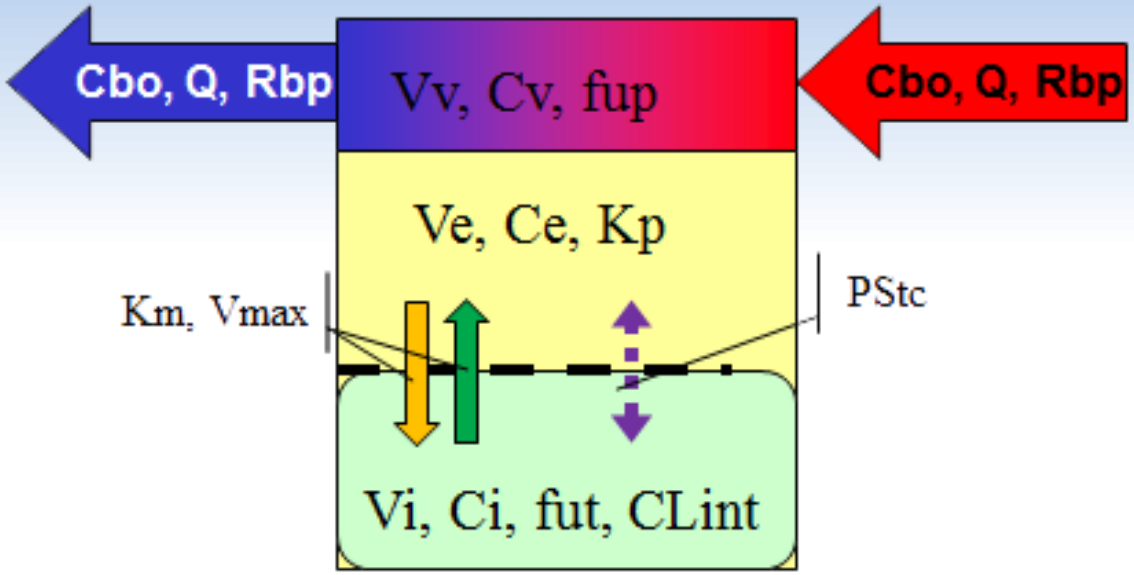
K_p : Tissue to Plasma Partition Coefficient

CL_{int} : Intrinsic Clearance in Tissue

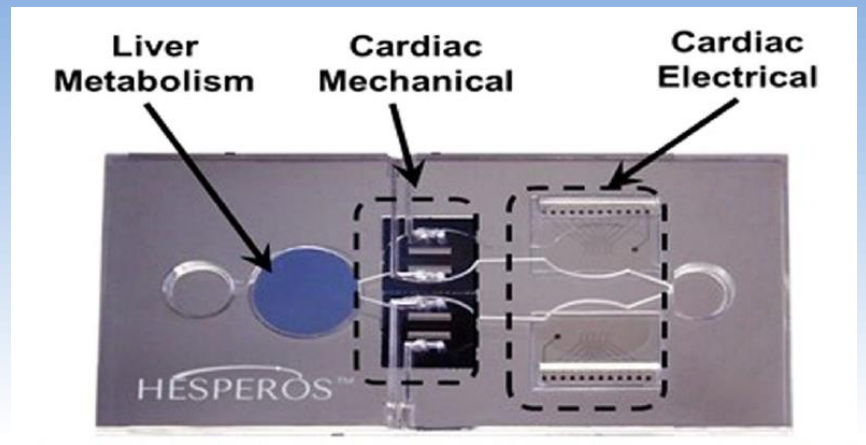
v_{metab} : Metabolism Rate in Tissue - based on unbound concentration

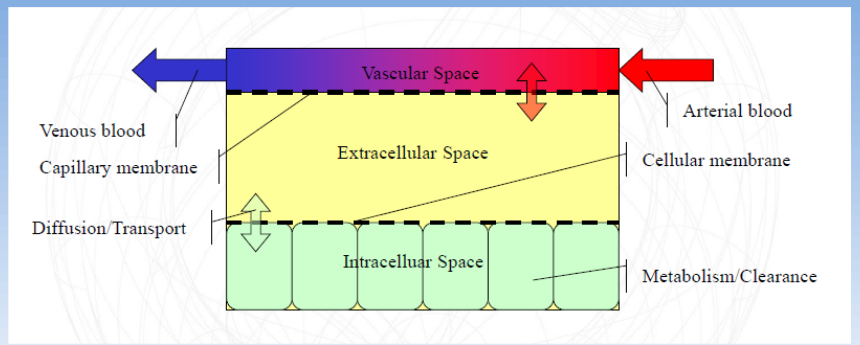
v_{trans} : Transport Rate - based on unbound concentration

PS_{TC} : Permeability-Surface Area Product

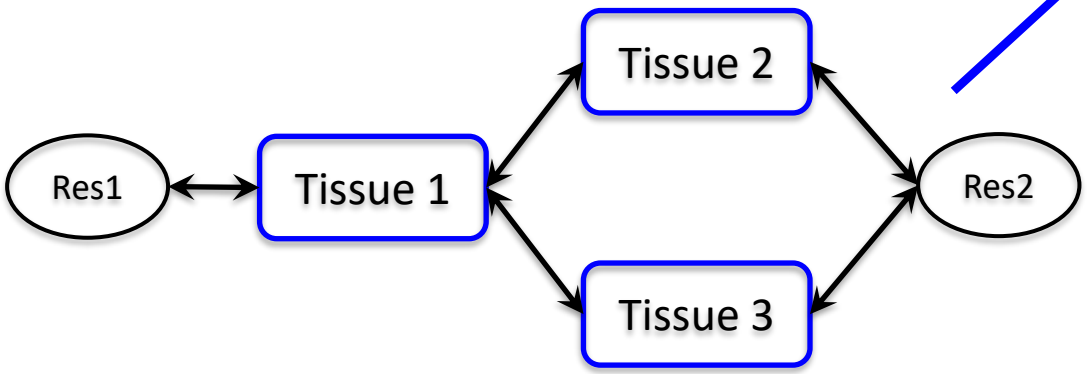


Mechanistic HoaC Model





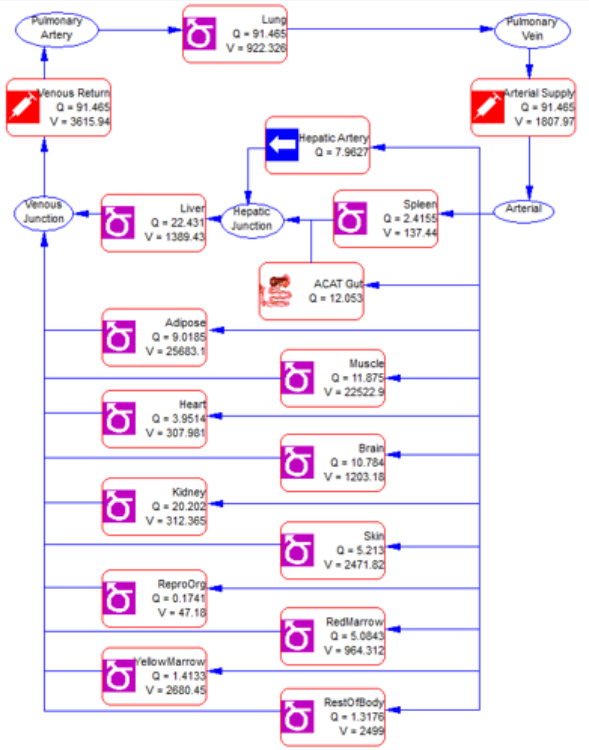
$$Vt\frac{dCt}{dt} = \left(Q \times Cbi - \frac{Q \times Ct \times R_{bp}}{Kp} - CL_{int,u}\left(\frac{Ct \times fu_p}{Kp}\right)\right)$$

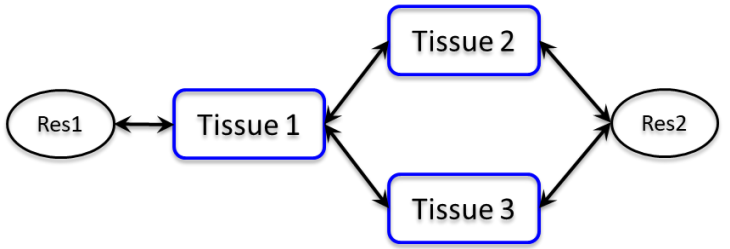


- Each compartment represents a tissue:
  - Specific volume(s)
  - Media Flow rate
  - Enzyme/transporter expression levels
    (*same as in vivo* – needs verification with standard substrate)
  - Volume fractions of lipids & proteins
    (*same as in vivo* – needs verification by predicting distribution of known compound)
  - Tissue: media partition coefficient (Kp_{media})
    - Dependent on drug properties:
      - logD vs. pH
      - pKa(s)
      - Plasma protein binding
      - Blood: plasma concentration ratio

Mechanistic HoaC vs. PBPK Model

- Possible differences in flow of media vs. plasma flow (depends on the HoaC setup)
- Tissue distribution and metabolism mechanisms – expected to be similar
- Possible binding to materials in HoaC system need to be considered





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Translating parameters from *in vitro* to *in vivo*

- Kps – account for differences between plasma *in vivo* and media *in vitro*
- PS_{TC} - initial assumption of constant Specific PS_{TC} (PS_{TC} per mL of tissue volume) - needs to be verified against test compounds
- Metabolism and/or carrier-mediated influx/efflux - initial assumption of *in vitro* expression = *in vivo* expression - needs to be verified against standard compounds

$$Kpu = V_{ew} + \frac{1/X_{[D],iw}}{1/X_{[D],p}} V_{iw} + \left(\frac{P \cdot V_{nlt} + (0.3 \cdot P + 0.7) \cdot V_{pht} \cdot}{1/X_{[D],p}} \right) +$$

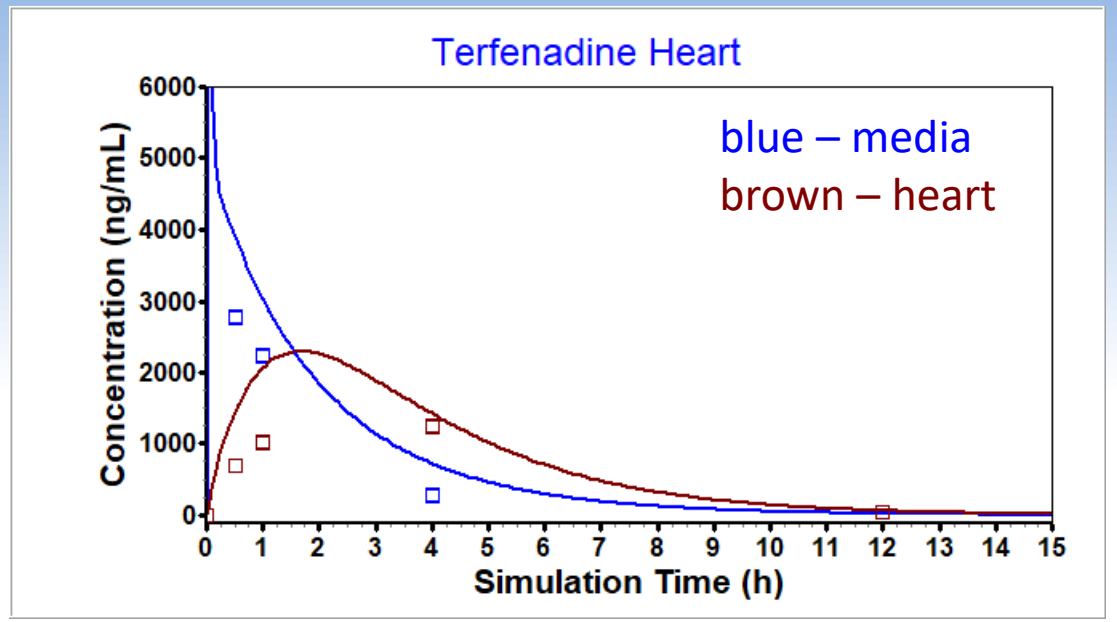
$$(Fn + Fa) \cdot \left[\frac{1}{fup} - 1 - \left(\frac{P \cdot V_{nlp} + (0.3 \cdot P + 0.7) \cdot V_{php} \cdot}{1/X_{[D],p}} \right) \right] \cdot RAtp +$$

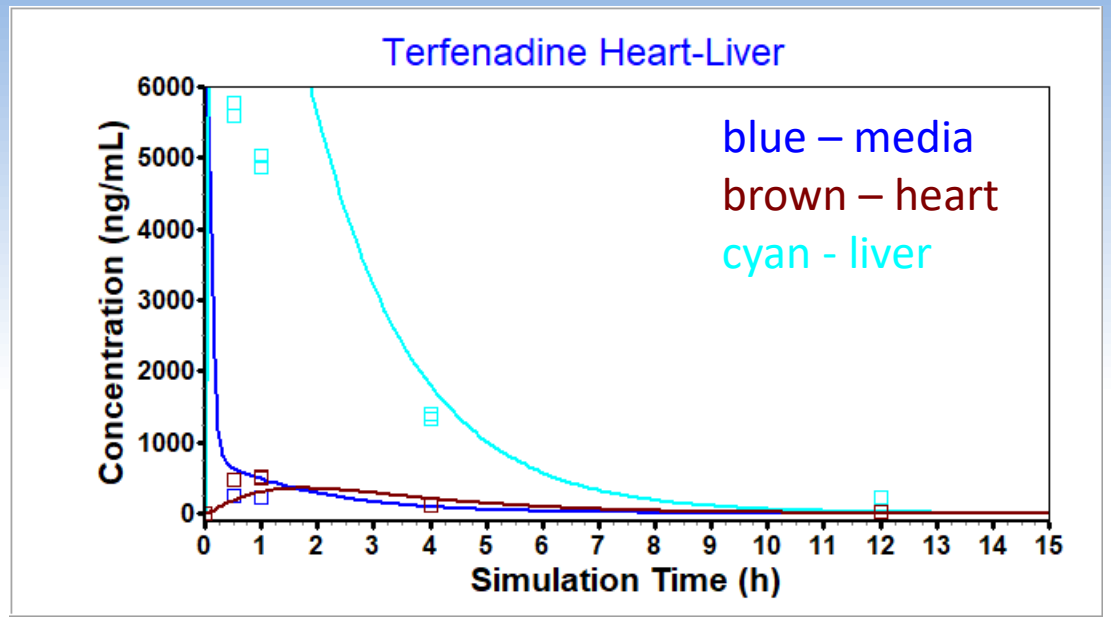
$$(Fc) \cdot \left(\frac{Ka \cdot [AP]_T ((1/X_{[D],IW}) - 1)}{(1/X_{[D],P})} \right)$$

$$PassiveDiffusion = PS_{TC} \left(C_{ect,u} - C_{ict,u} \right)$$

Mechanistic HoaC Model

(approximate results – preliminary model)





Fitted parameters:

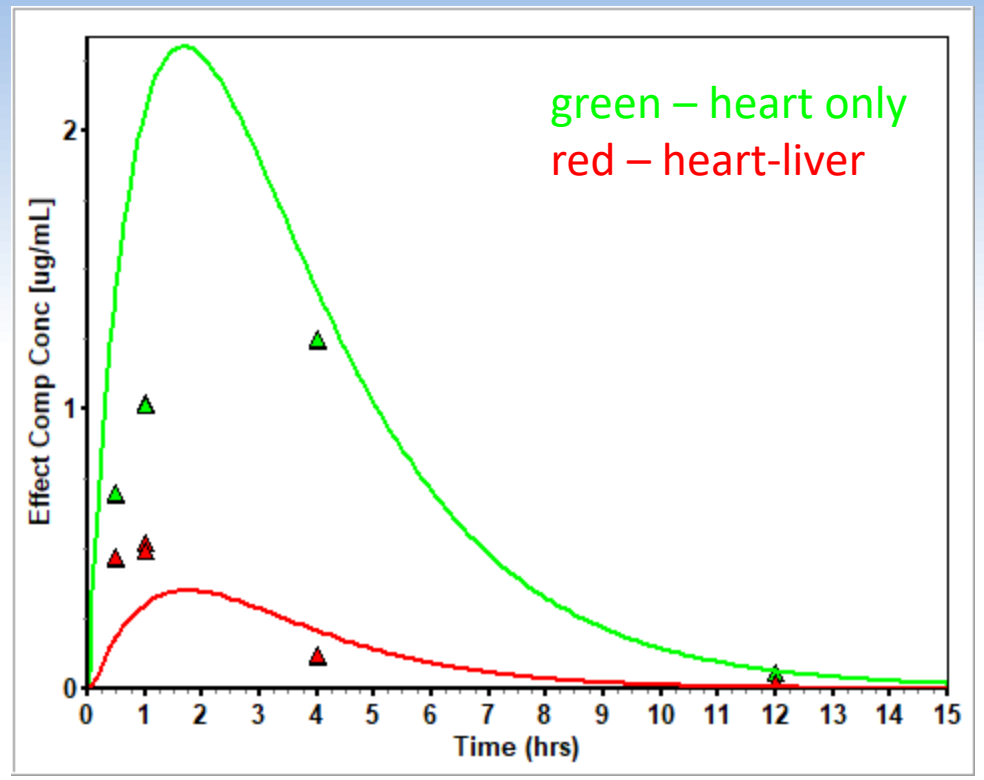
- Distribution to Heart tissue
  - Fitted Pstc for permeability-limited tissue
- Non-specific binding
- Distribution to Liver tissue
  - Fitted Kp
- Elimination by CYP3A4
  - Km predicted by ADMET Predictor®, Vmax fitted

Observed data:
McAleer – Sci Reports 2019, 9:9619

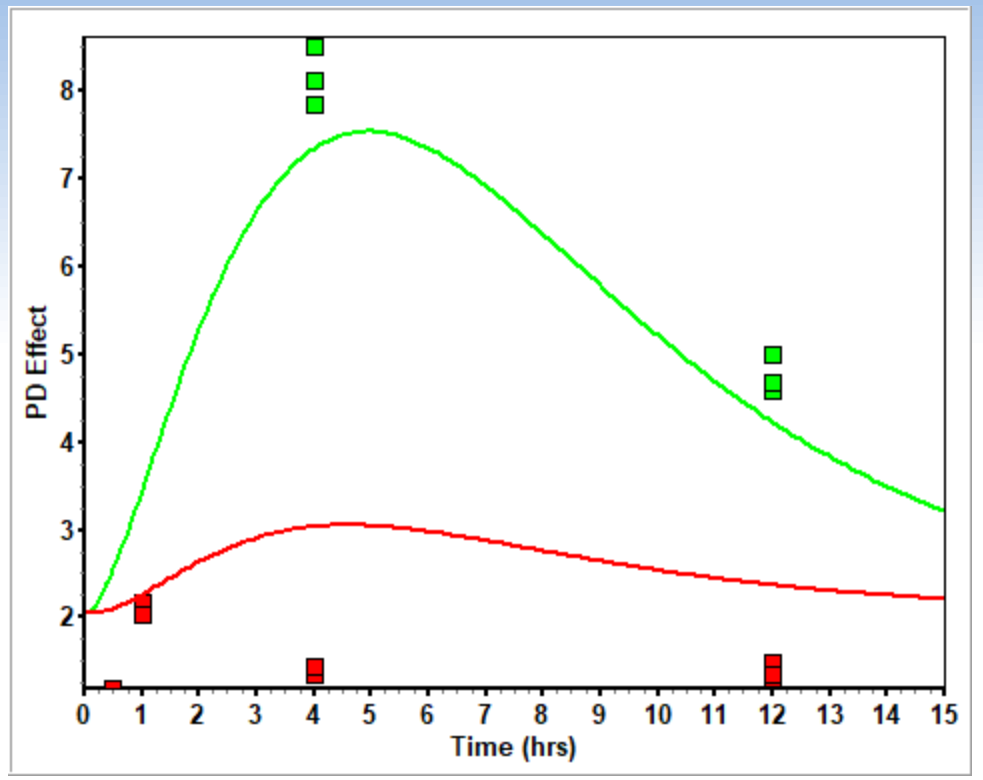
PD Modeling

(approximate results – preliminary model)

Heart concentrations



PD Effect



PD Model

Observed data:
McAleer – Sci Reports 2019, 9:9619

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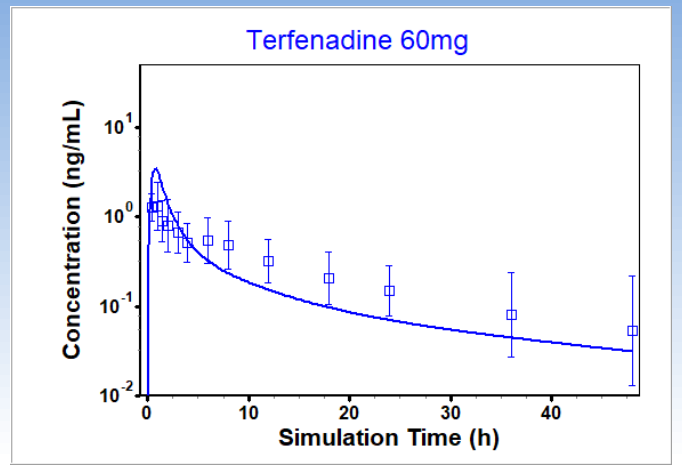
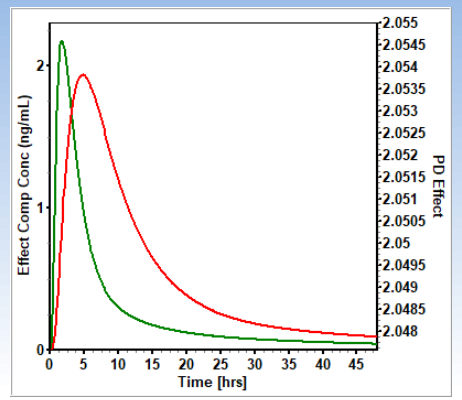
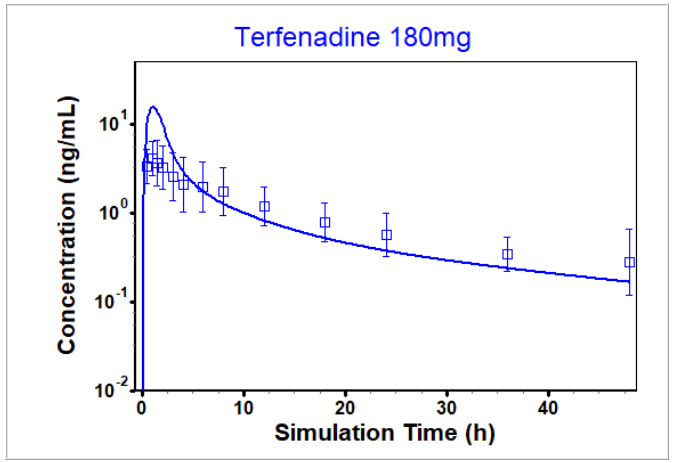
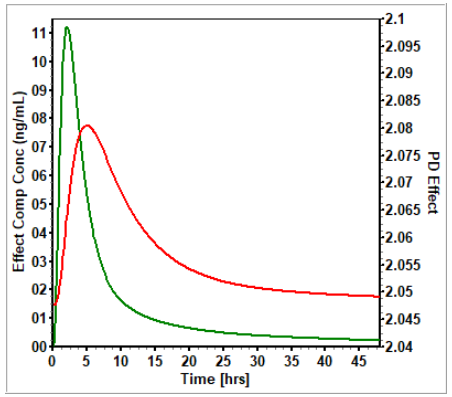
Simulate PK/PD *in vivo*









Observed data:
Okerholm – Biopharm Drug Dispos 1981, 2, 185-190

Parameter scaling:

- Kps estimated based on *in vitro* Kp fitted for Liver compartment
  - The *in vitro* Kp,u was scaled to *in vivo* Kp
  - Found the method that most closely predicted the *in vivo* liver Kp
- Distribution into the Heart scaled from *in vitro* distribution (PStc) fitted for Heart compartment
  - Assumed Pstc per mL tissue is constant
- Fraction unbound in enterocytes predicted in MembranePlus™
- Remaining properties predicted by ADMET Predictor®
- Clearance had to be scaled – the results are shown with *in vivo* Vmax increased 100x from the fitted *in vitro* Vmax
  - Enzyme activity different than *in vivo*?
  - Different rate limiting process *in vitro*?
  - Impact of significant binding to plates?
  - Caused by approximate media flow?

Summary

- A mechanistic simulation of HoaC system has a potential for full PK/PD extrapolation with a PBPK model.
- The use of a PBPK model allows linking the PD effect to the specific tissue concentration.
- The preliminary simulations showed potential for adequate prediction of tissue distribution.
- A mechanistic model for HoaC system could be linked with other *in vitro* assays (i.e. HLM clearance measurements) to identify and predict impact of individual processes.

Questions?

For further information visit:

https://hesperosinc.com/

https://www.simulations-plus.com/

Hesperos Overview

- Over 20 US patents have been licensed by Hesperos thereby documenting the innovation and novelty of this platform. This also provides full freedom to operate in this space & a strong defendable IP position.
- Winner of the 2015 London-based *Lush Prize* for creating an alternative to animal testing for industry.

- Have won multiple SBIR grants including a $2M Phase II and recently a $4M phase IIB award to Bridge the “valley of death”.
- Established R&D contracts with multiple national and international Pharma companies.
- Moved into a new 14,100 sq. ft. state of the art facility in August 2019.
- Have recruited excellent staff for company, 32 at present.
- No products will be offered at this time, only services based on compounds sent to our Orlando facility.

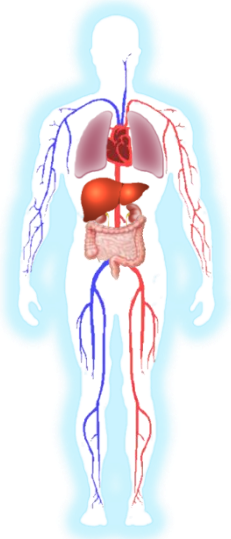
Clinically Relevant Functional Readouts

- Mechanical or electrical readouts of cellular functions such as:
  - ***muscle contraction***
  - ***electrical activity*** from neurons and cardiac cells
  - motoneuron → muscle: ***NMJ physiology*** and other combinations
  - barrier integrity (TEER) and active transport for barrier tissues
- Allows ***functional*** analysis of cellular health non-invasively for acute, but more importantly, for ***chronic*** monitoring of human-on-a-chip systems
- ***Reduces substantially***, if not eliminates, the need for measuring ***biomarkers*** in these systems for certain organ mimics. Normally need to measure multiple biomarkers by molecular techniques and put them together ***to extrapolate functional*** activity, with these systems can ***measure directly***.
- Allows ***mechanistic*** determination of toxicity and for target identification.
- Facilitates ***physiological*** determination of drug efficacy and safety

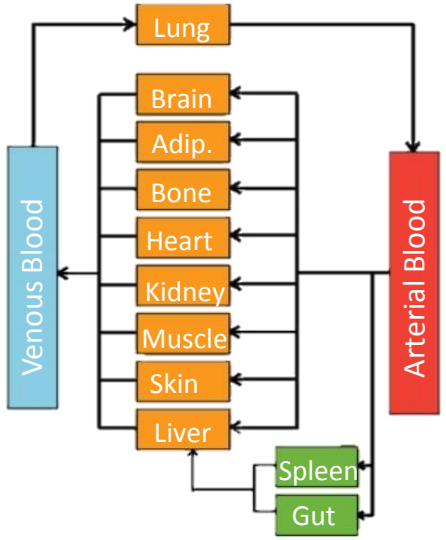
Human-on-a-Chip Systems for Disease Modeling

HESPEROS™

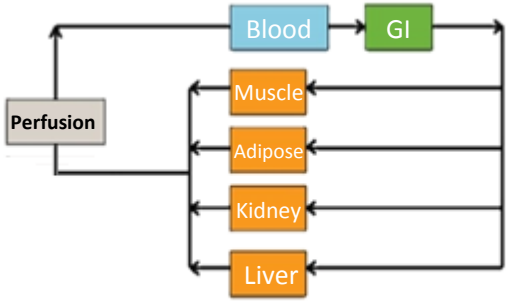
Human Body



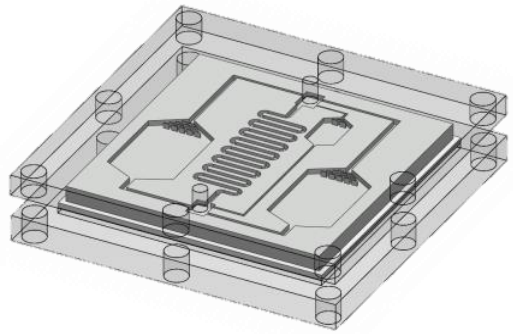
PBPK



Chip PBPK

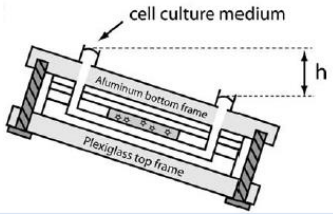


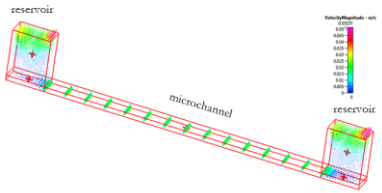
Serum-Free Human-on-a-Chip



Gravity-induced flow through a microfluidic device on a rocking platform

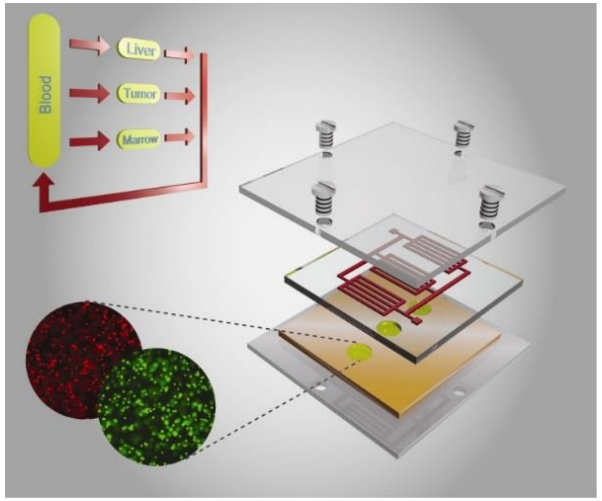
- Pumpless operation
- Minimizes bubble formation

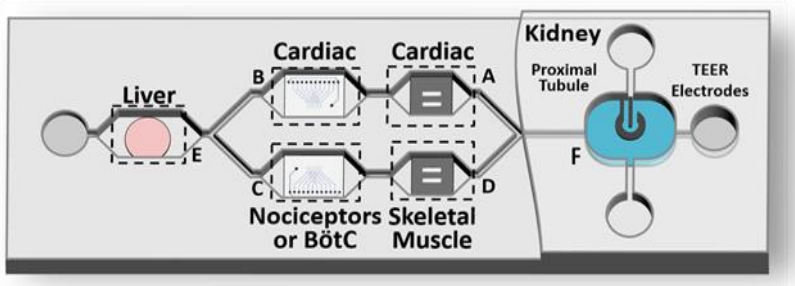




Medium recirculation with gravity-induced flow. Tilting of the device causes liquid to flow between the wells. In a timed manner, the rocking platform changes the angle and medium flows in the opposite direction. [1]

OVER 20 PUBLICATIONS USING THE PUMPLESS SYSTEM





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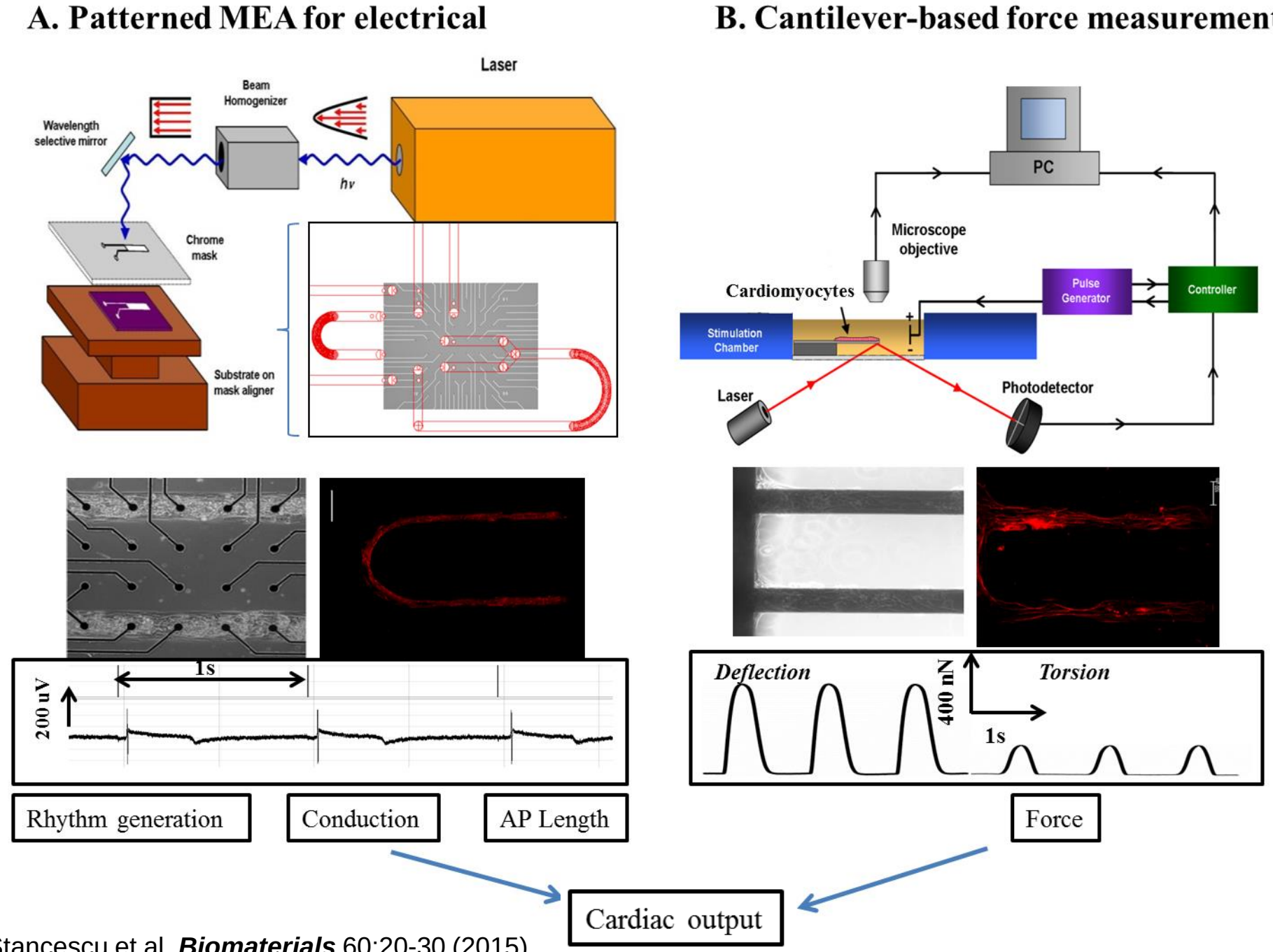
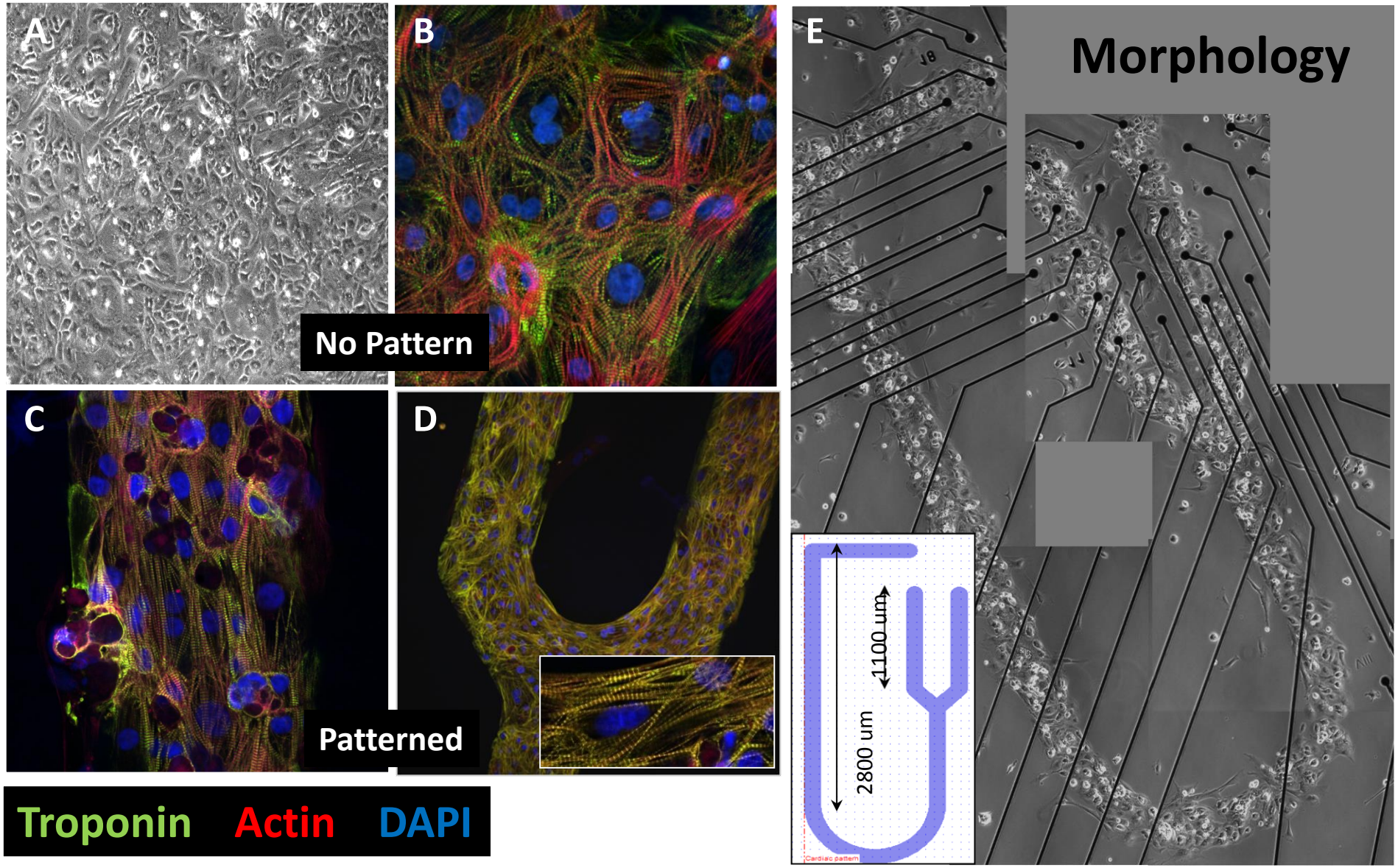
JH Sung, C Kam, ML Shuler, Lab on a chip,10: 446 (2010)
Castellanos M, et al., *Proc Natl Acad Sci U S A*. 101(17):6681-6 (2004)
Sweeney LM, et al., *Toxicol In Vitro*. 9(3):307-16 (1995)

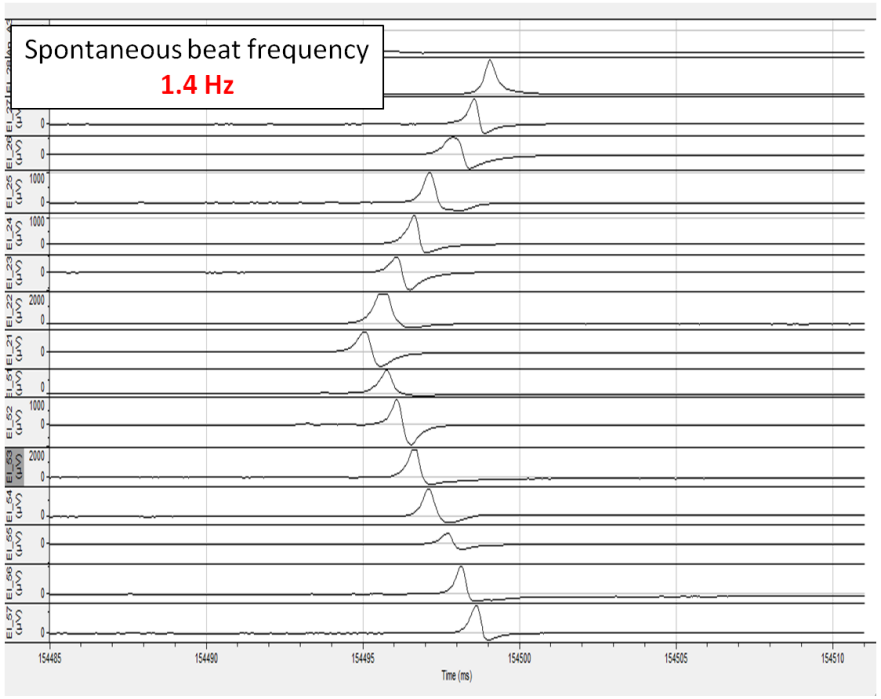


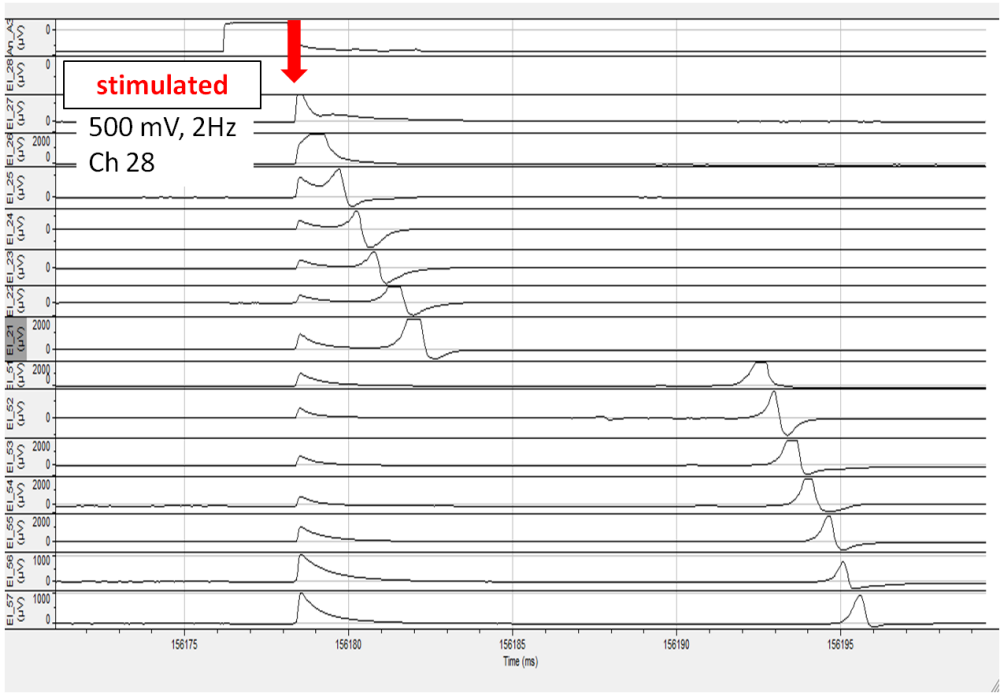
Stancescu et al, ***Biomaterials*** 60:20-30 (2015).

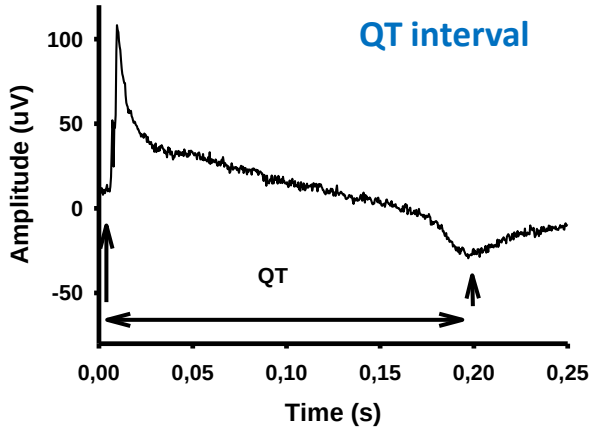
Morphology and Immunocytochemistry of Patterned and Non-Patterned Cardiomyocytes in serum-free medium

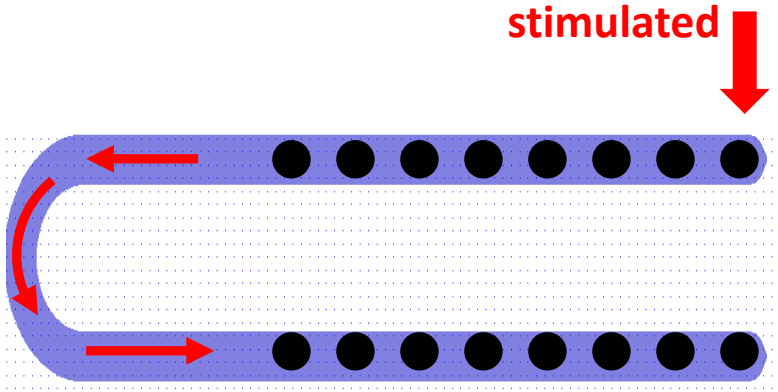


Electrophysiology – cardiomyocytes on MCS MEA Conduction velocity measurement







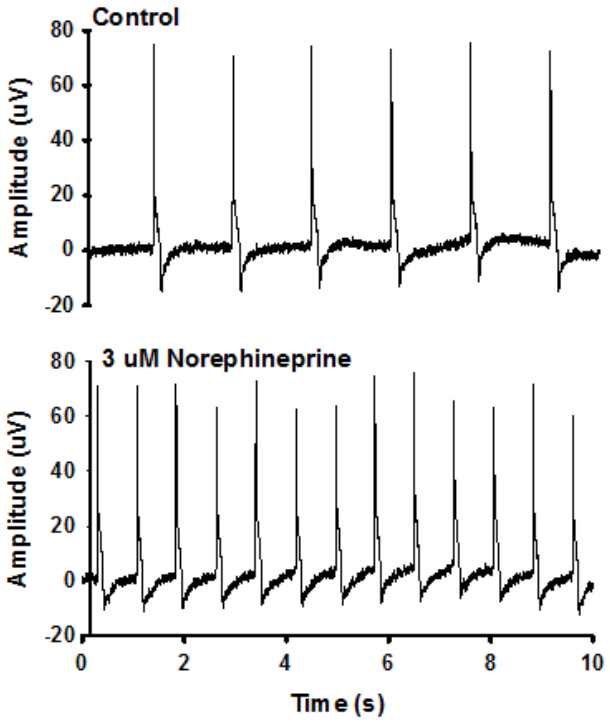


Calculated Conduction velocity = 0.42 m/s

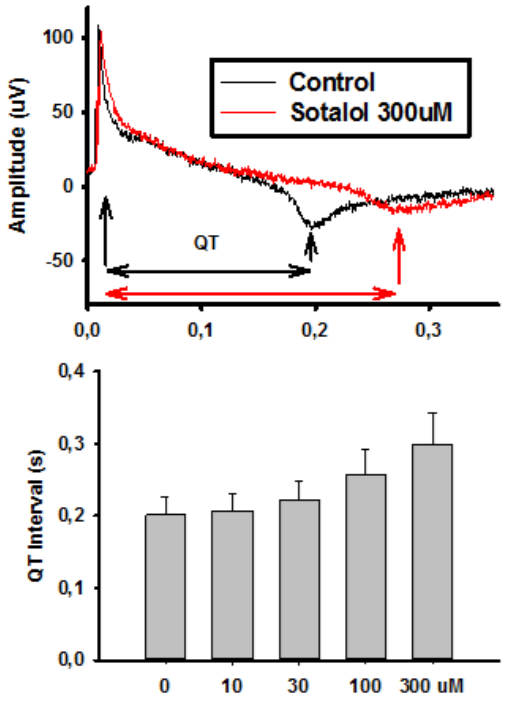
Sample Data Evaluation of Cardiac System

Drugs	Mechanism
Sotalol	Antiarrhythmic Class III: K+ channel blocker Beta-blocker
Verapamil	Antiarrhythmic Class IV: Ca2+ channel blocker
Norepinephrine	α-adrenergic agonist

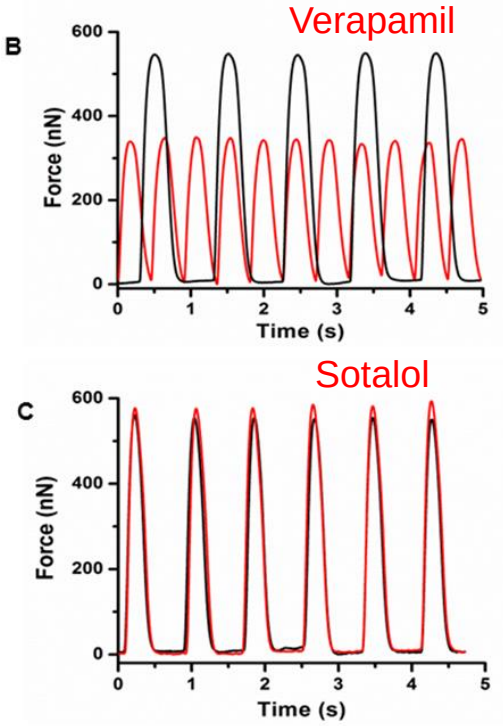
Frequency



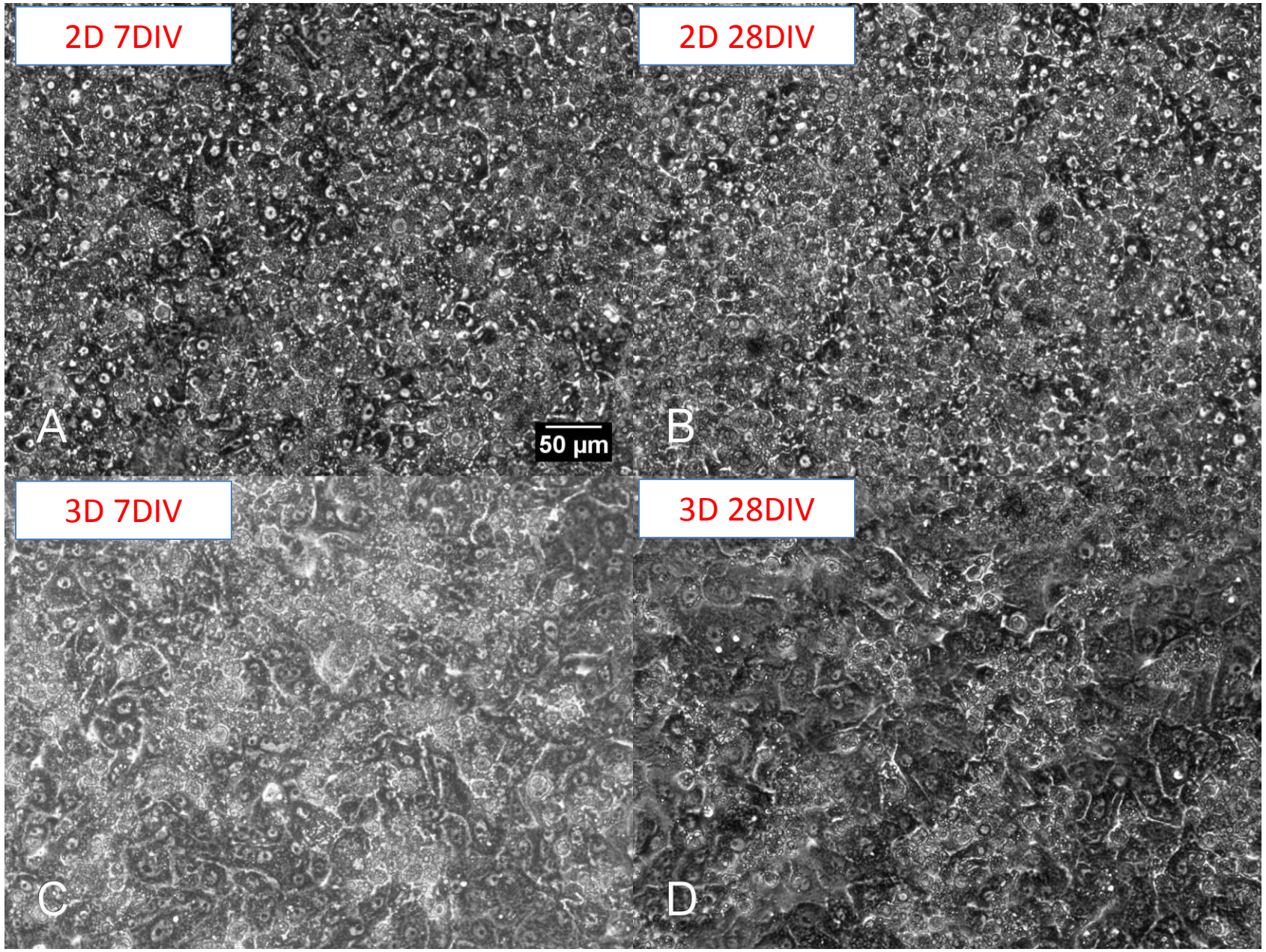
QT interval



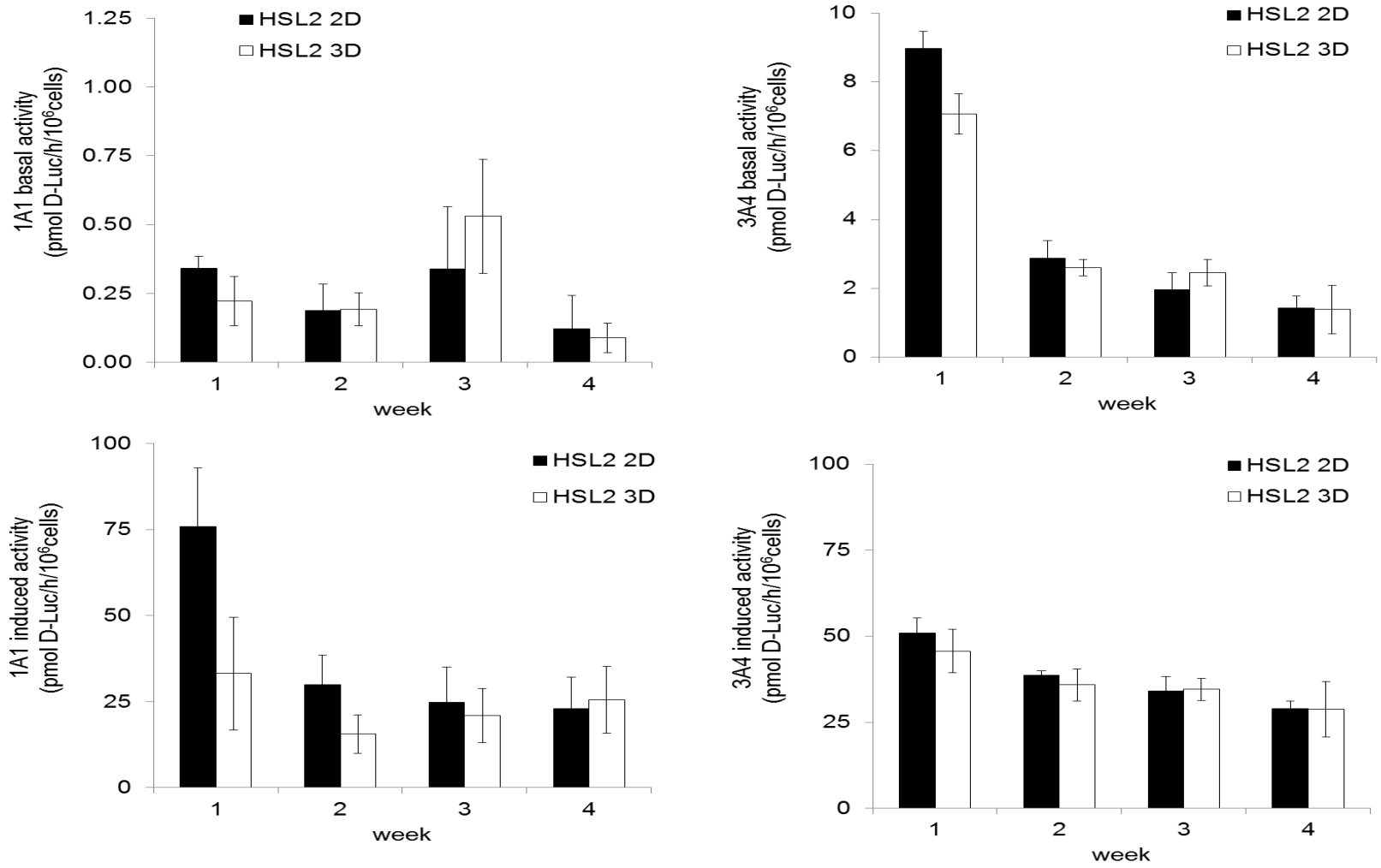
Force



Morphology of Primary Human Hepatocytes in Serum-free Medium at 7 and 28 Days in Culture



Basal and Induced CYP Activity Levels of 2D and 3D hepatocyte cultures up to 28 days in serum-free medium

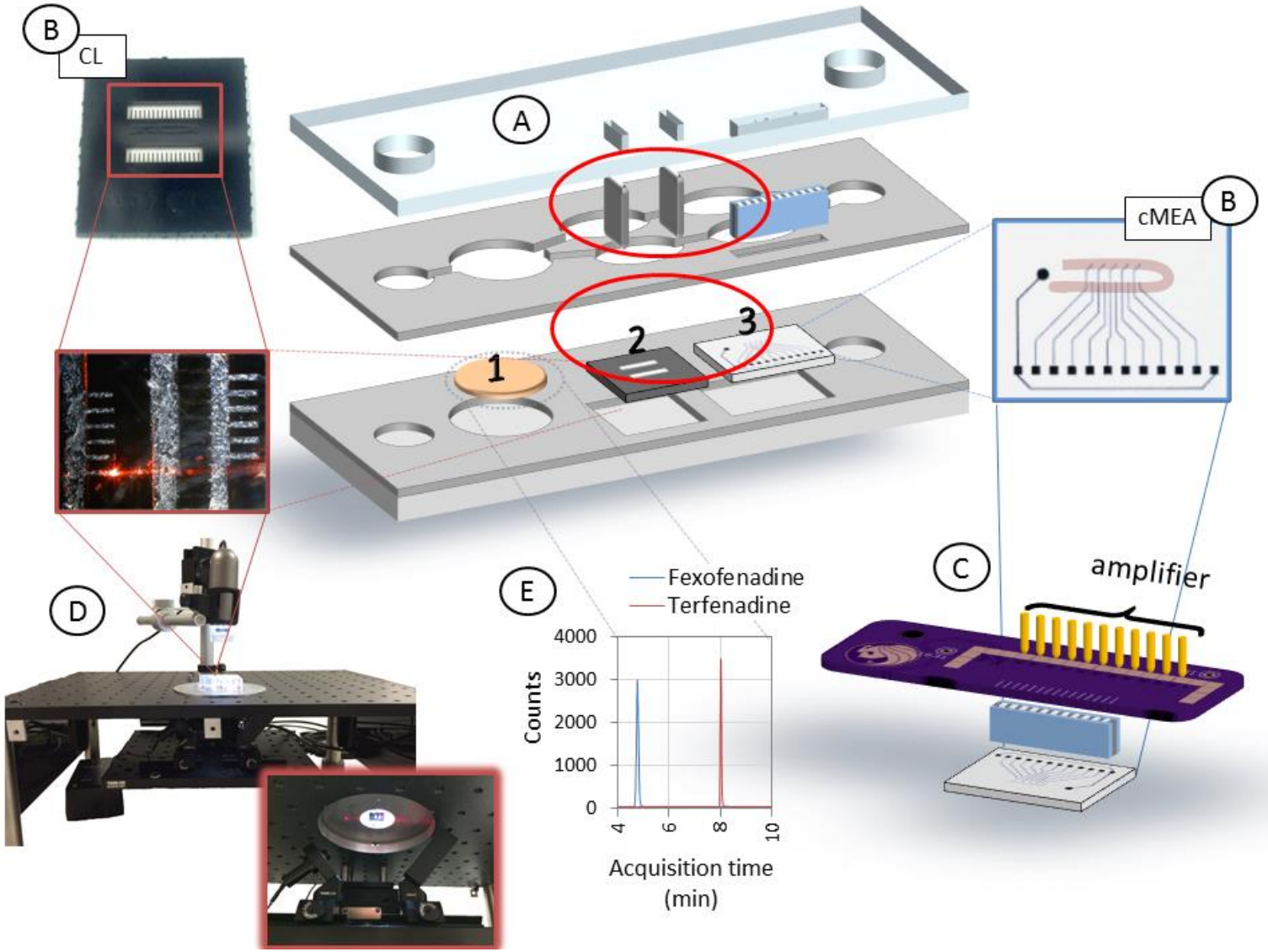


Hepatocytes in Serum-free medium at 28 DIV possess reasonable CYP activity levels which are inducible

C. Oleaga,etal., “A functional long-term serum-free human hepatic in vitro system for drug evaluation,” ***Biotechnol Prog***, Online first: DOI: http://dx.doi.org/10.1002/btpr.3069 (2020)

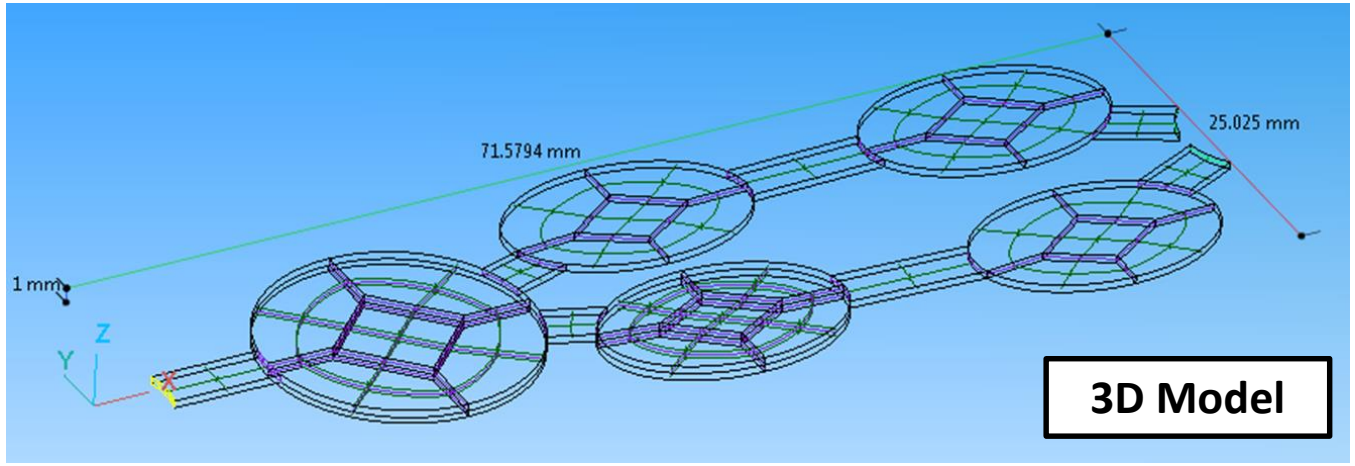
HEART-LIVER

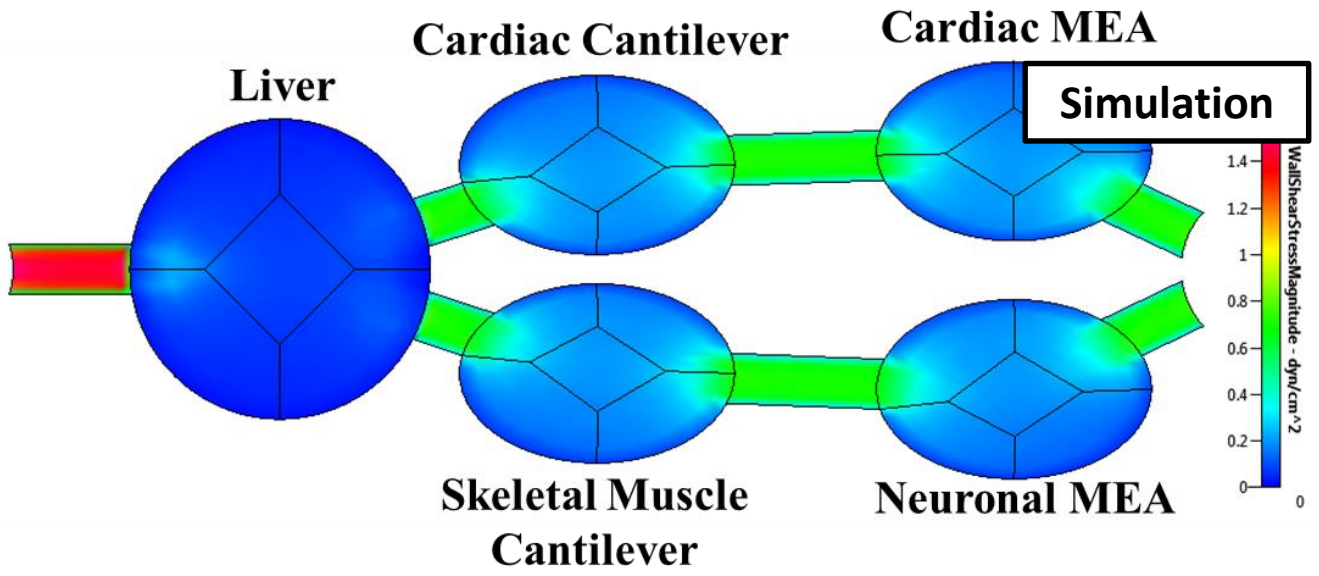
Cardiac and liver co-culture in a pumpless microfluidic system



Simulation-Assisted System Design

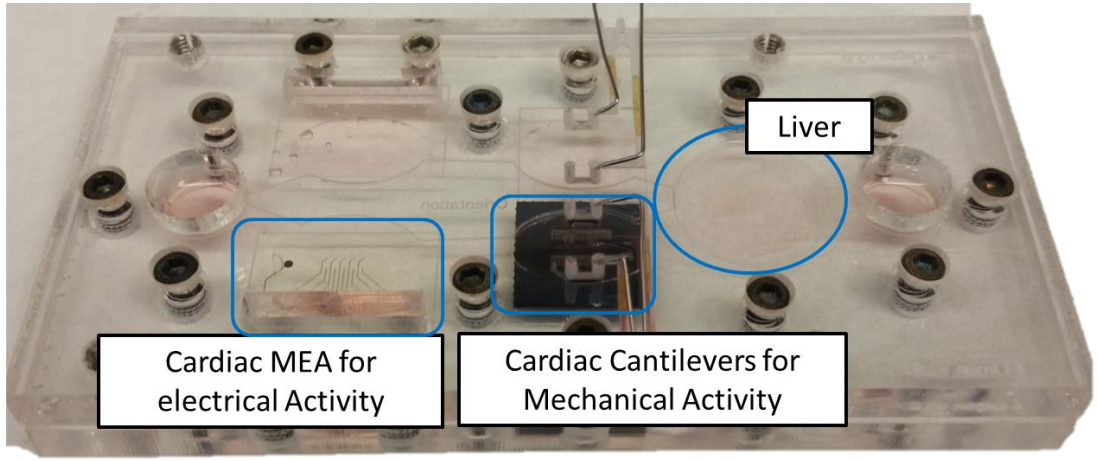
Combination of microfluidic computational fludic dynamics (CFD) modeling and electrical-like simulation





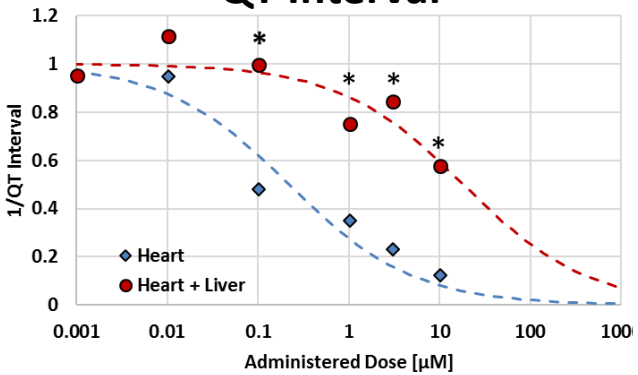
Flow rates and shear stresses on cells

Heart-Liver Systems with Recirculating Serum-Free Medium



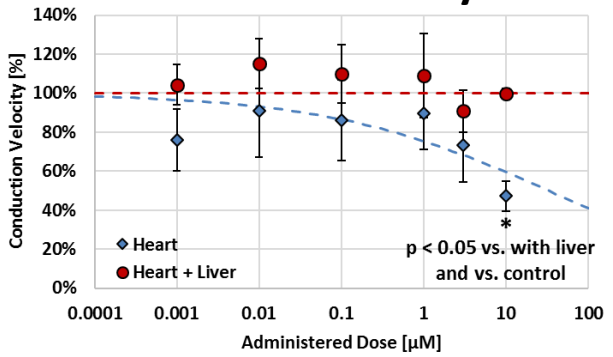
Heart + liver system derived from 4-organ system with similar flow characteristics

QT Interval



Terfenadine Addition Results in Elongation of QT Interval in the Absence of a Liver Component

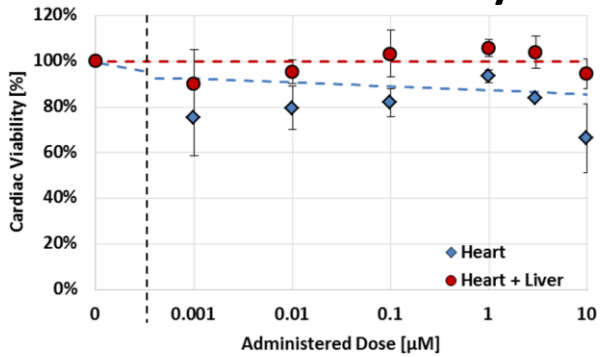
Conduction Velocity



Terfenadine Addition Reduces Conduction Velocity in the Absence of a Liver Component

IC_{50} = 33 µM (Heart)
IC_{50} = NA (Heart + Liver)

Cardiac Viability



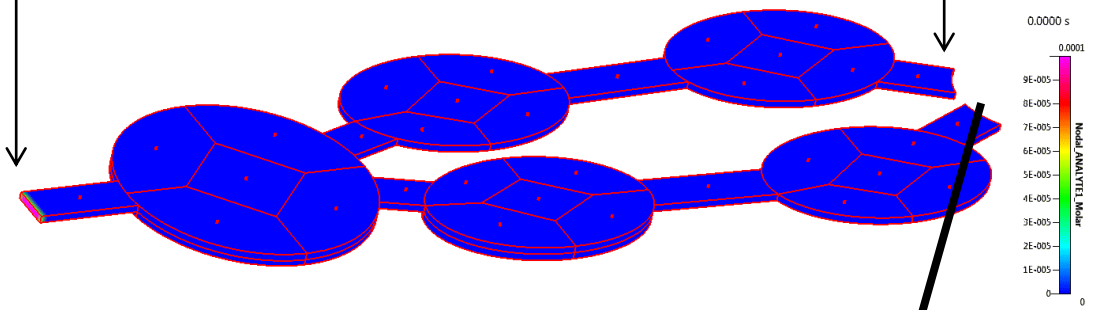
Without liver, terfenadine slightly reduces cardiac viability at 10 µM terfenadine

$IC_{50,1}$ > 10 µM (Heart)
IC_{50} > 10 µM (Heart + Liver)

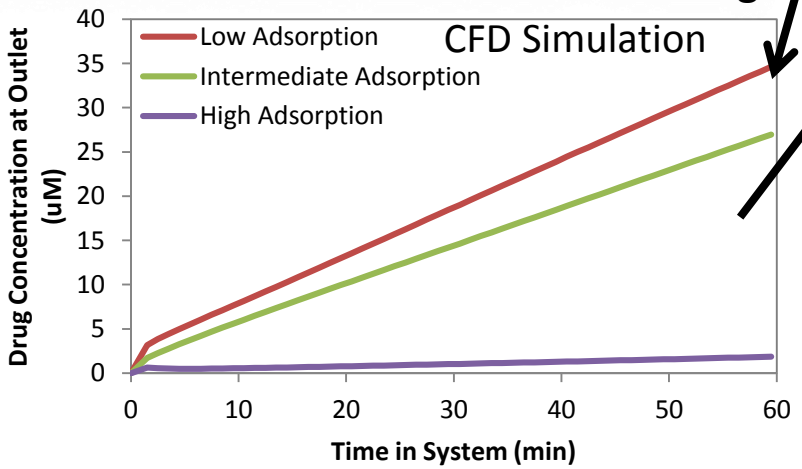
Lipophilic Compound Concentration in Systems 5 min to 24 hours

Compound (330 uM) added to this reservoir for 100 uM final concentration

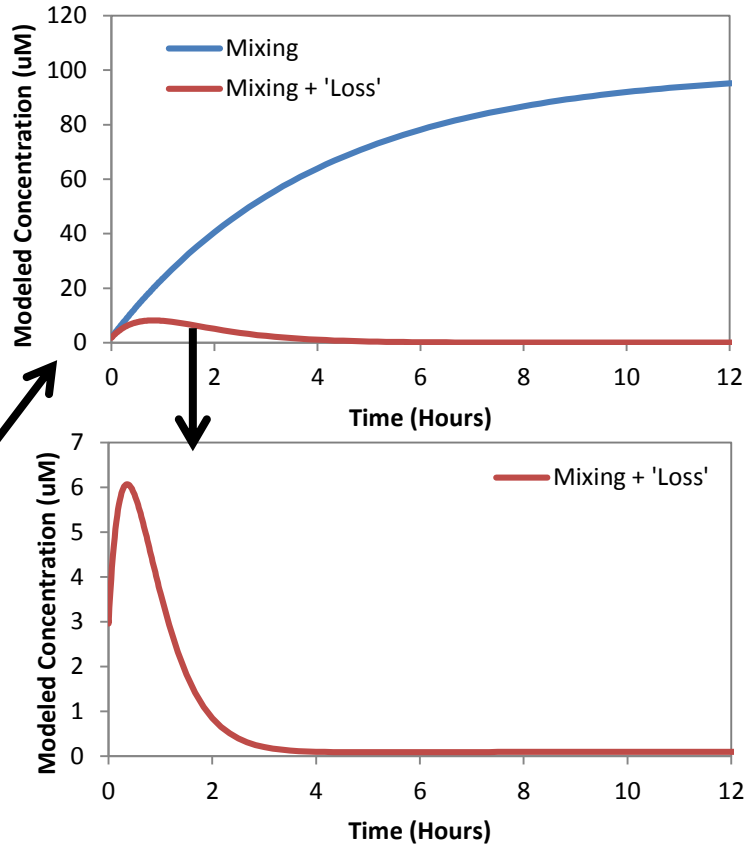
Withdrawal from this reservoir



Simulation of Drug addition to one side with initial perfusion due to volume difference + Rocking



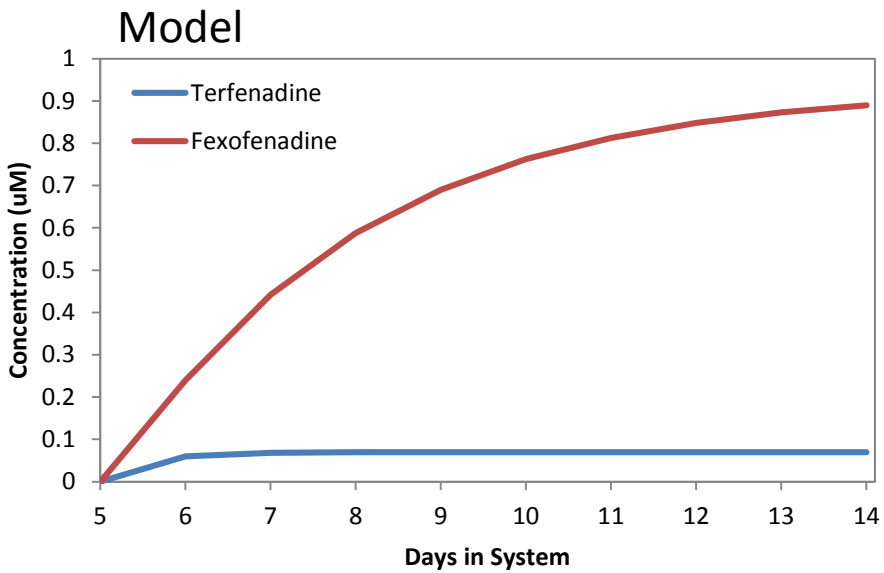
Numerical Simulation

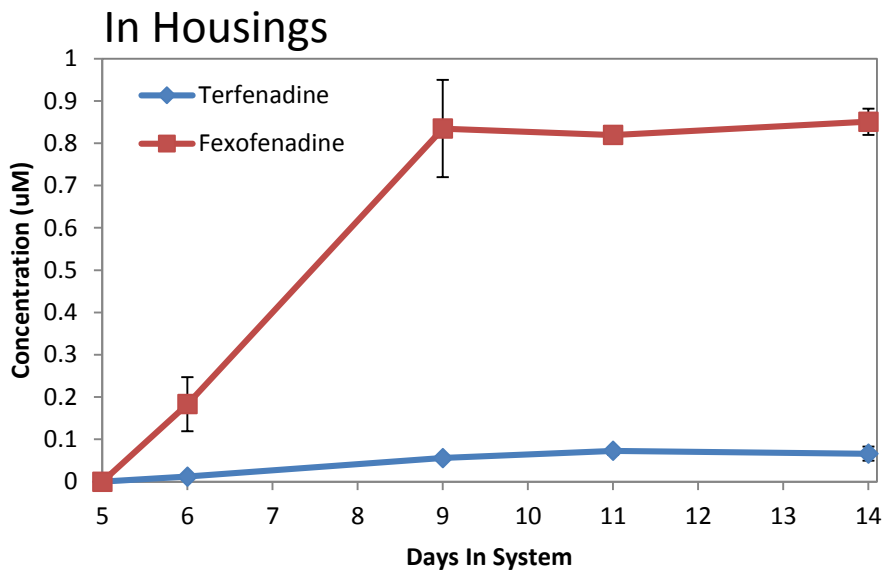


Concentration at reservoir opposite liver dependent on mixing along with adsorption, metabolism, and bioaccumulation (“loss”)

Chronic 4 Organ System: HPLC-MS

0.2 uM Daily Addition of Terfenadine





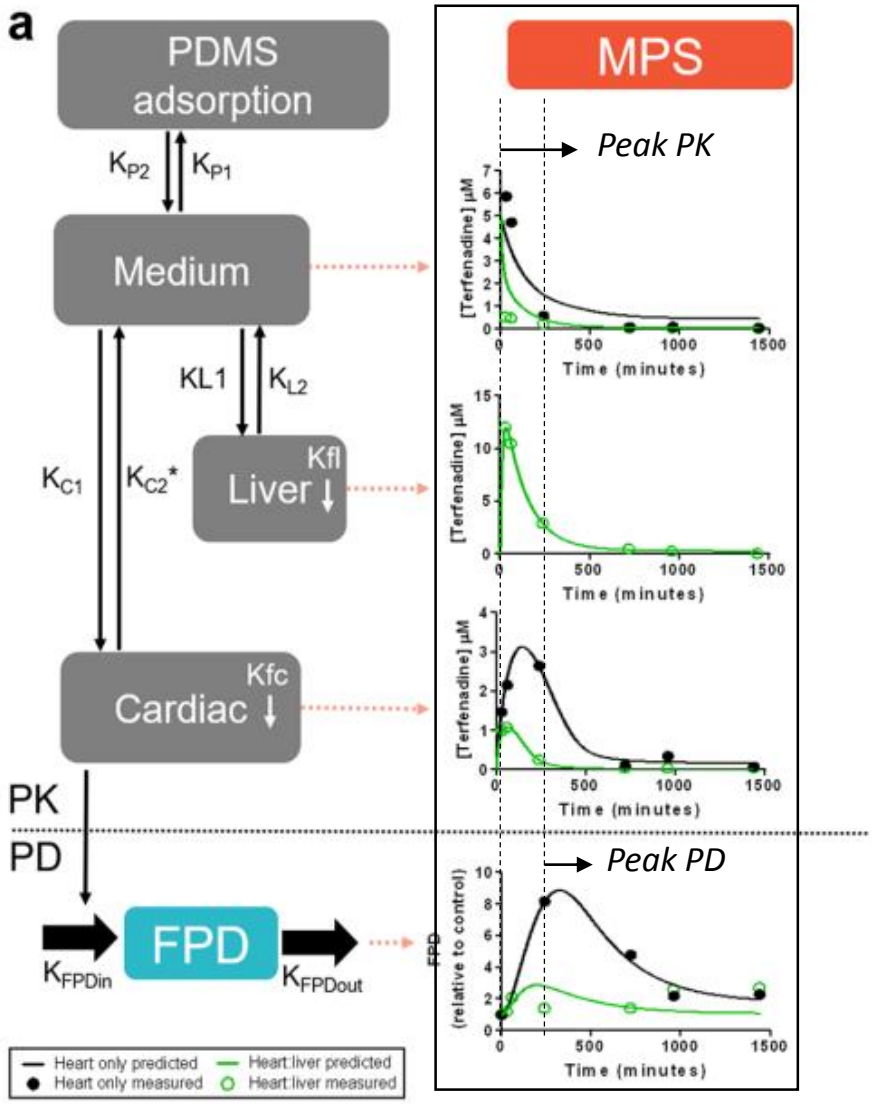
$f = 0.3$, $X = 0.8$

The measured concentrations of terfenadine and fexofenadine in housing systems with chronic addition of drug leveled to a steady state concentration, **as predicted by modeling**

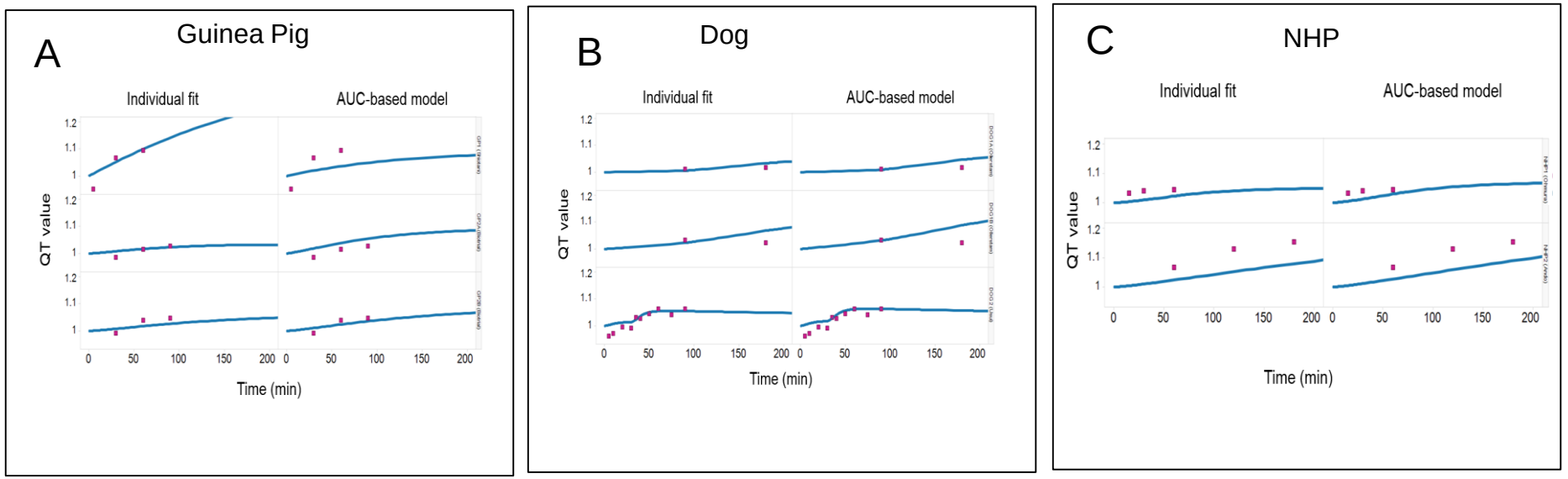
Equilibrium between removal from feeding and addition + metabolism

PK-PD Relationship Approach

- Previous data from Terfenadine (Scientific Reports paper) showed a time between medium concentration and the FPD elongation
  - Similar to monkey data in vivo
- Multi-compartment model incorporating PDMS adsorption, medium, and the tissues was implemented
- A PK model was implemented in that study to obtain the relationship between medium concentration and bioaccumulation in the in vitro systems (K_{C1}, K_{C2})



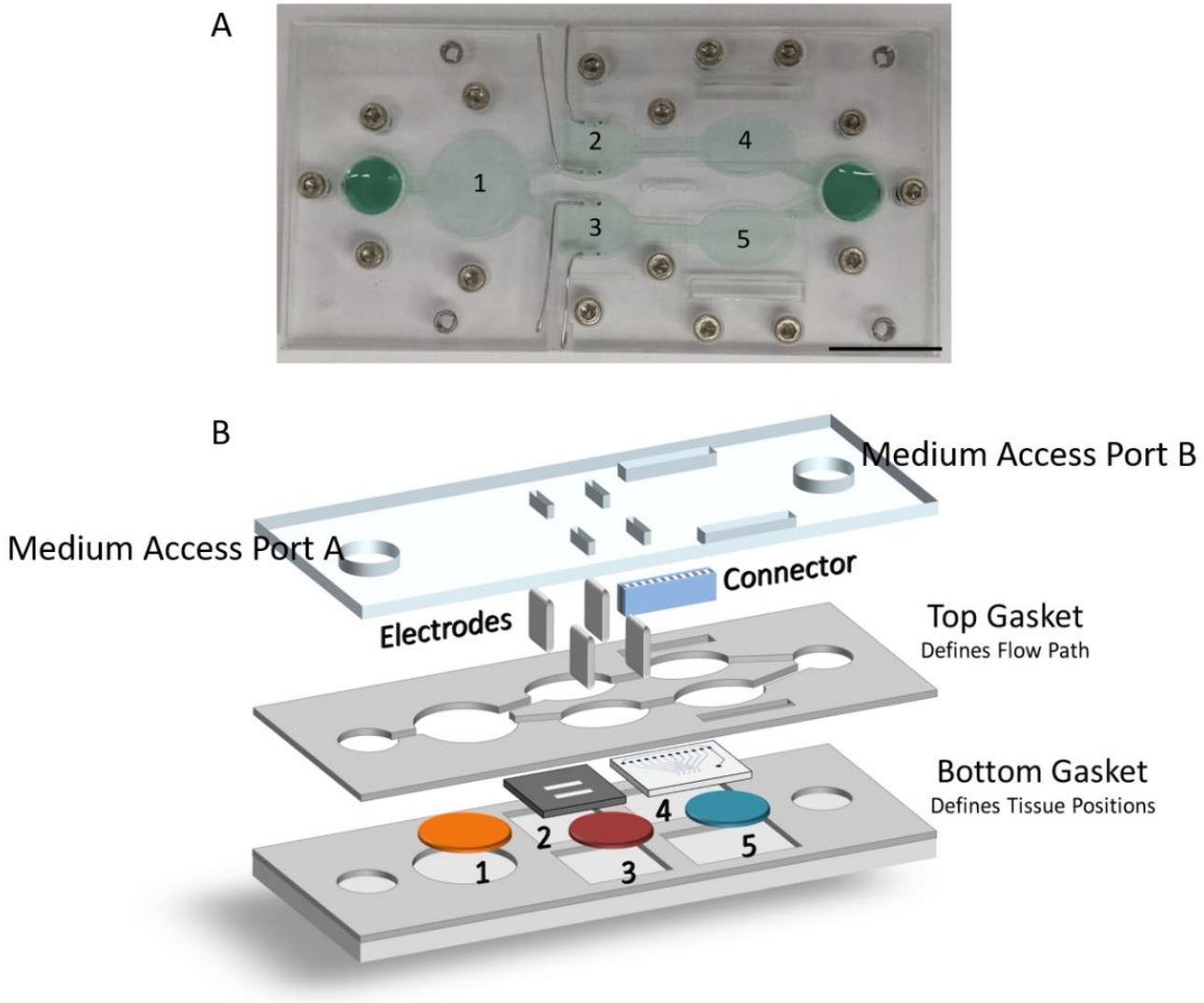
Correlation of In Vitro PKPD Model with In Vivo Animal PKPD Models



Since in vivo animal data has been correlated with clinical data we should be able to correlate our in vitro models with clinical data as well both retrospectively and prospectively

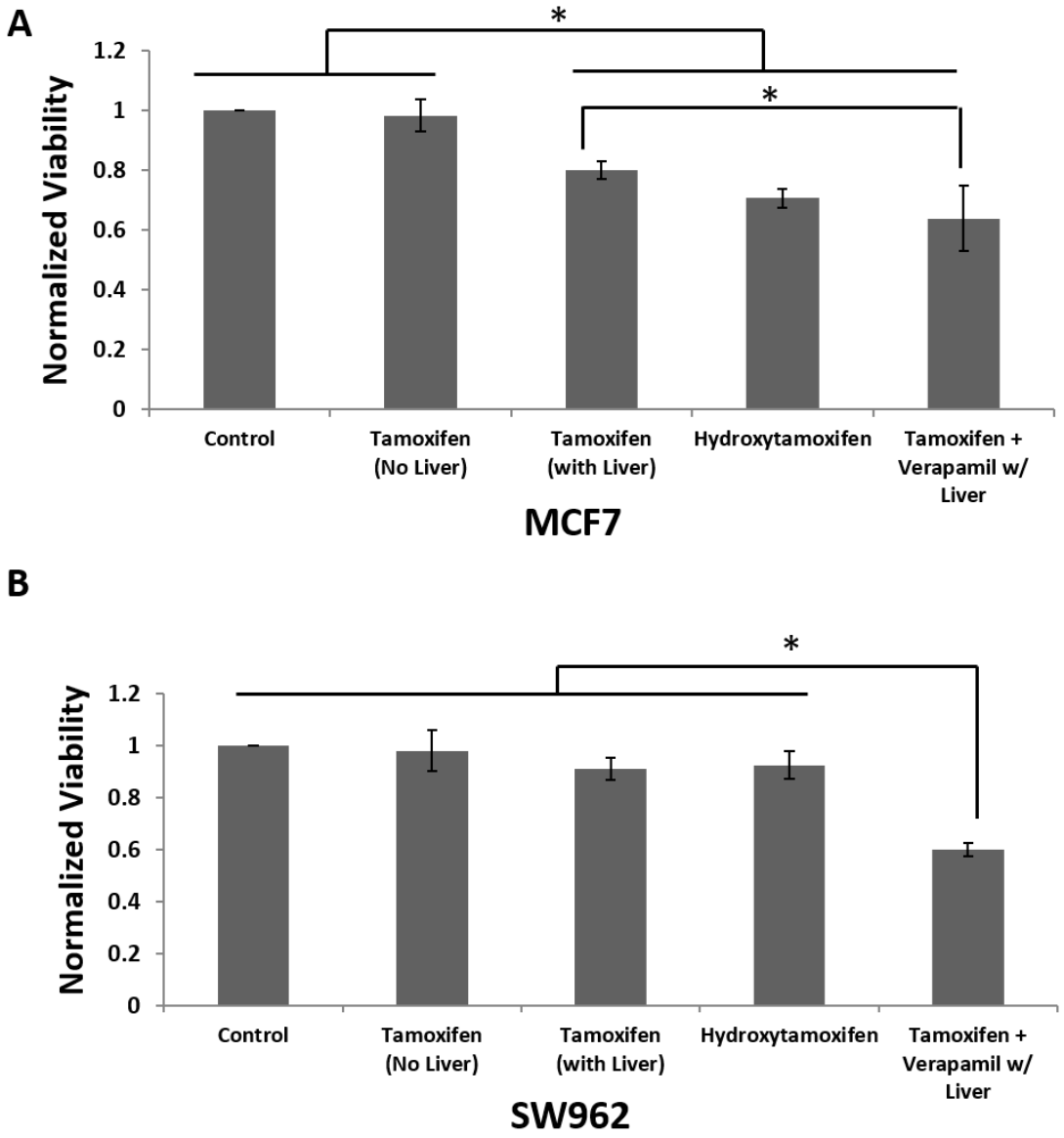
McAleer et al., ***Nature Scientific Reports***, 9:9619, (2019)

Cancer, Cardiac and Liver System for Efficacy and Tox



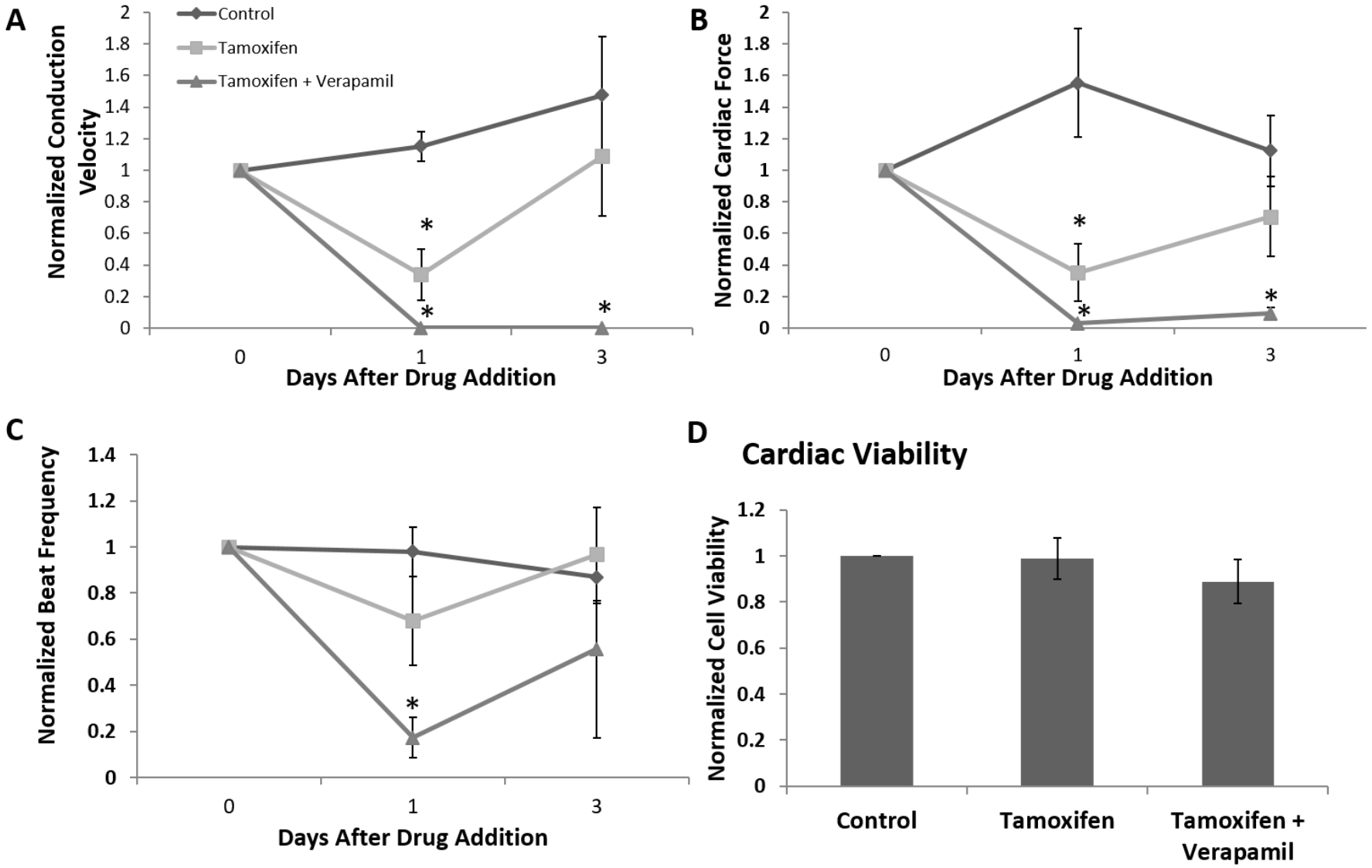
Five chamber reconfigurable multi-organ system. Scale bar is 2 cm. B) Schematic representation of the MPS assembly and design used in the system 2 study of tamoxifen. Chamber 1 houses hepatocytes on coverslips Chambers 2 and 4 are cardiac cantilevers and MEAs respectively. Chambers 3 and 5 are for cancer cells SW962 and MCF7. Drugs were applied to Medium Access Port A and initially pass over the liver to mimic aspects of first pass metabolism. Electrodes are embedded for the option of using broadfield stimulation to elicit contraction.

Tamoxifen, with and without metabolism, and the effect on cancer viability



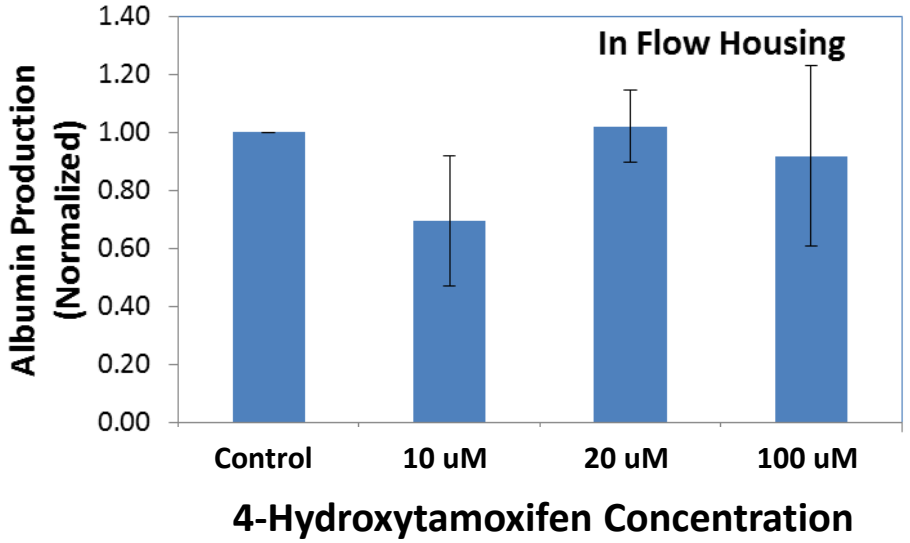
Error bars are represented as SEM. *p < 0.02.

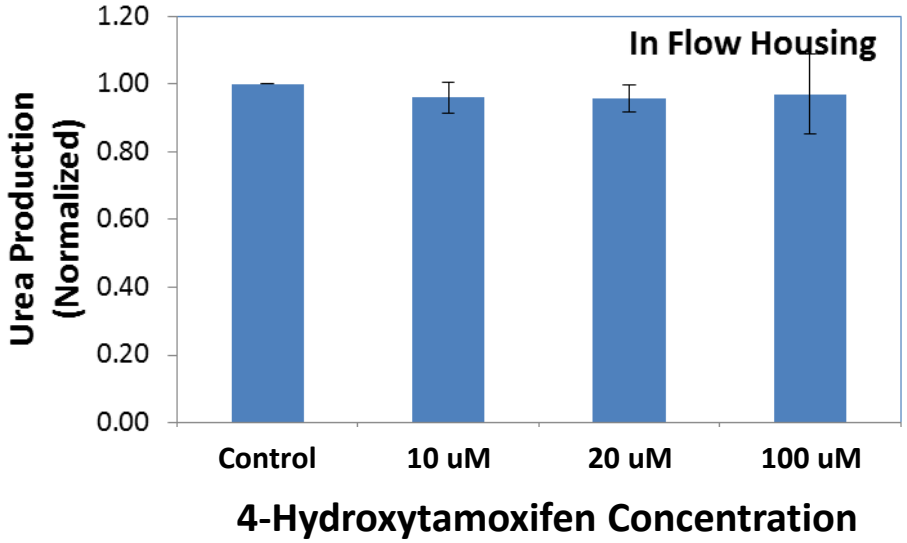
Sentinel Monitoring of Cardiac Function
Cardiac, Cancer, Liver Systems



Error bars are represented as SEM. *p < 0.02

Liver Function in Housings Cancer, Cardiac, Liver System

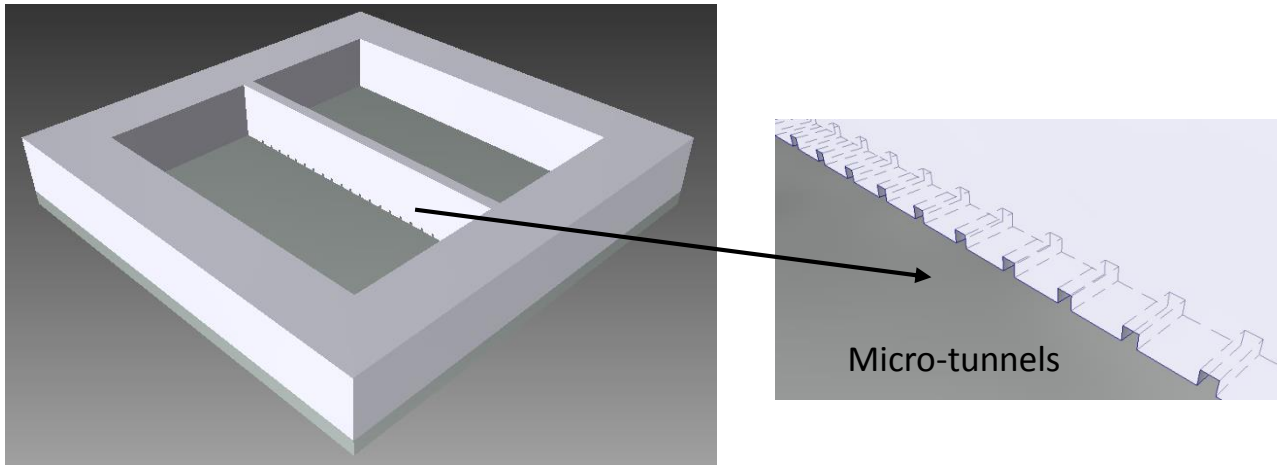


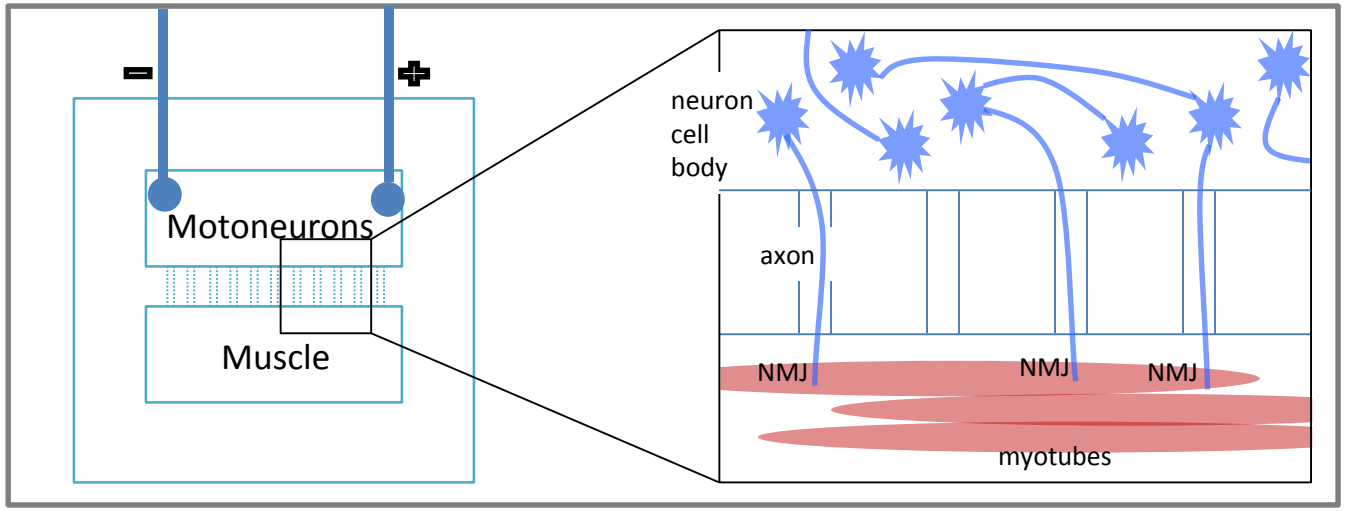


4-Hydroxytamoxifen inside the systems did not affect albumin or urea production after a 24 hour treatment

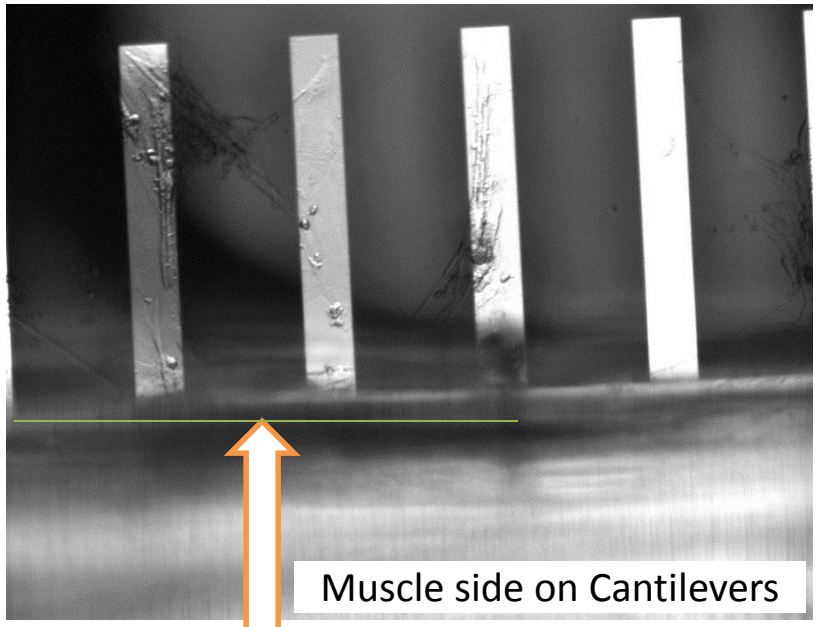
McAleer et al., ***Sci. Transl. Med***. 11, (2019)

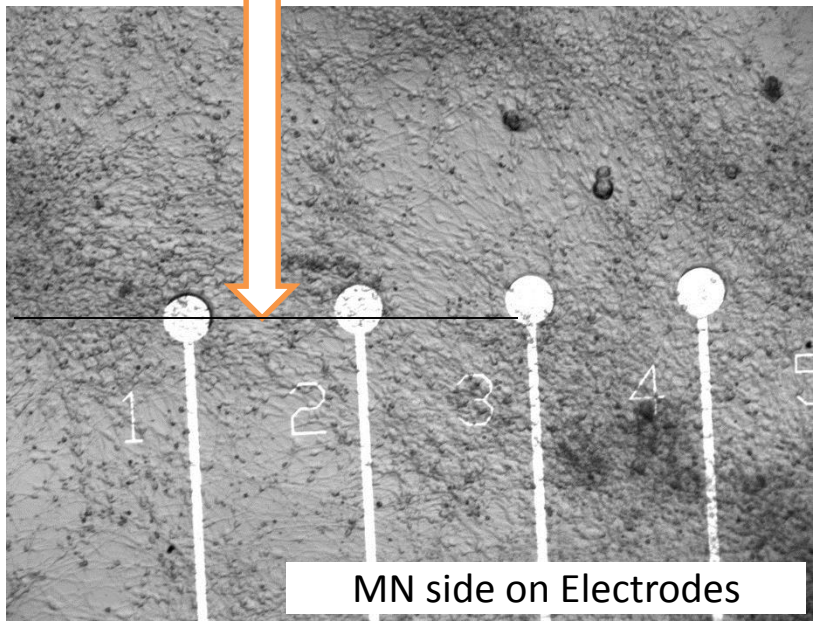
Neuromuscular Junction (NMJ) Platform

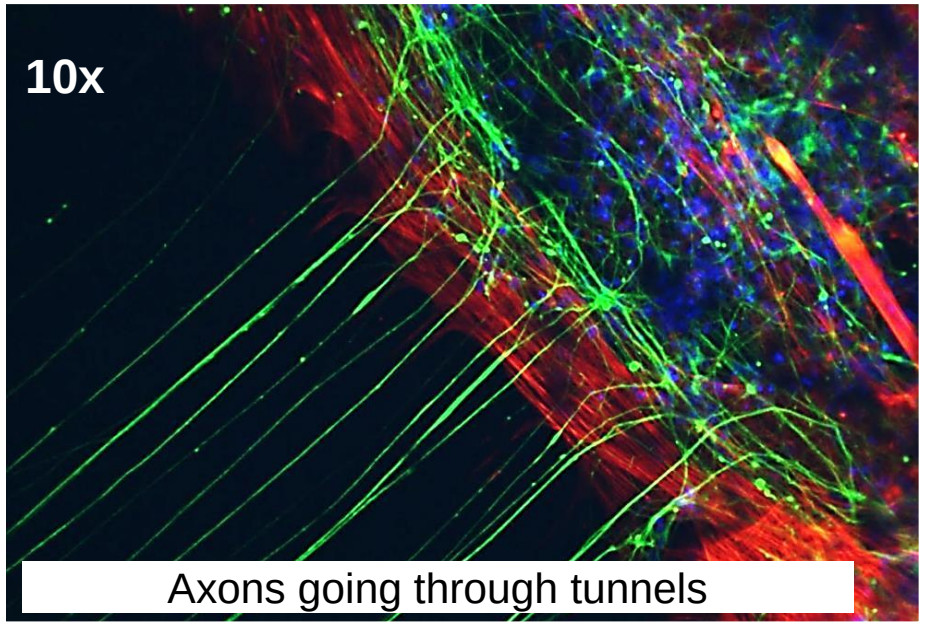


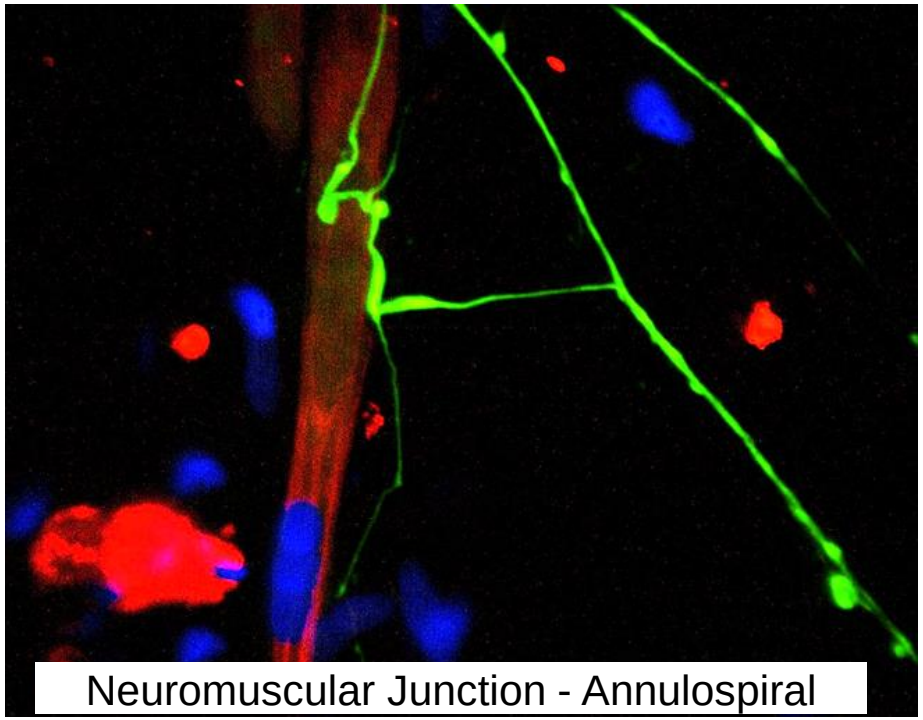


- PDMS molded chambers bonded to glass coverslips
- Two chambers separated by micro-tunnels
- Motoneurons send axons through tunnels and form NMJs
- Electrical stimulation and drugs partitioned by barrier

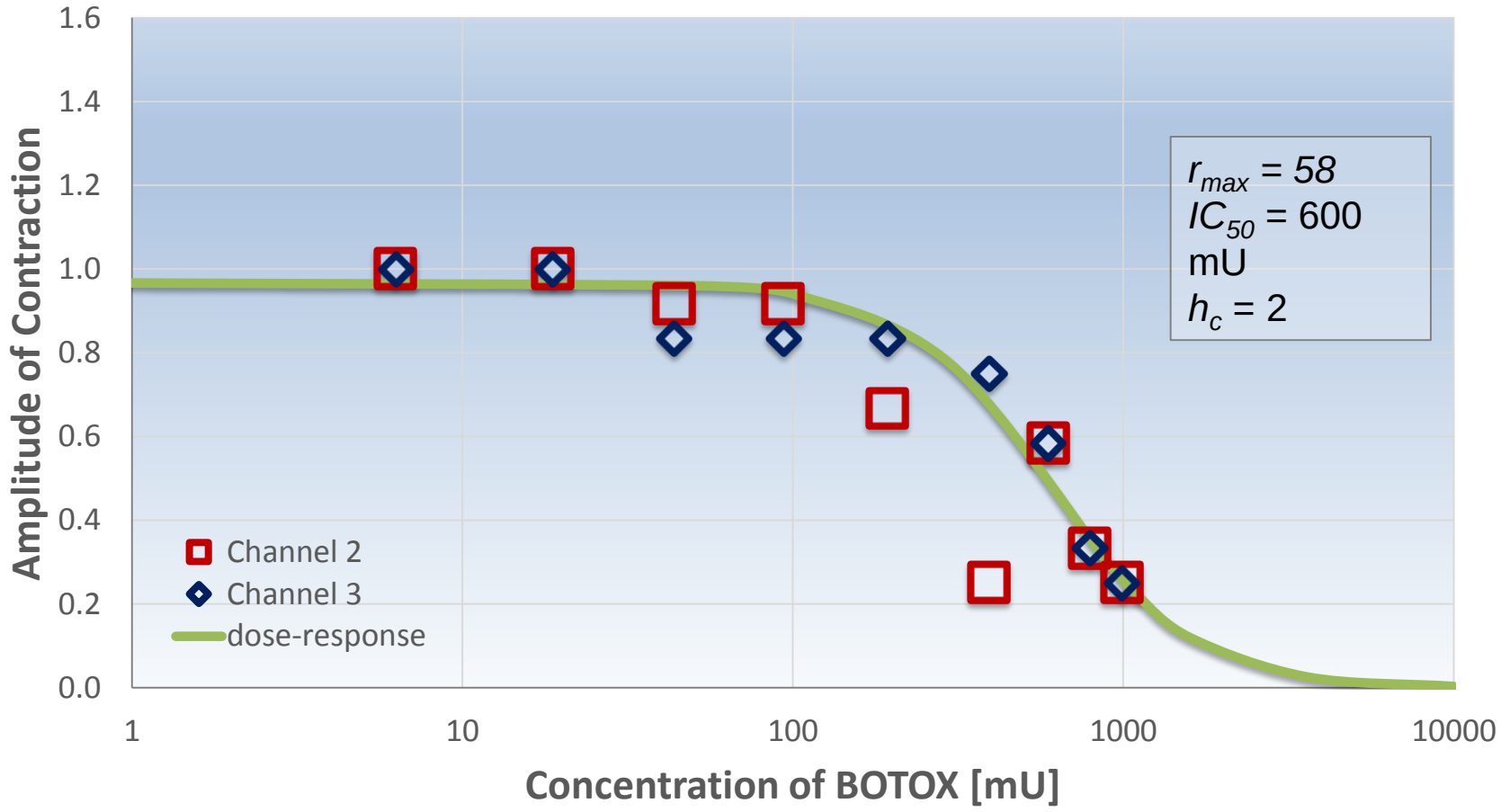

Muscle side on Cantilevers


1
2
3
4
MN side on Electrodes


10x
Axons going through tunnels

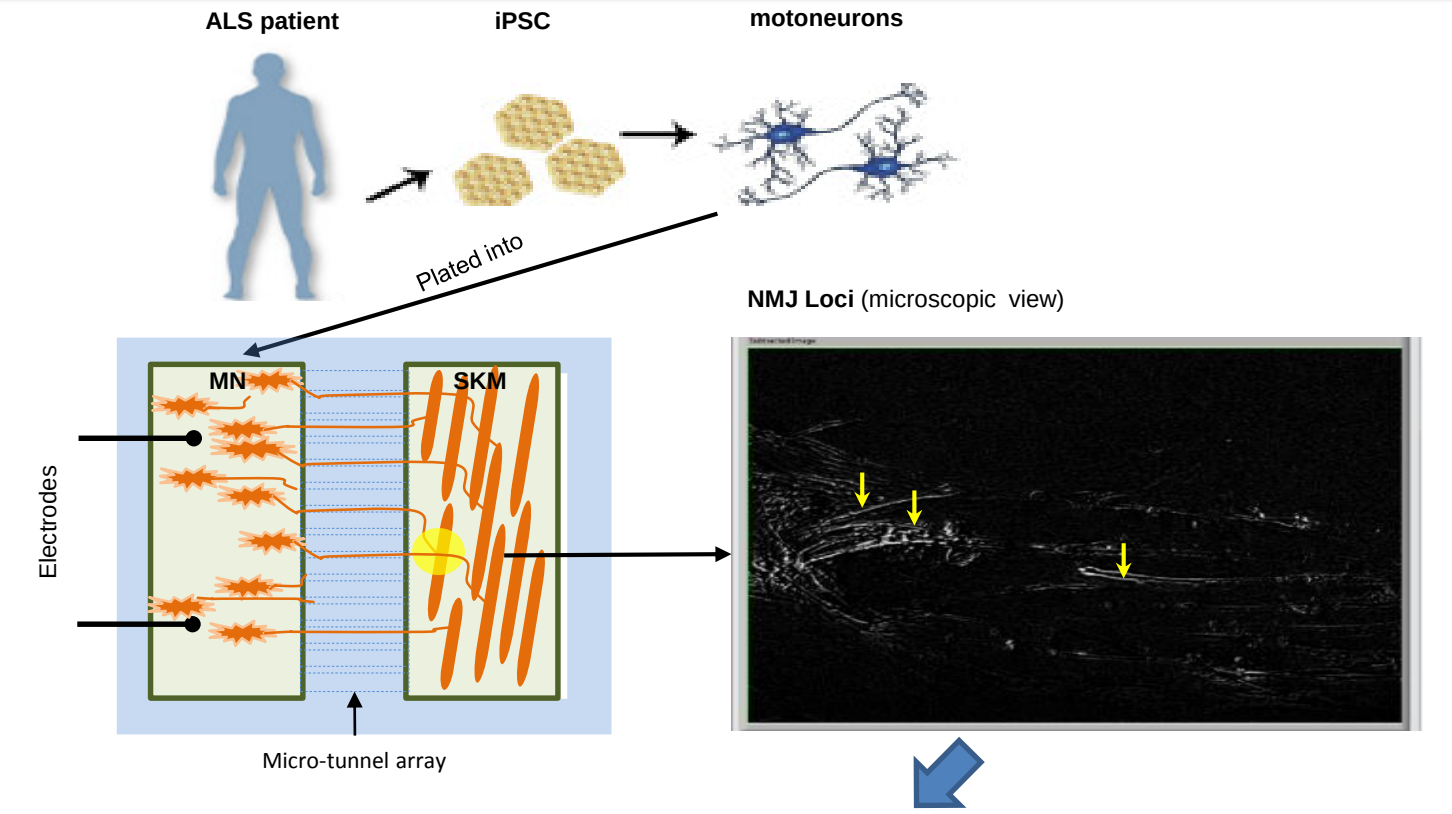

Neuromuscular Junction - Annulospiral

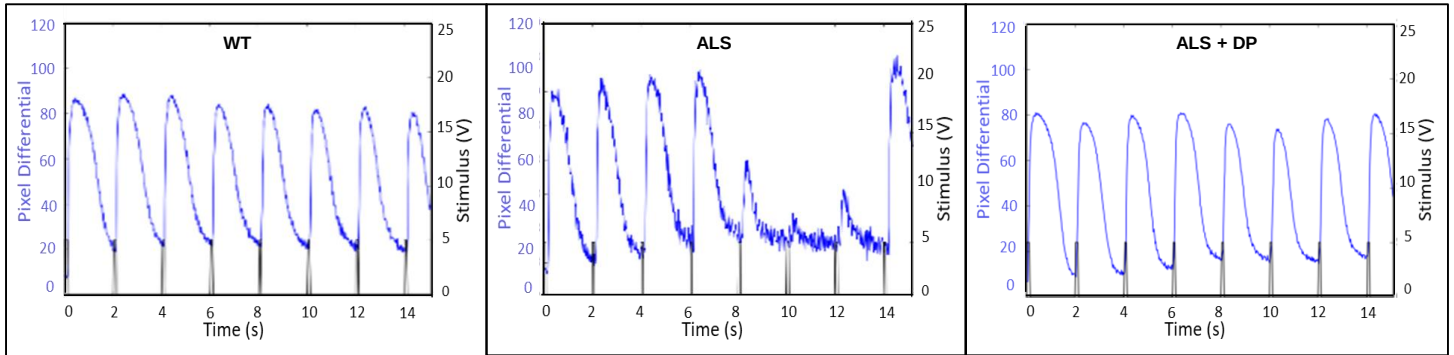
Monophasic Dose-Response for BOTOX at 0.33 Hz



Santhanam et al, ***Biomaterials***, 166:64-78 (2018)

A Human-Based Functional NMJ System for Personalized ALS Modeling and Drug Testing

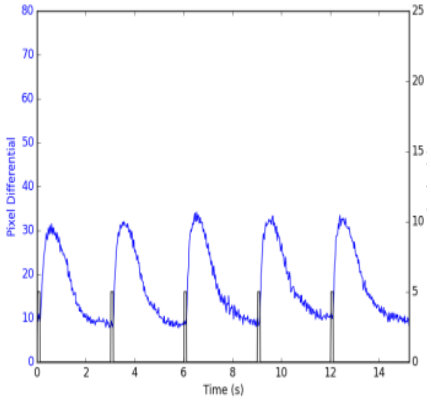




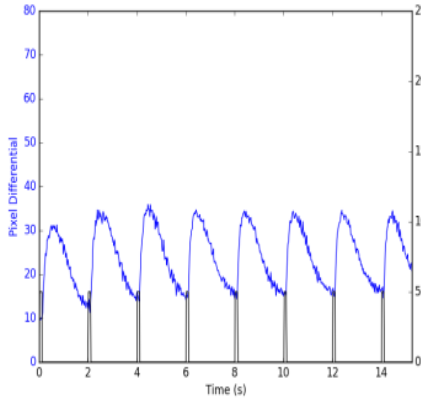
Parameters Analyzed

- **Number of functional NMJs/chamber (before and after extensive stimulation)**
- **NMJ stability (post-NMJ/pre-NMJ)**
- **NMJ function under different stimulation frequencies (0.33 Hz, 0.5 Hz, 1 Hz, 2 Hz)**
  --- NMJ fidelity (number of muscle contractions induced by MN stimulation/total number of stimulations)

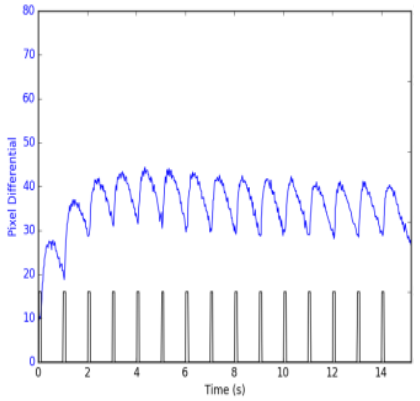
0.33 Hz



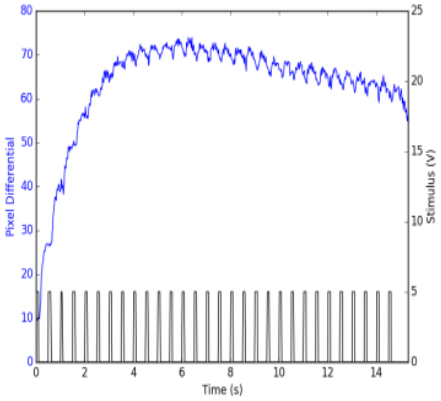
0.5 Hz



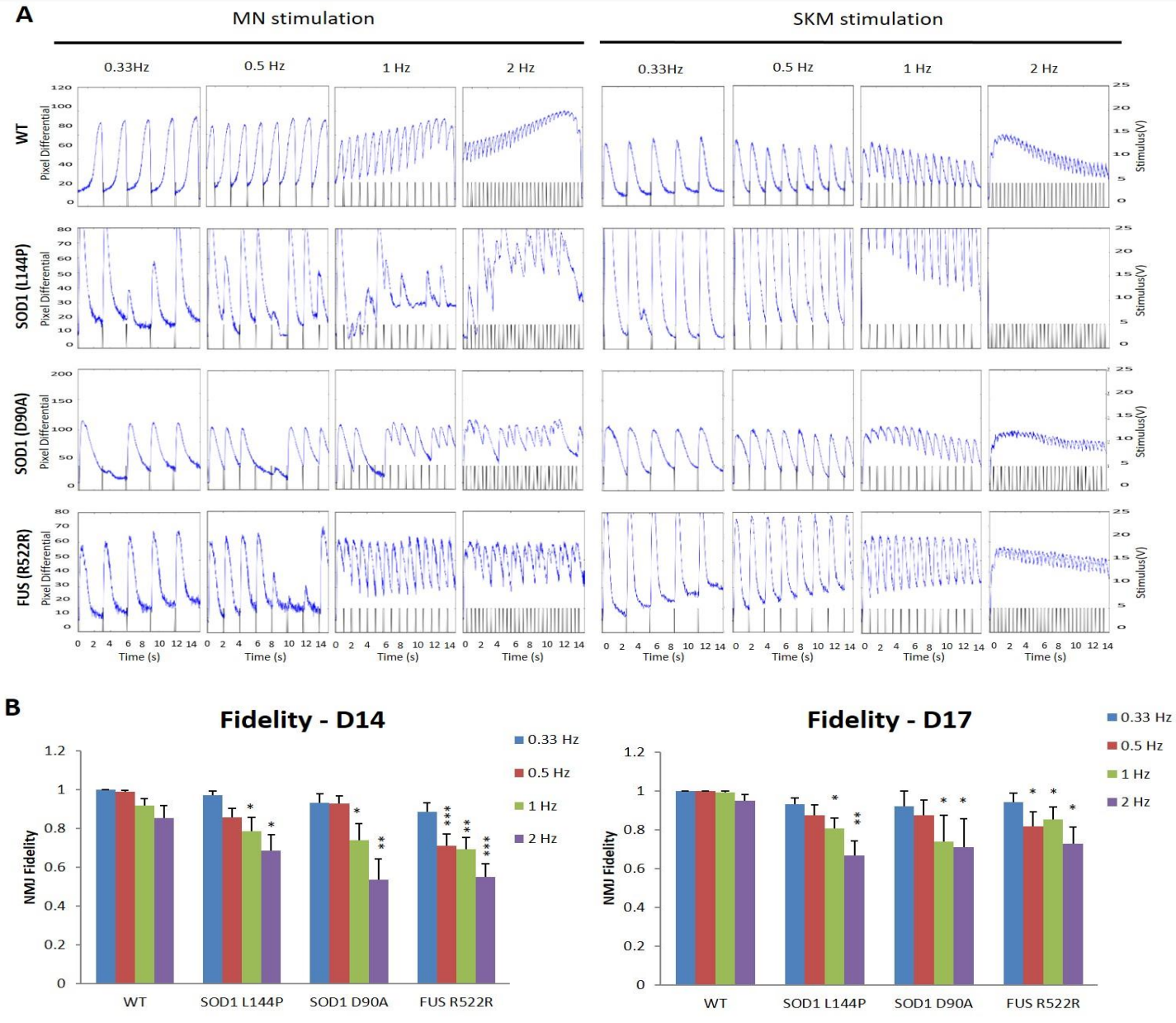
1 Hz



2 Hz

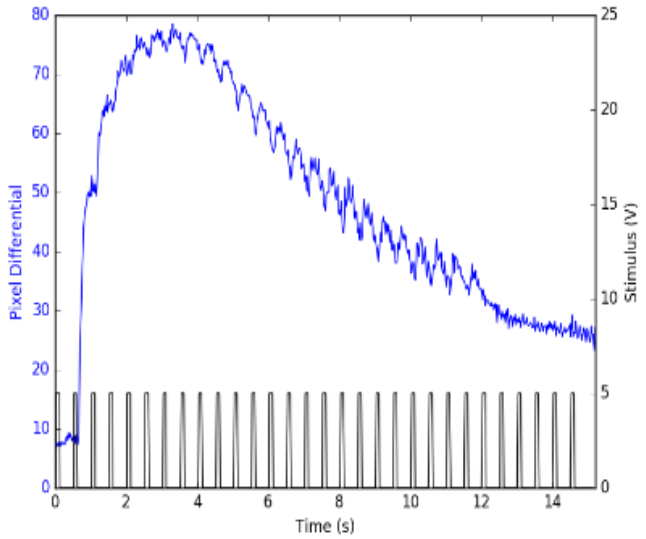


Compromised NMJ fidelity in ALS-NMJs



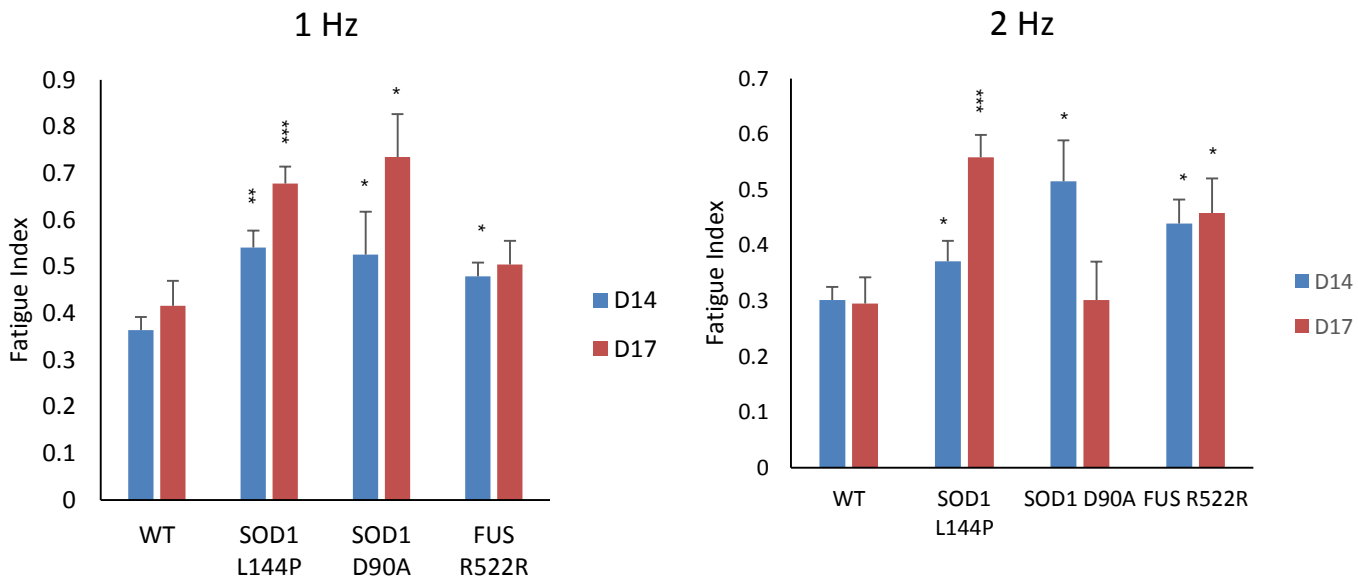
Increased NMJ fatigue index in ALS-NMJs

A

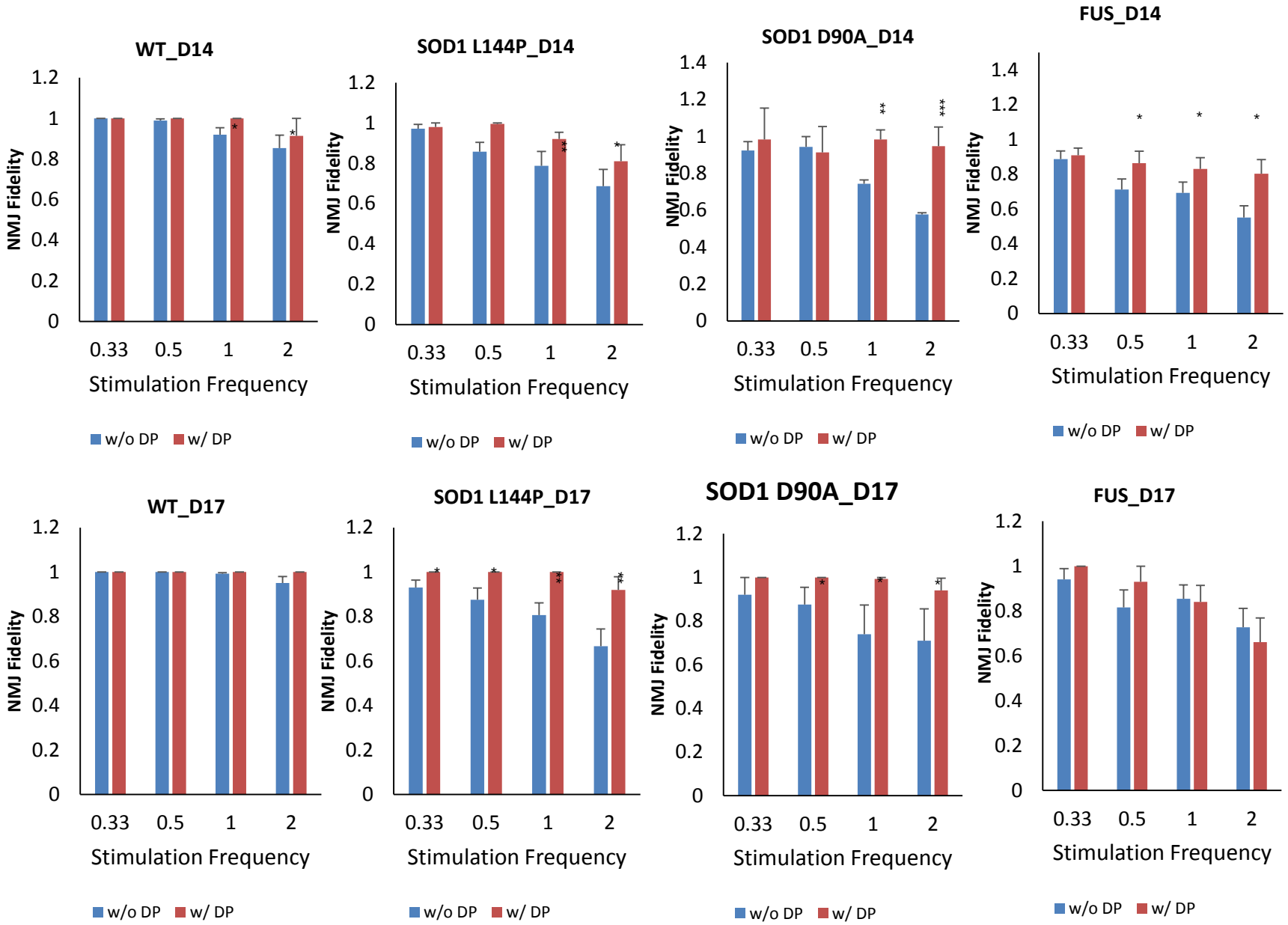


Fatigue Index
=
1- [Area Under Curve/(Peak Force*Time)]

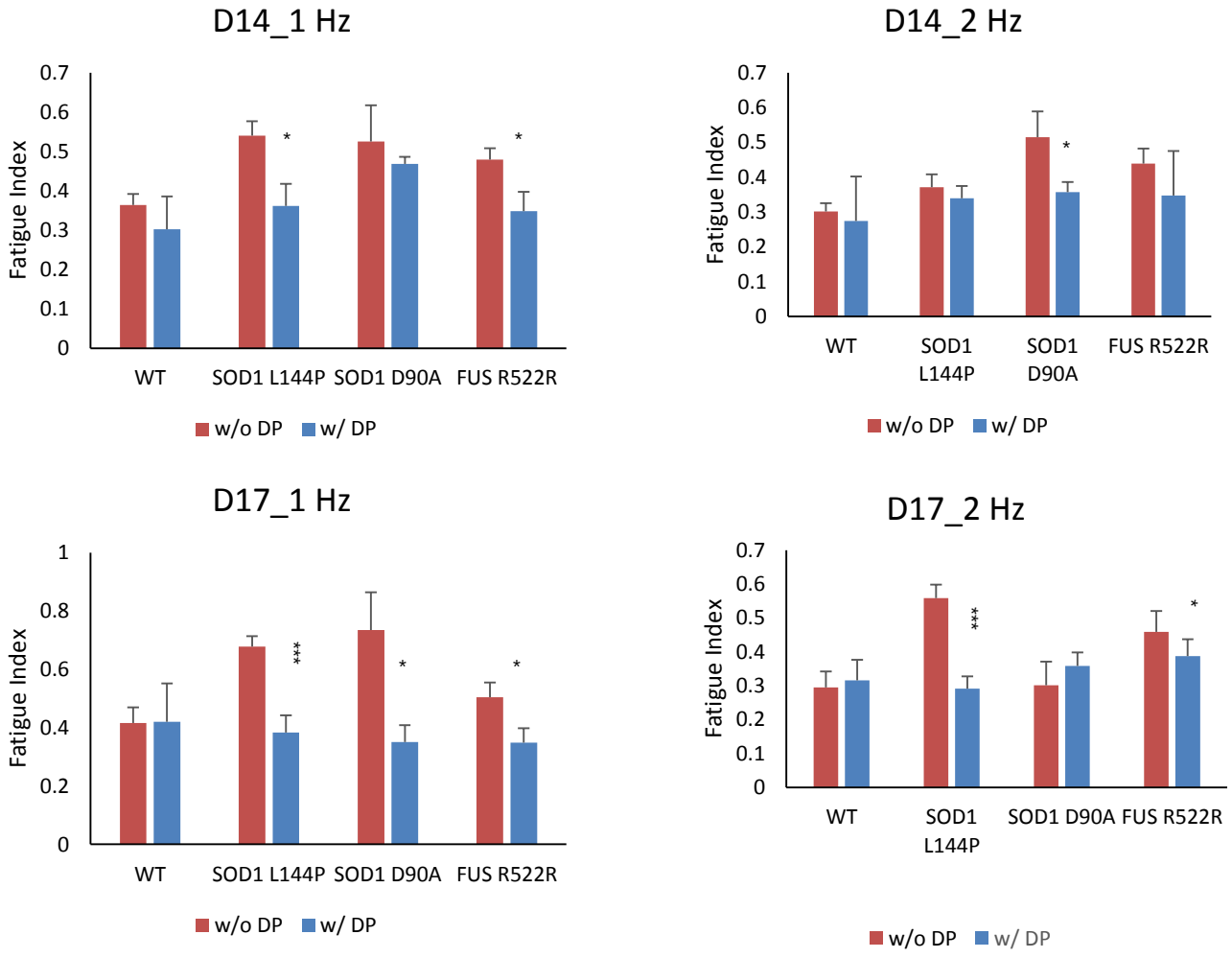
B



Rescue of NMJ fidelity by DP



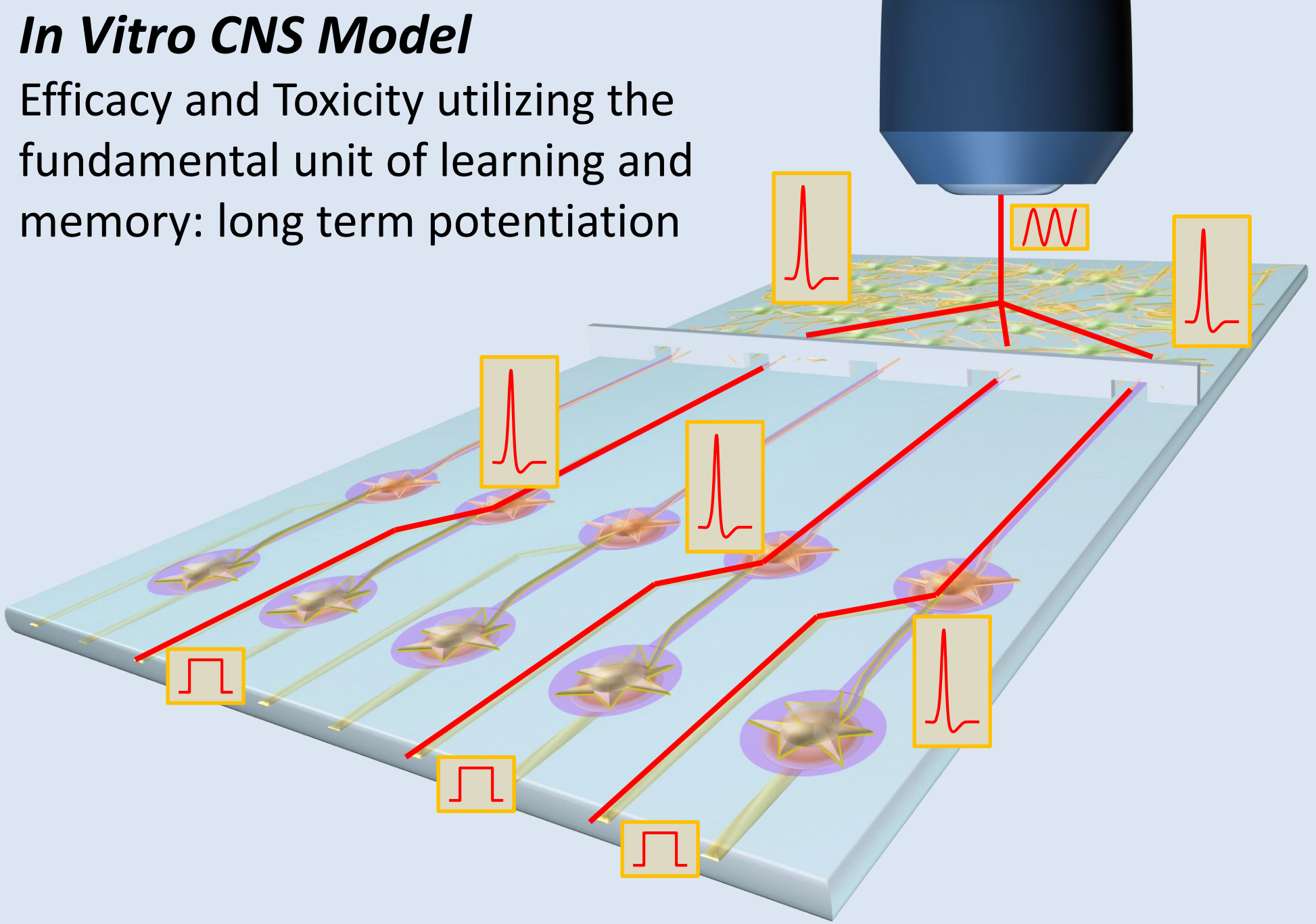
Rescue of NMJ fatigue by DP



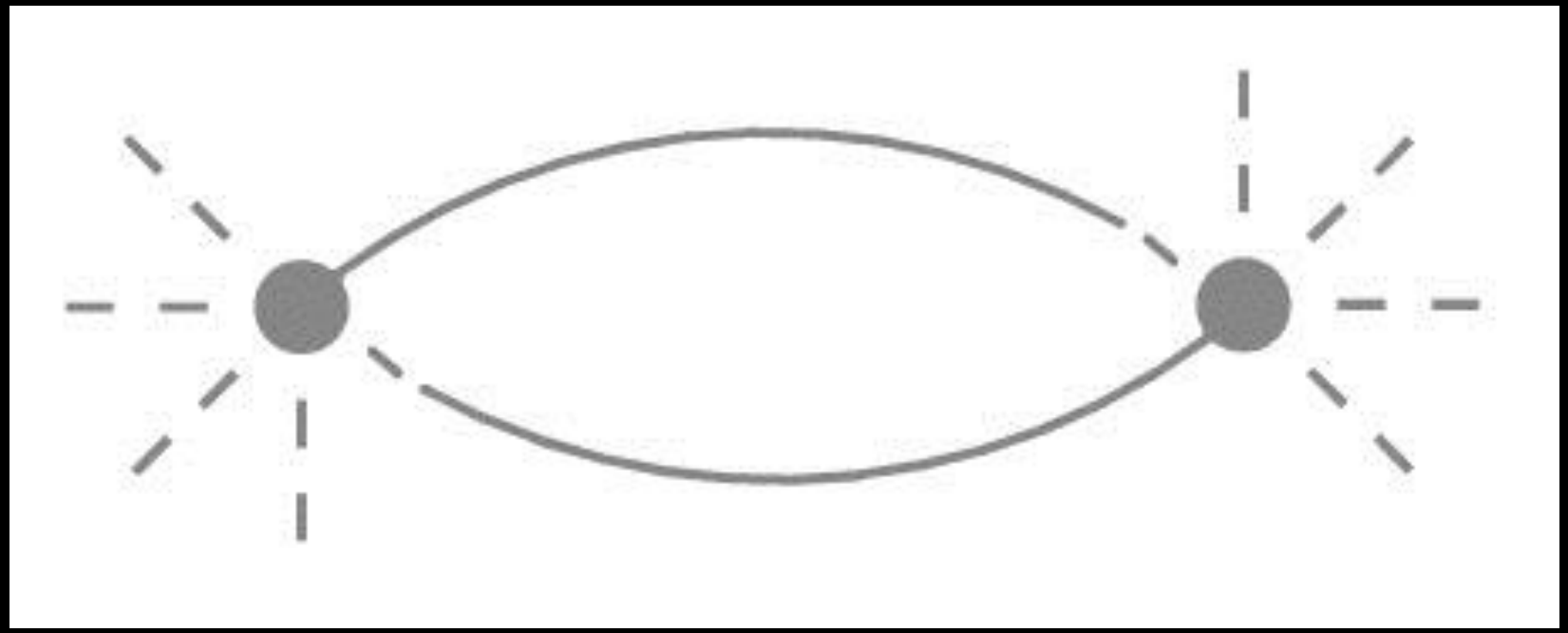
X. Guo,etal., “A human-based functional NMJ system for personalized ALS modeling and drug testing,” ***Adv. Therapuetics*** In Press, Online First: DOI: 10.1002/adtp.202000133, 2020)

In Vitro CNS Model

Efficacy and Toxicity utilizing the fundamental unit of learning and memory: long term potentiation

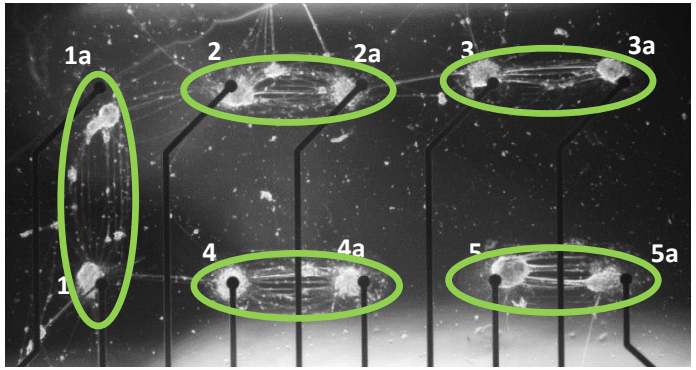


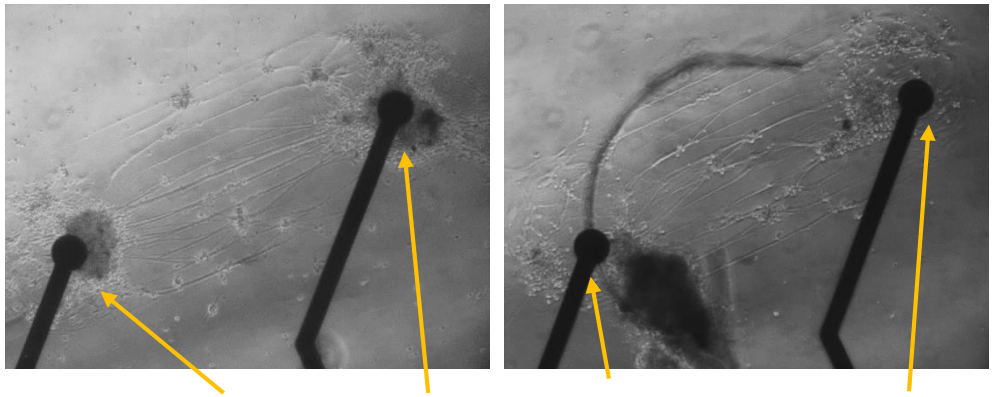
Fundamental unit of long-term potentiation



Patterned Neural Networks on MEAs - Long Term Potentiation (LTP)

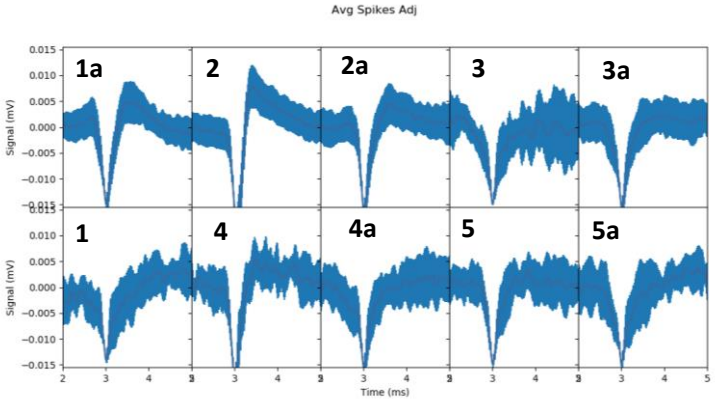
Whole-MEA View





- High magnification phase images indicating long-term pattern conformity and network formation

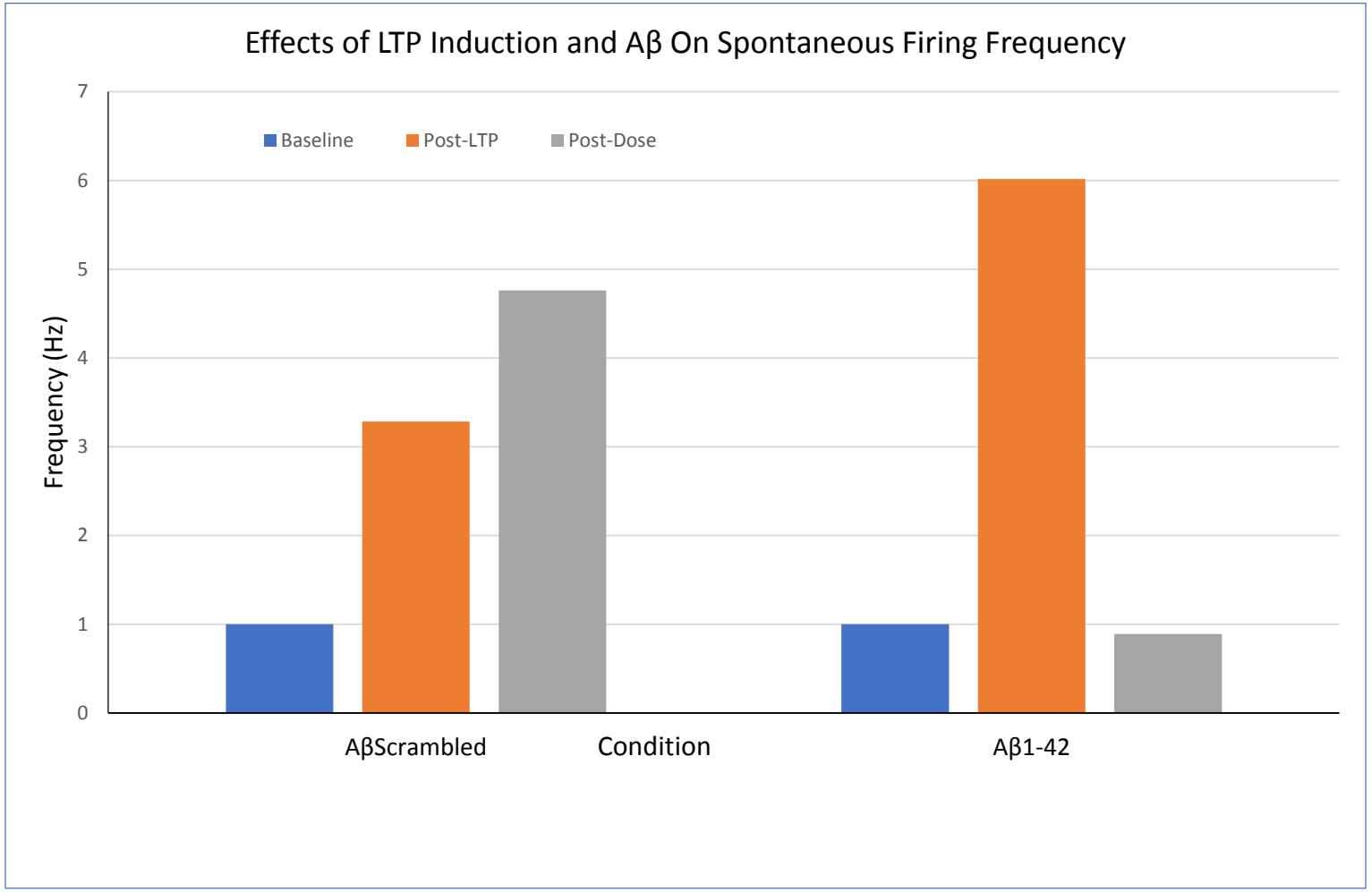
Ave. Electrode Signal Recordings



Depolarizing Potentials Recorded from MEAs

- 5 network pairs per MEA
- 45 days *in vitro*
- Spontaneous action potentials recorded on electrodes from paired neural circuits

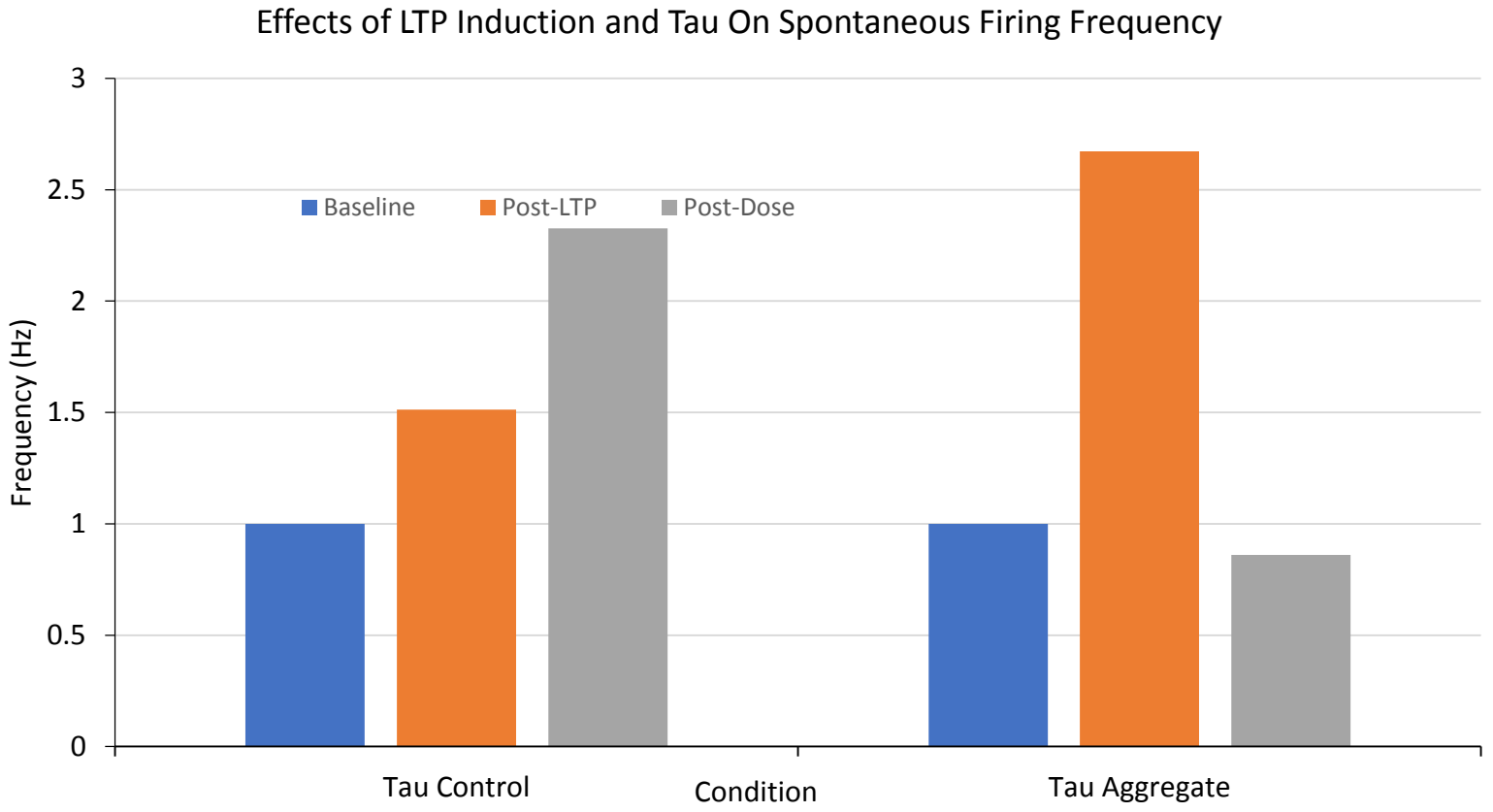
Effects of $A\beta_{1\text{-}42}$ on Cortical Neuron Spontaneous Firing Frequency



- LTP can efficiently be induced in cortical neurons grown on MEAs
- Dosing MEAs for one hour with $A\beta_{1\text{-}42}$ after LTP induction abolishes the LTP effects compared to $A\beta_{scrambled}$ treated MEAs

N=4, nested replicates

Effects of Tau Aggregate on Cortical Neuron Spontaneous Firing Frequency

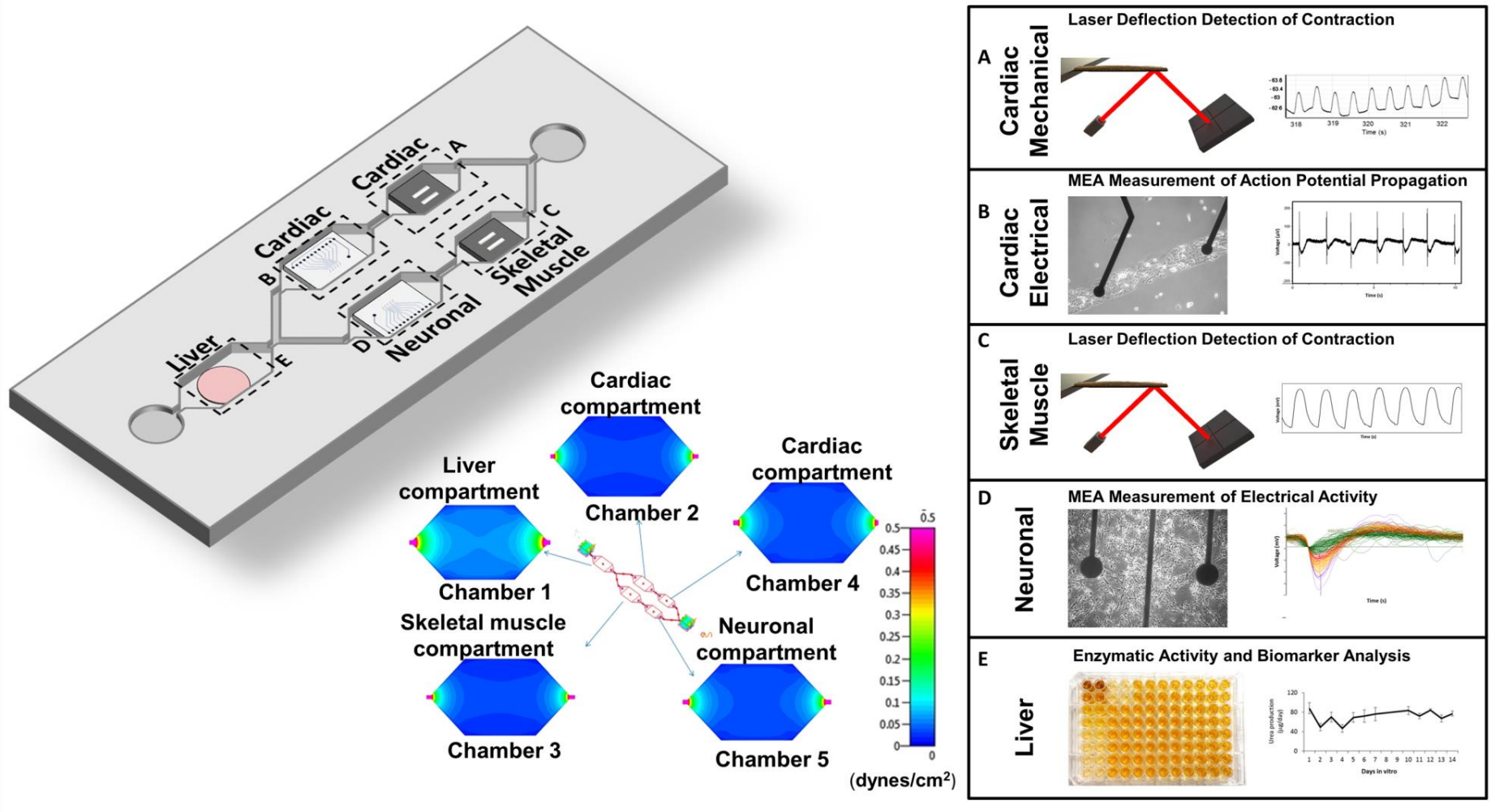


- Dosing MEAs for one hour with tau aggregates after LTP induction abolishes the LTP effects compared to tau buffer control MEAs

N=4, nested replicates

J. Caneus, etal., "A Human Induced Pluripotent Stem Cell-Derived Cortical Neuron Human-on-a chip System to Study Aβ42 and Tau-induced Pathophysiological Effects on Long-Term Potentiation," ***Alzheimer's & Dementia: Translational Research & Clinical Interventions***, Online First: May 28: (2020) DOI: 10.1002/trc2.12029.

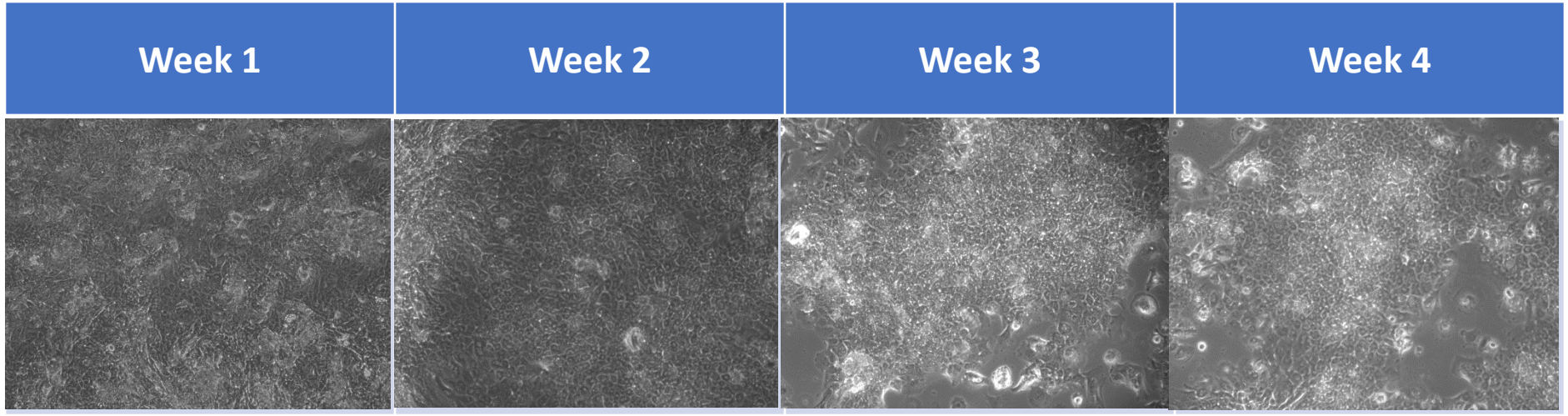
Functional Measurements in 4-Organ Systems

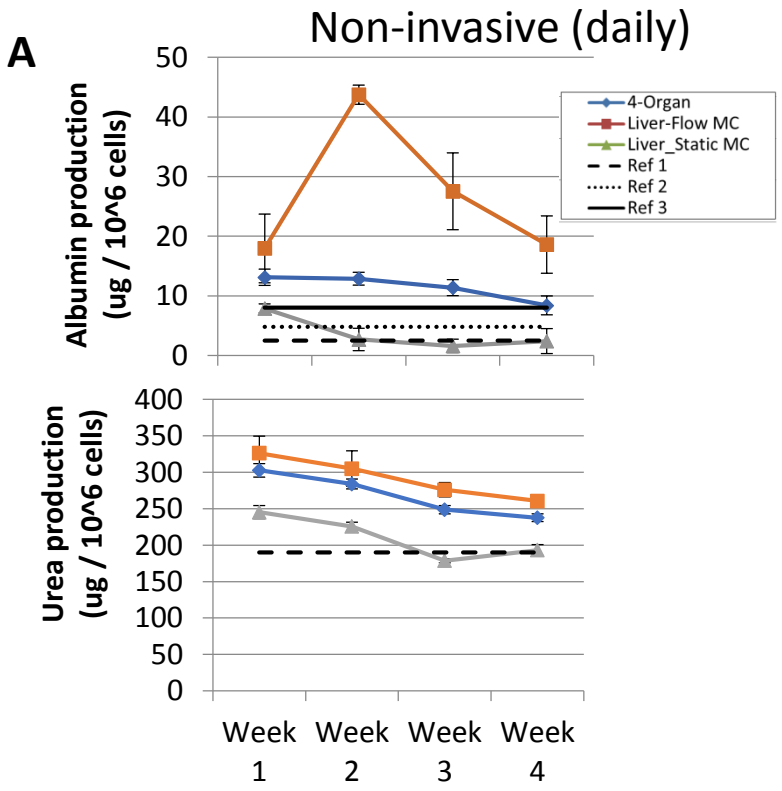


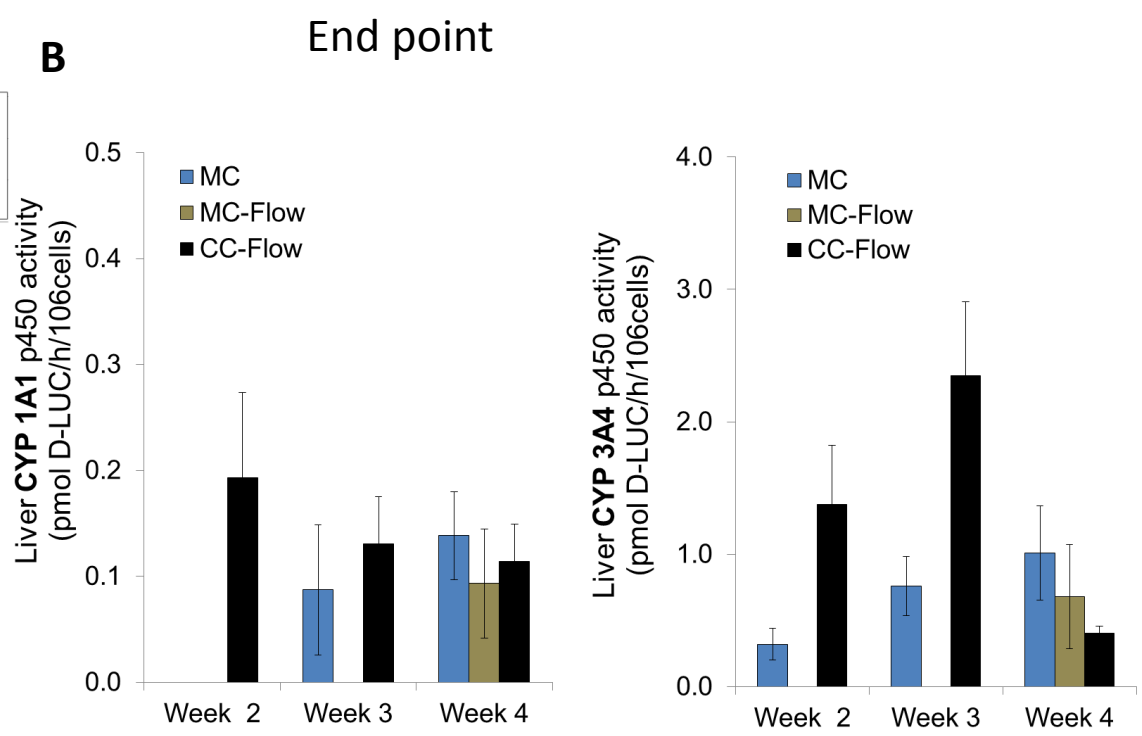
Oleaga et al. 2016. ***Nature Scientific Reports*** "Multi-Organ toxicity demonstration in a functional human *in vitro* system composed of four organs"

Oeaga, et al. 2019. ***Advanced Functional Materials*** "Long-Term Electrical and Mechanical Function Monitoring of a Human-on-a-Chip System"

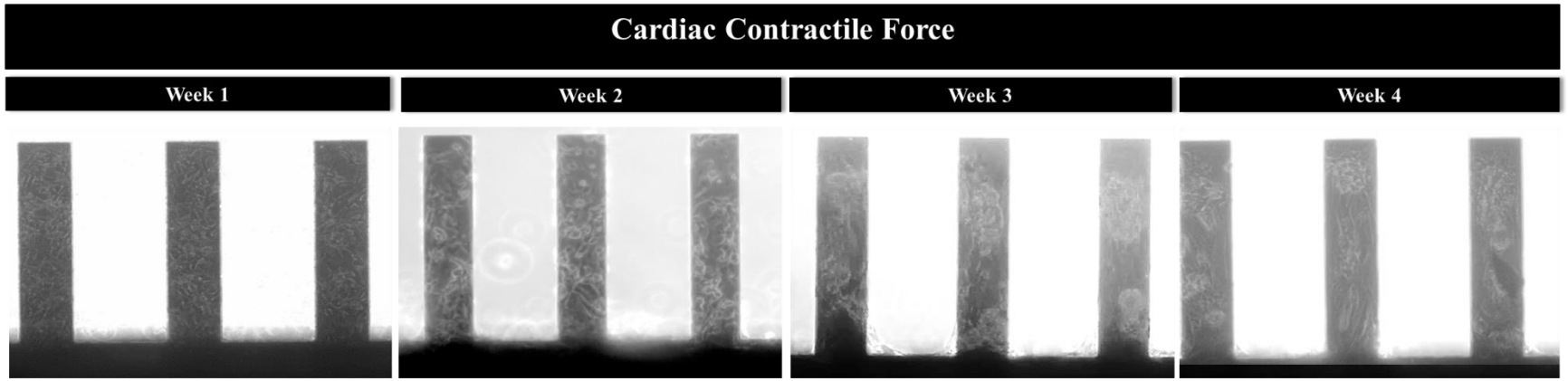
Liver Module

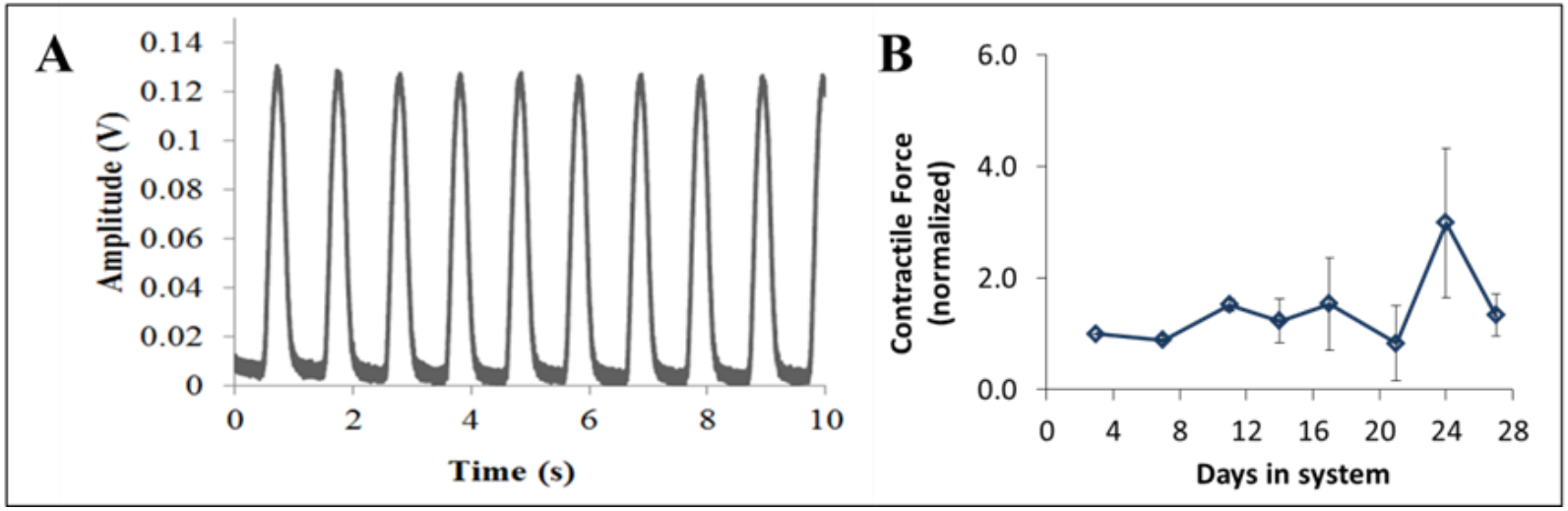




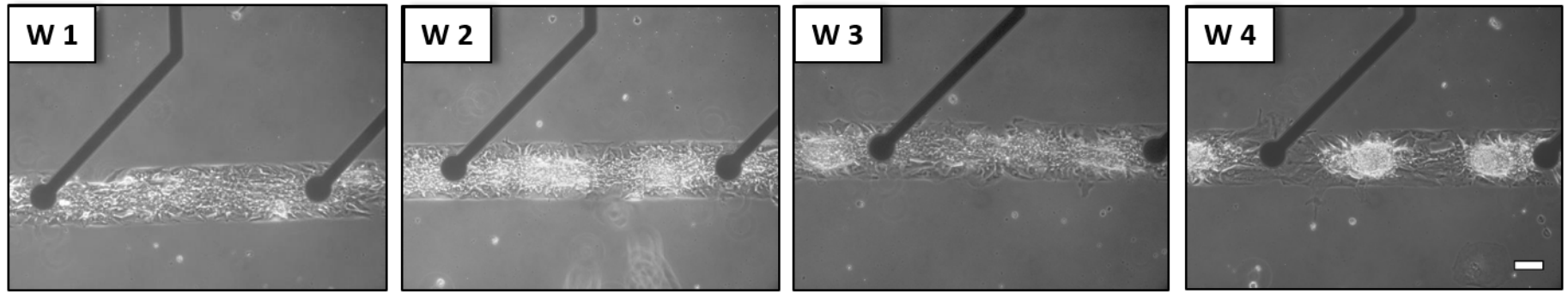


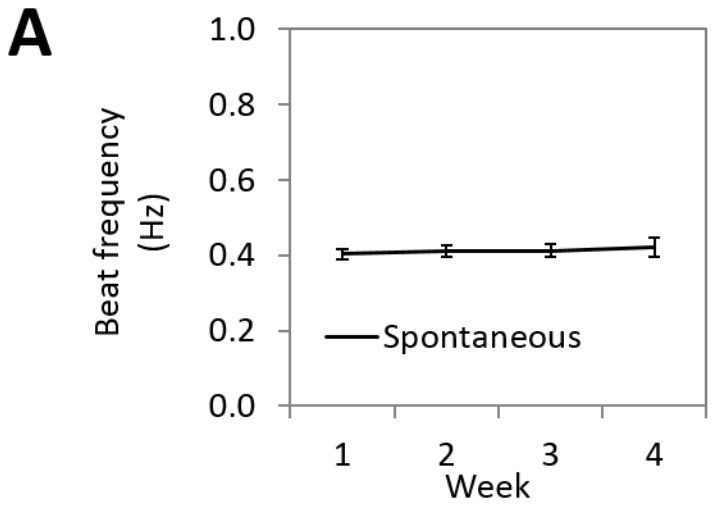
No statistical differences compared to MC static at 28 days

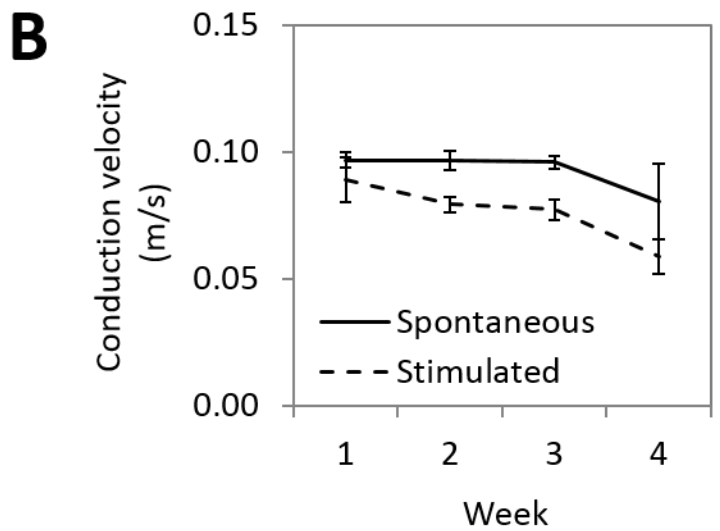


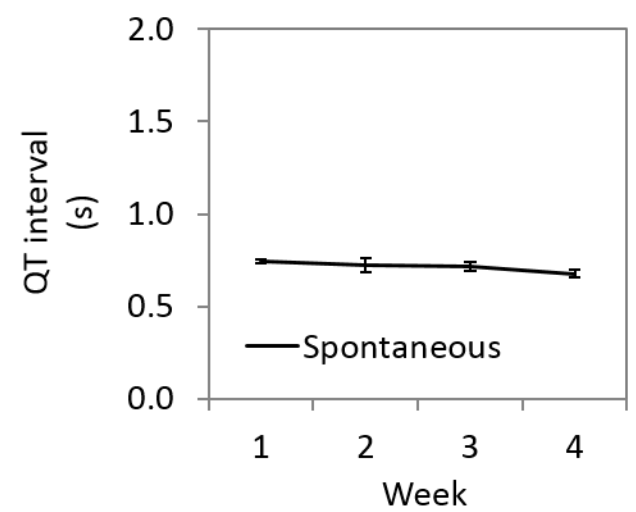


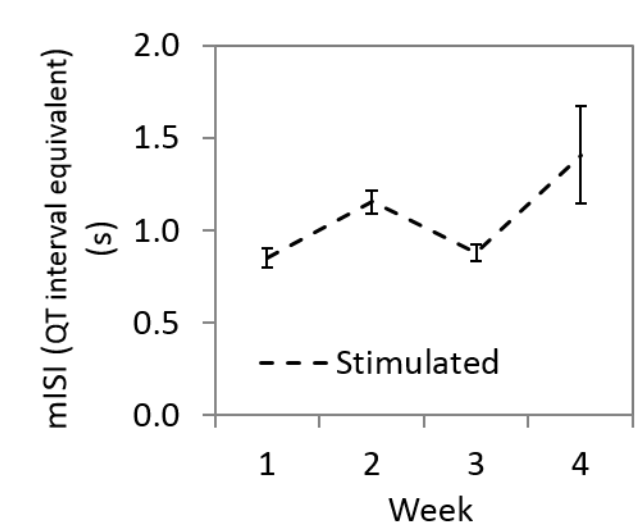
The cardiac module maintained its contractile properties during the 28 days. (A) Example of spontaneous contractile activity output and (B) stable contractile activity indicates stable force generation for cardiomyocytes throughout the 28 day period.

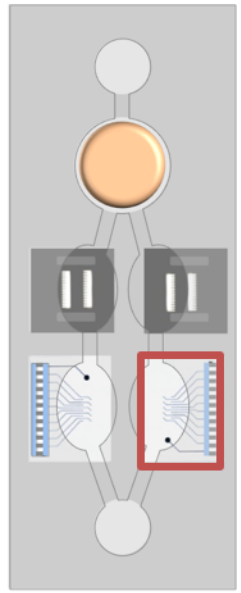




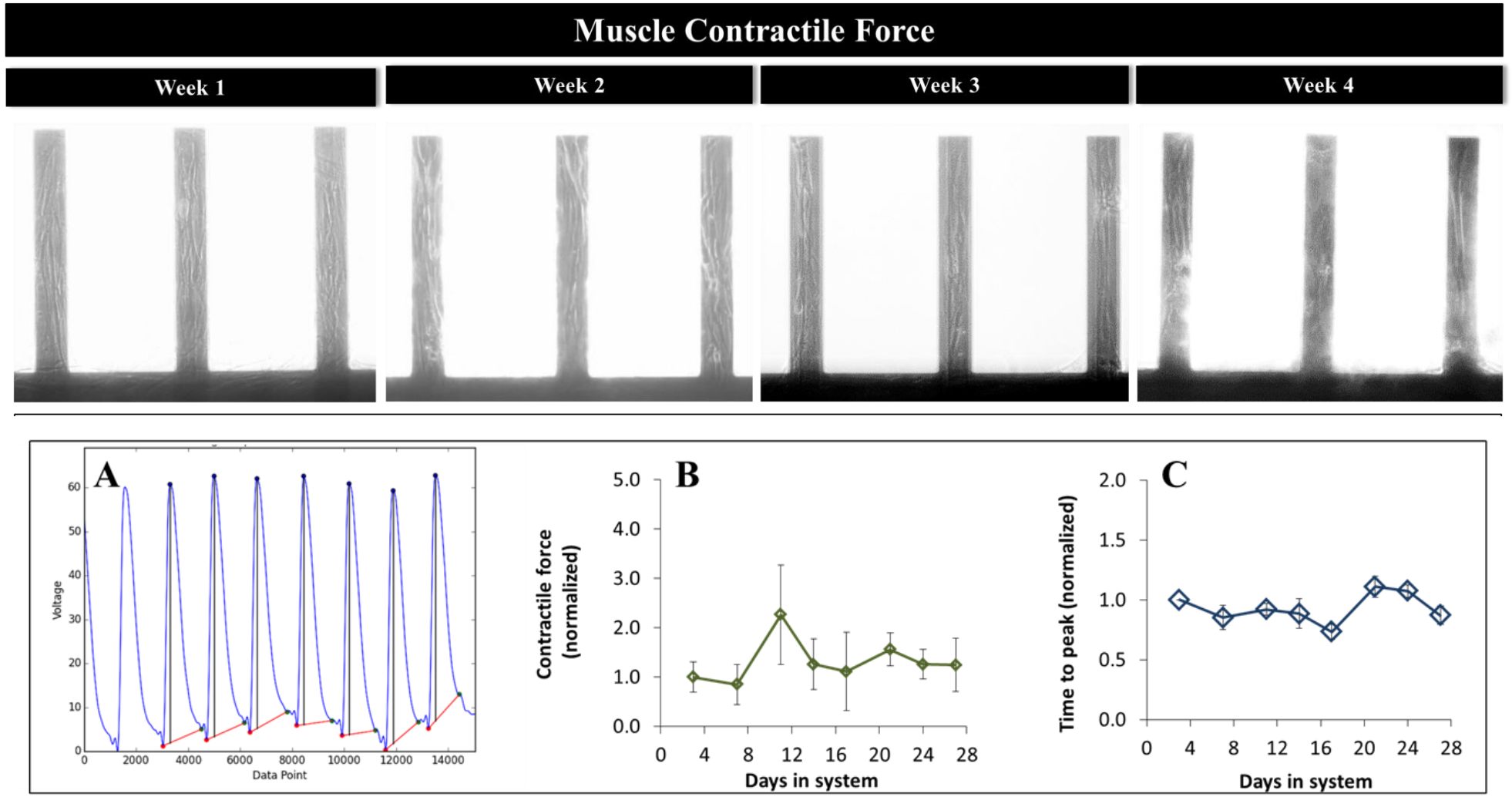




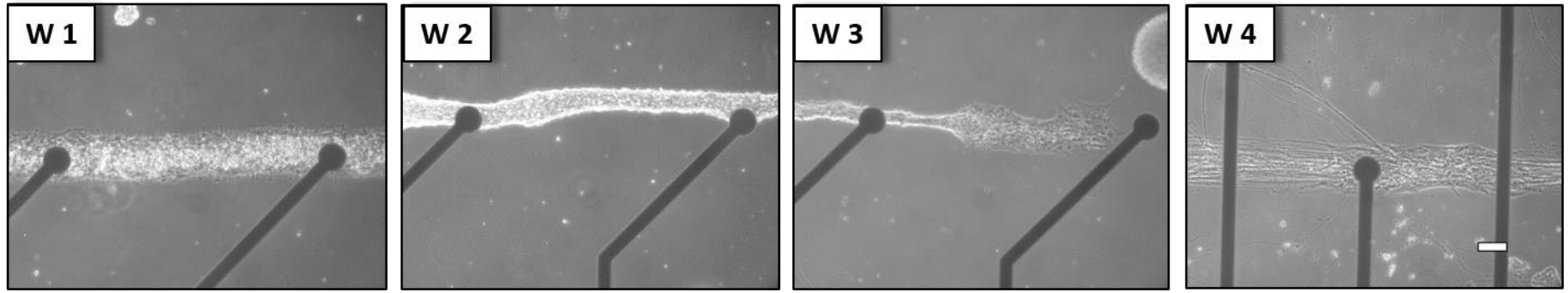


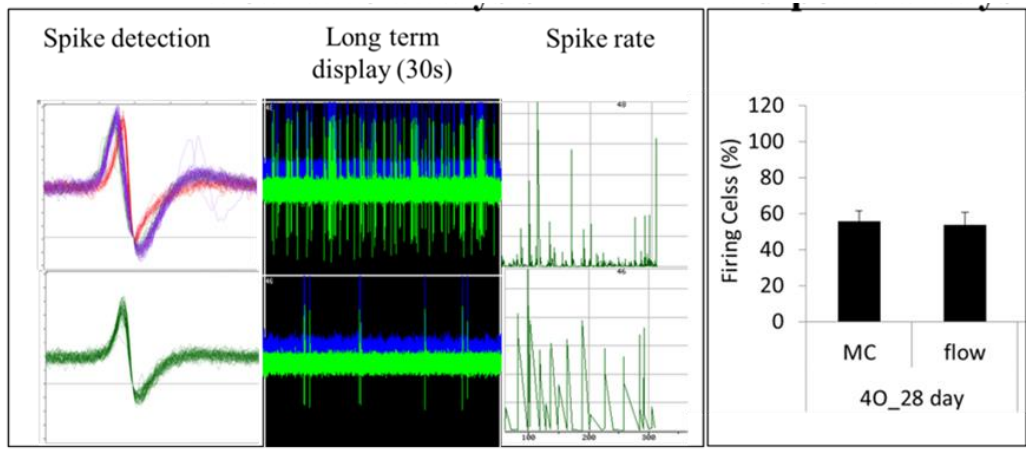


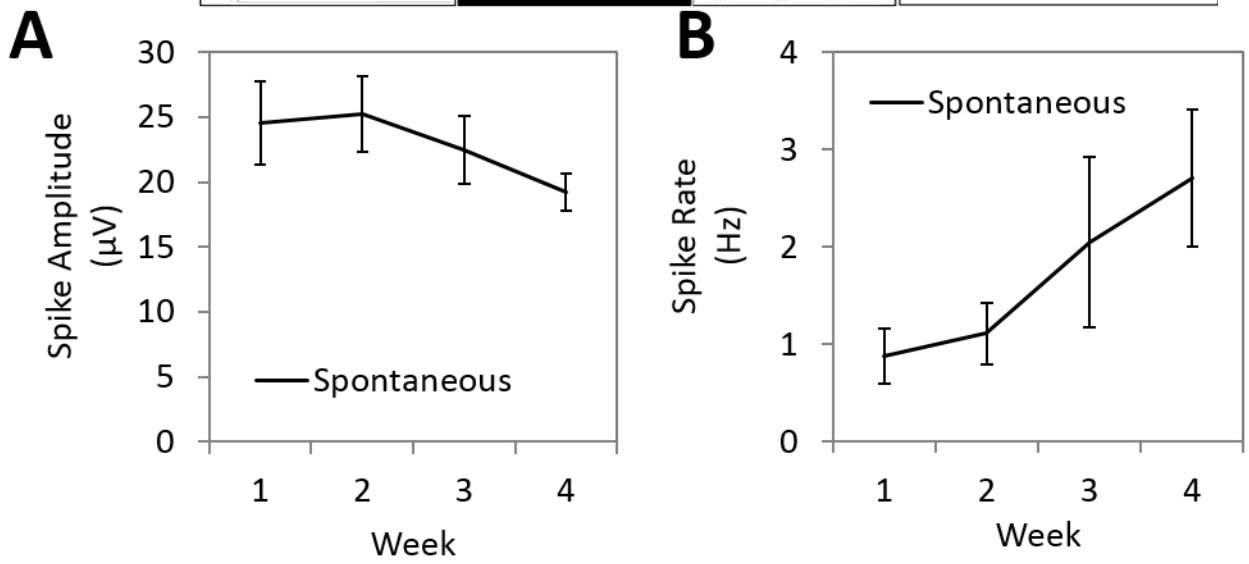
CARDIOMYOCYTES

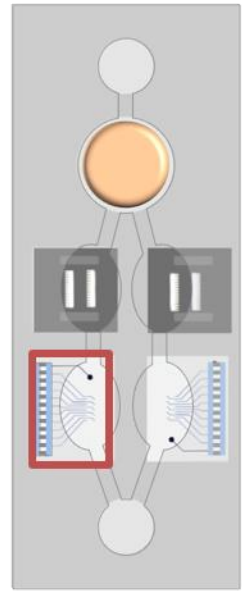


The muscle module maintained its morphological characteristics for the entire 28 days under flow. (A) Example of muscle contractile recordings, (B) stable contractile force and (C) time to peak indicates stable function of the myotubes throughout the 28 day period.



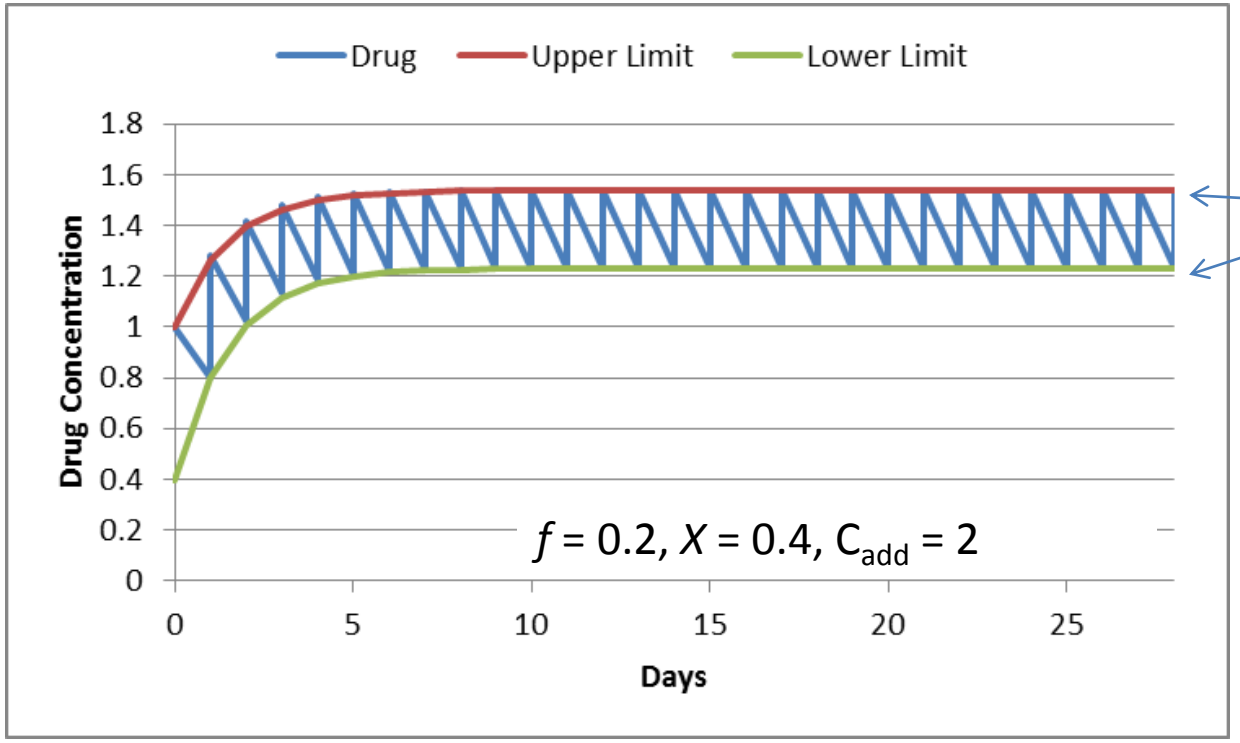






MOTONEURONS

Iterative Data: Drug Concentration



Match Equations

Steady state concentration of drug After medium change

$$C_{drug,ss,I} = \frac{C_{add}X}{1-(1-f)(1-X)}$$

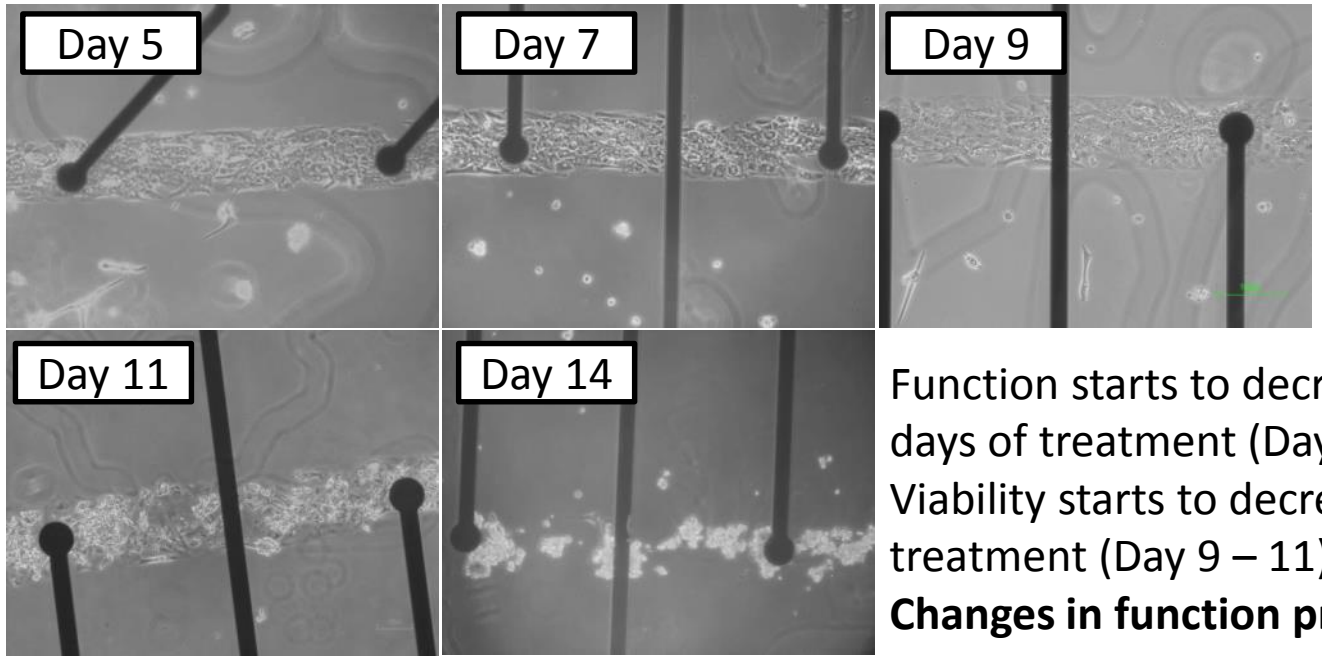
Right before medium change

$$C_{drug,ss,F} = \frac{C_{add}X(1-f)}{1-(1-f)(1-X)}$$

Upper and Lower limit follow “Carburization” like curve – exponential with time constant of

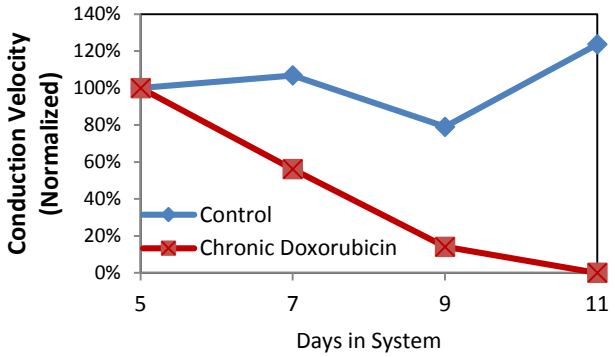
$t_c = \pi(1-f)(1-X)$

Chronic Low-Dose Doxorubicin

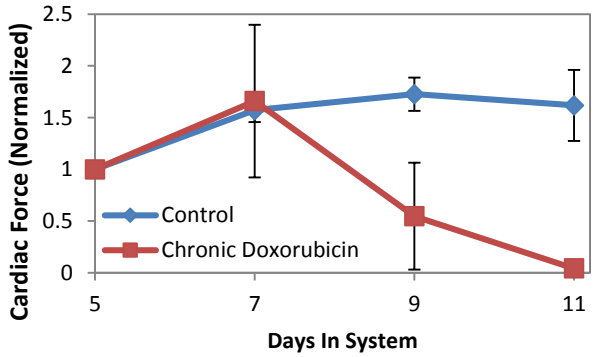


0.5 µM Doxorubicin repeated dose started at day 5 and continues to day 14

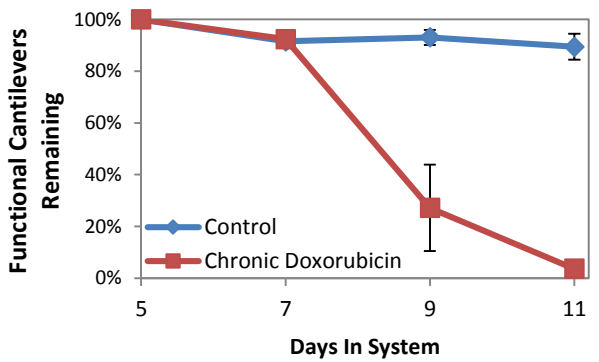
Function starts to decrease between 2 and 4 days of treatment (Day 5 – 7)
Viability starts to decrease around 4 to 6 days of treatment (Day 9 – 11)
Changes in function precede changes in viability



Steady decrease in Conduction velocity

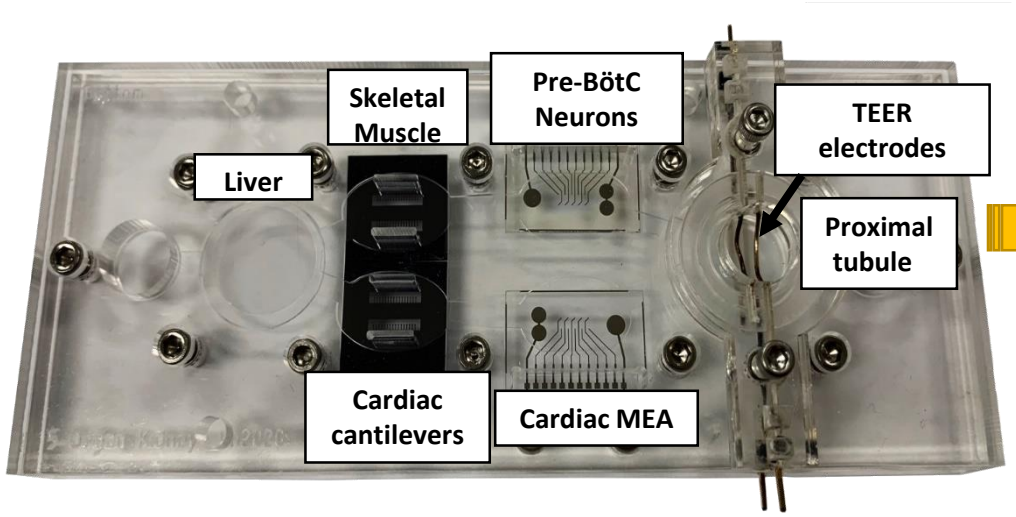


Weakening of contractile force



Steady loss of contractile functionality

Develop an opioid overdose (OD) model using a multi-organ human-on-a-chip system



- Evaluate the acute and chronic effects of OD and OD treatments such as naloxone
- Evaluate treatment efficacy and toxicity for cardiac, liver, skeletal muscle, and kidney
- Evaluate opioid OD and OD treatments in the presence of recirculating monocytes
- Evaluate opioid OD and OD treatments in the presence of disease comorbidities including cardiomyopathy and acute infection

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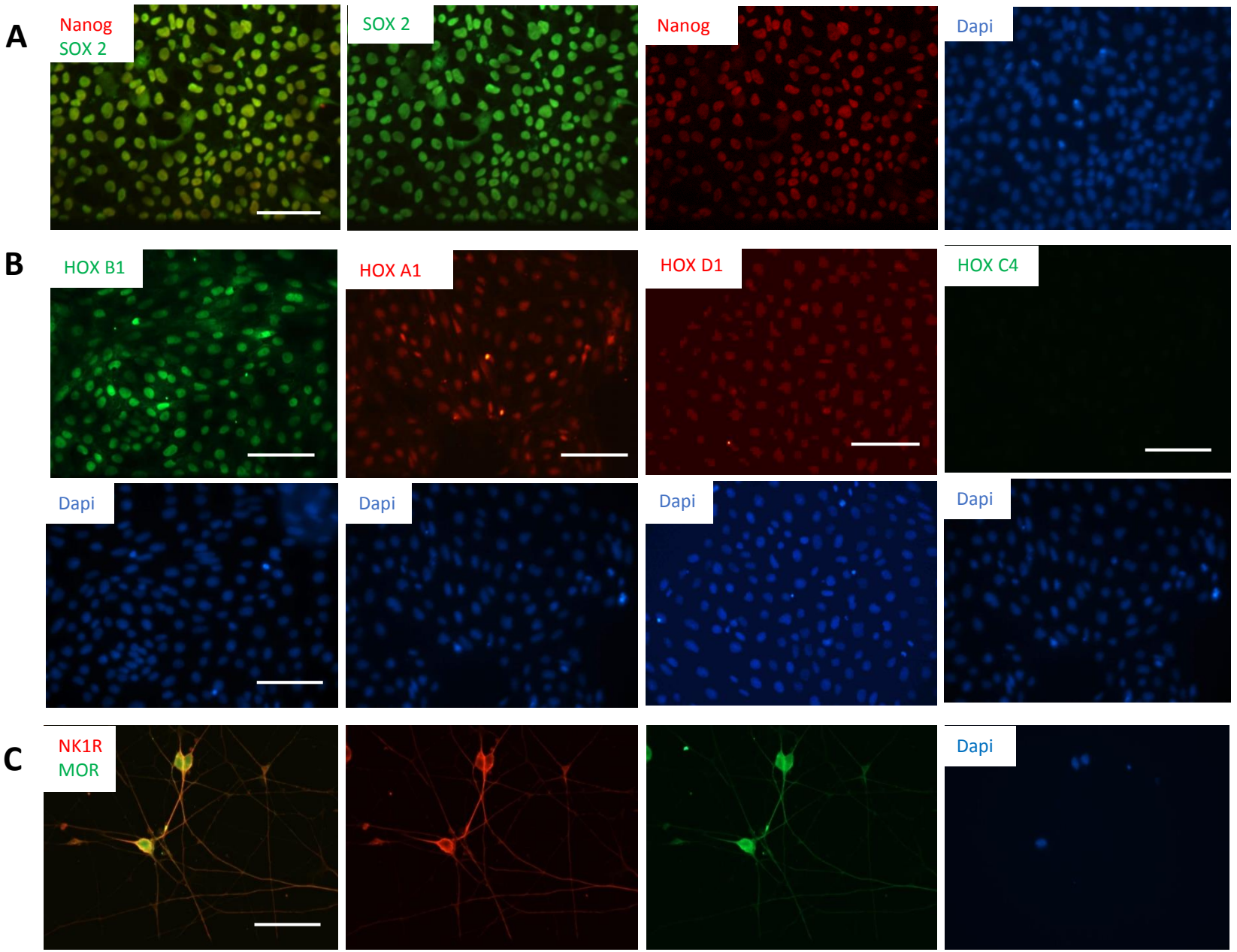
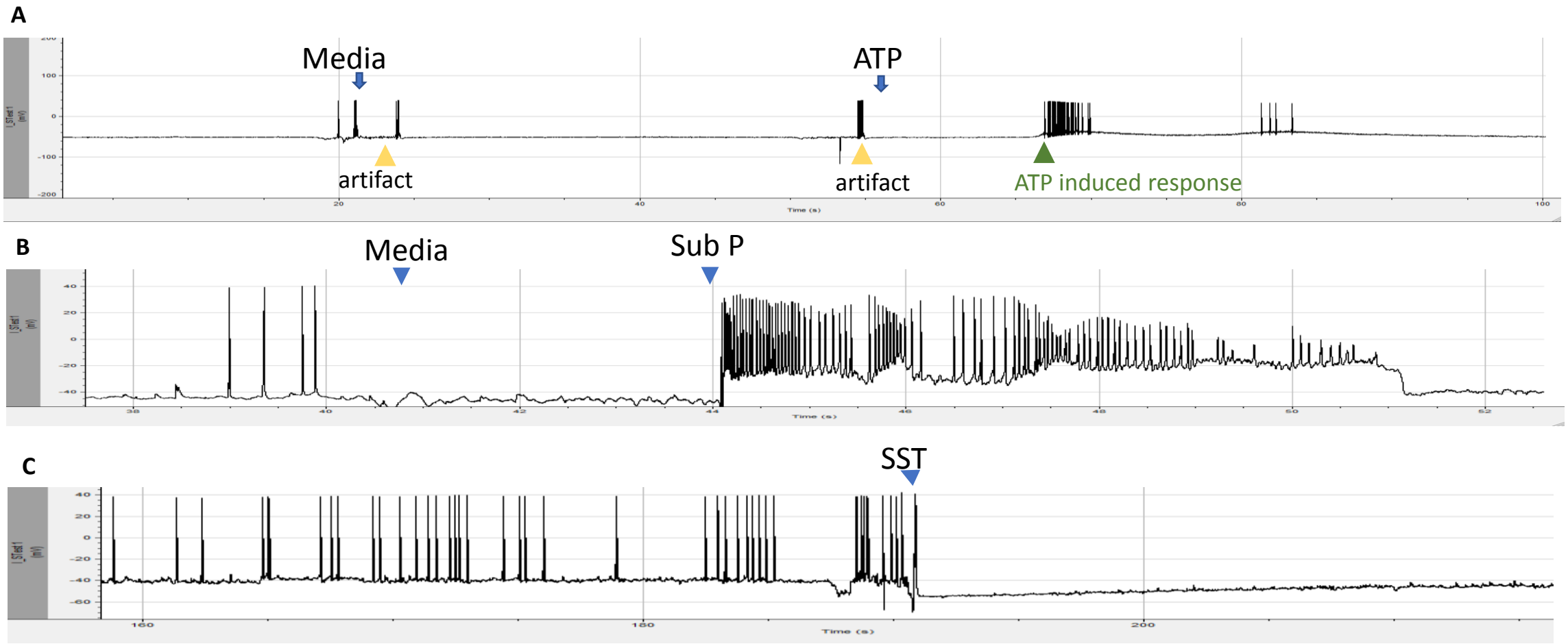
Expression of stage markers correspondent to Pre-BotC differentiation



Differentiation of pre-BotC neurons from hiPSC characterized by immunocytochemistry. A) HiPSCs stained positive to pluripotent markers Nanog and SOX2. Scale bar: 100 µm. **B)** Cells at early stage of differentiation expressed the set of rohmbomeric genes correspondent to the derivative region for preBotC complex during development: positive to HoxA1, HoxB1 and HoxD1 but negative to HoxC1. Scale bar: 100 µm. **C)** Differentiated neurons plated at low density were stained positive for the typical preBotC markers NK1R and MOR. Scale bar: 50 µm.

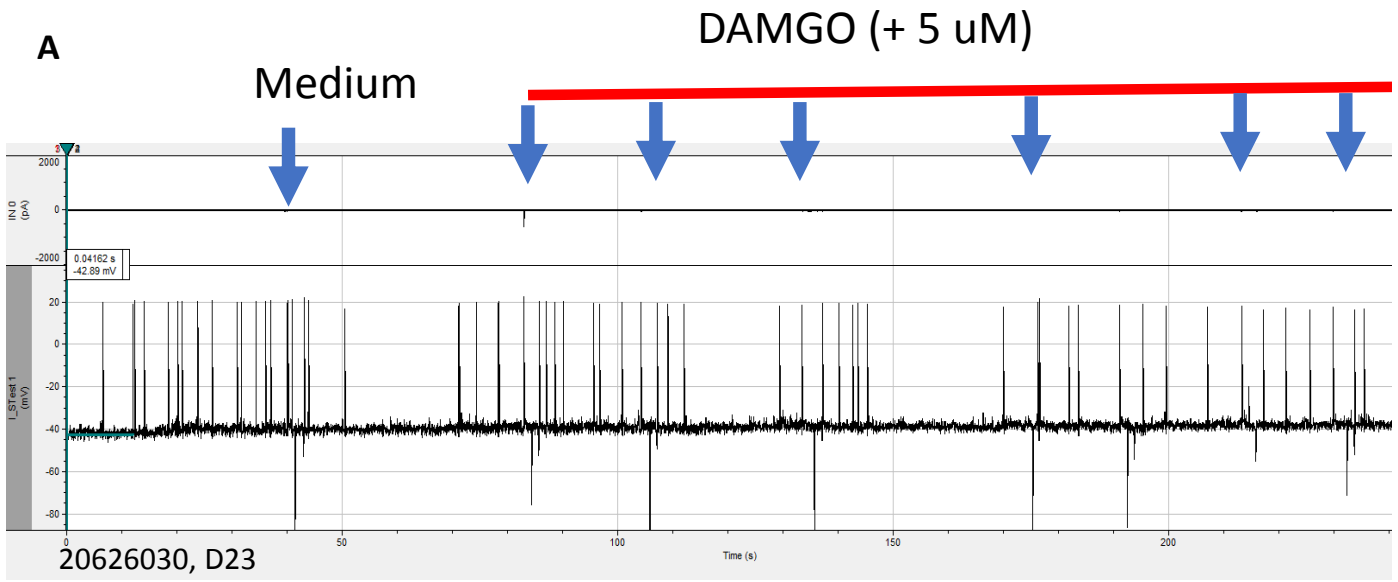
→ **Successfully differentiated Pre-BotC neurons from human iPSC**

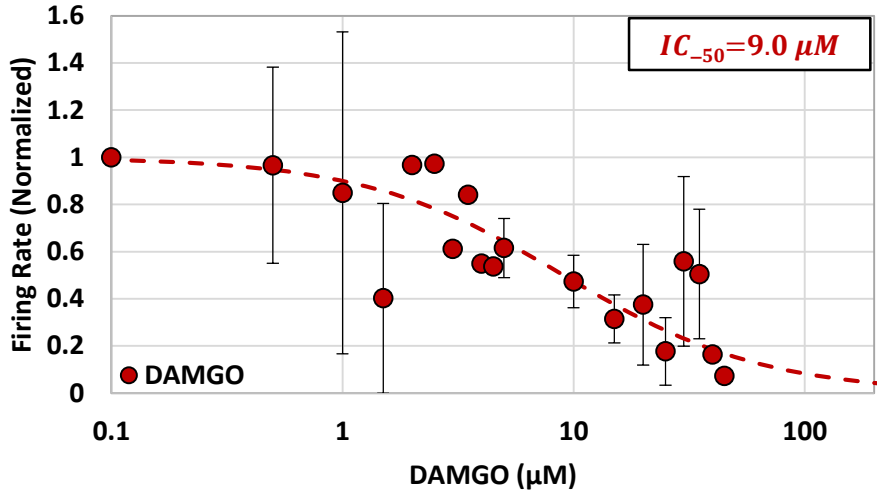
Patch clamp recordings from iPSC-Pre-Botc neurons: ATP, Substance P (Sub P) and Somastatin (SST)



Patch clamp analysis of iPSC-pre-BotC neurons. A) Under gapfree condition, dosing of ATP (100 µM) induced depolarization and burst of action potential. **B)** Treatment with Substance P (Sub P) increases iPSC-Pre-BotC neuronal activity. **C)** Treatment with Somatostatin (SST) inhibits iPSC-Pre-BotC neuronal activity

iPSC-PreBotc neurons demonstrated reduction of neuroactivity in response to DAMGO



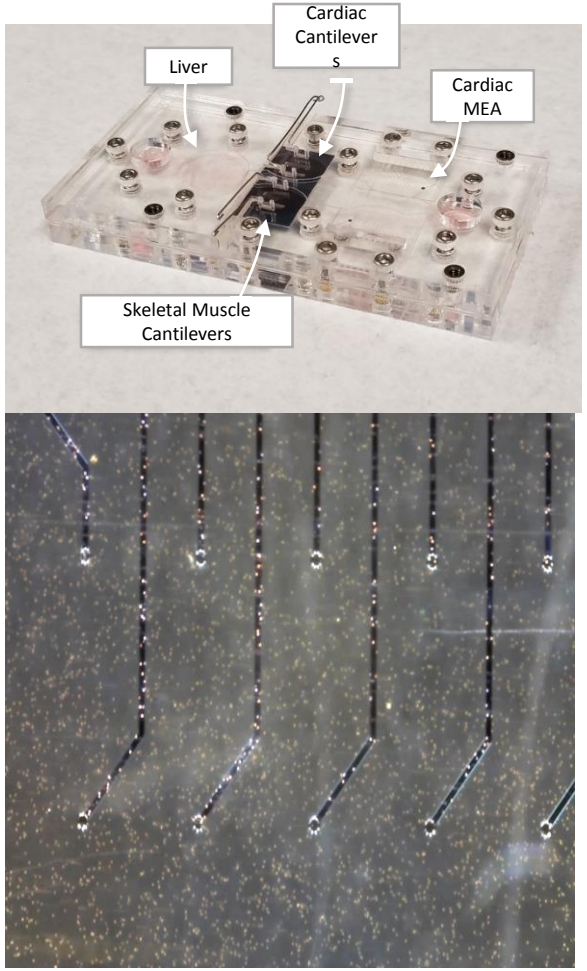


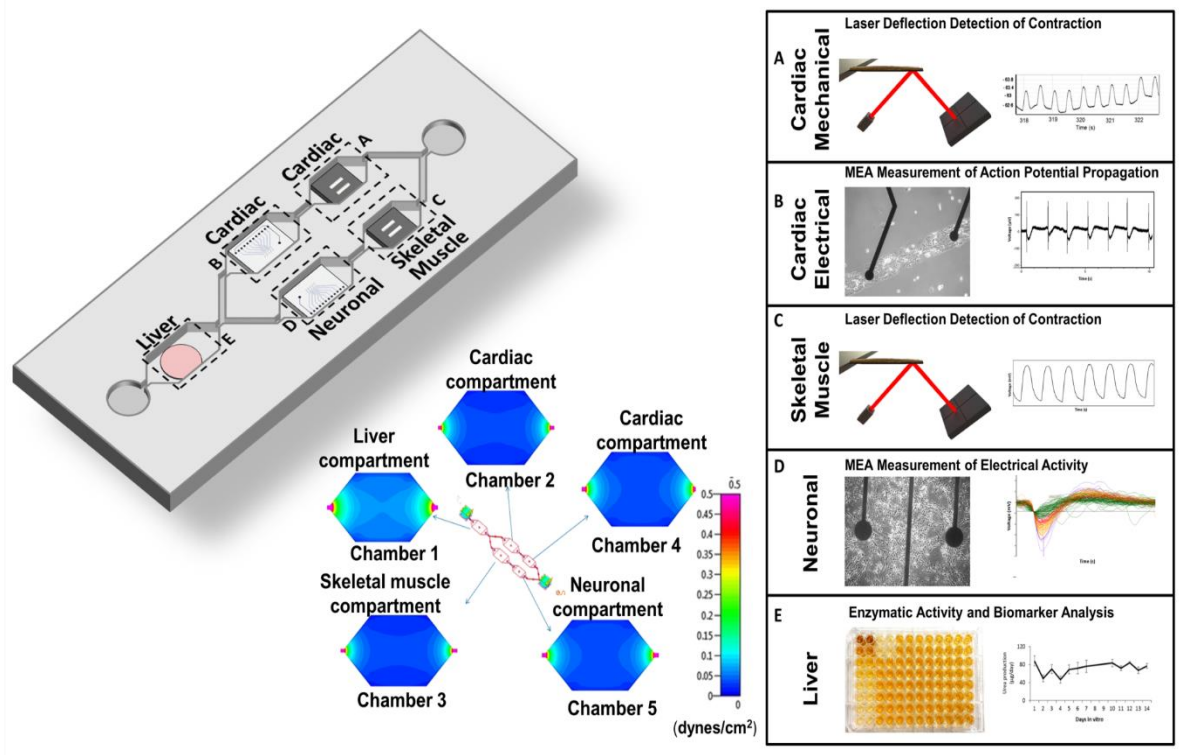
iPSC-pre-BotC neuronal response to DAMGO. A) Patch clamp analysis showed decrease iPSC-Pre-BotC neuronal activity after DAMGO treatment **B)** DAMGO dose-response curve for iPSC-Pre-BotC neurons

DAMGO's effect:

- Decrease Pre-BotC neuronal activity

Our Multi-Organ MPS Device Supports Recirculating Immune System Cells



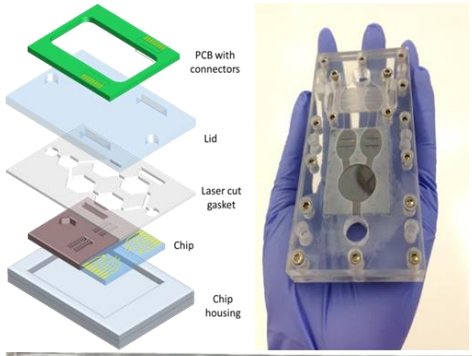


T. Sasserath etal., "Differential Monocyte Actuation in a Three-Organ Functional Innate Immune System-on-a-Chip," ***Advanced Science***, (2020)

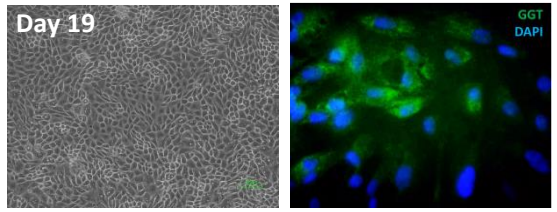
Barrier Tissue Organ Systems

Cornell University

Skin in 4-Organ System

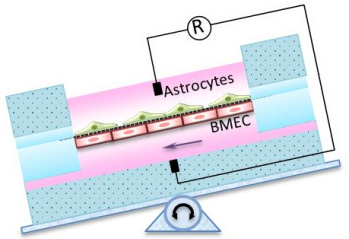


Proximal Tubule System

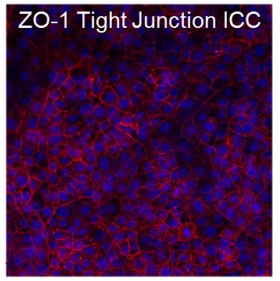


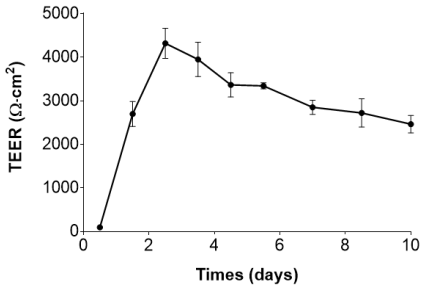
- Human proximal tubule cells grown on membranes under continuous flow maintain conformal monolayer
- Cells stain for kidney cell marker **Gamma-glutamyl transpeptidase (GGT)**

Human Blood-Brain Barrier System



Rocker platform

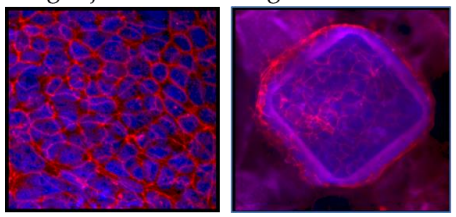


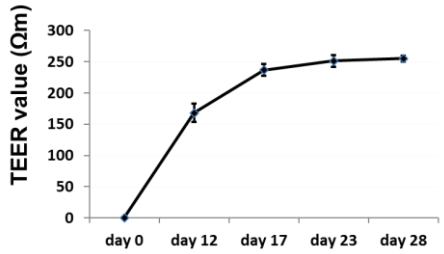


Gastrointestinal Tract Barrier System

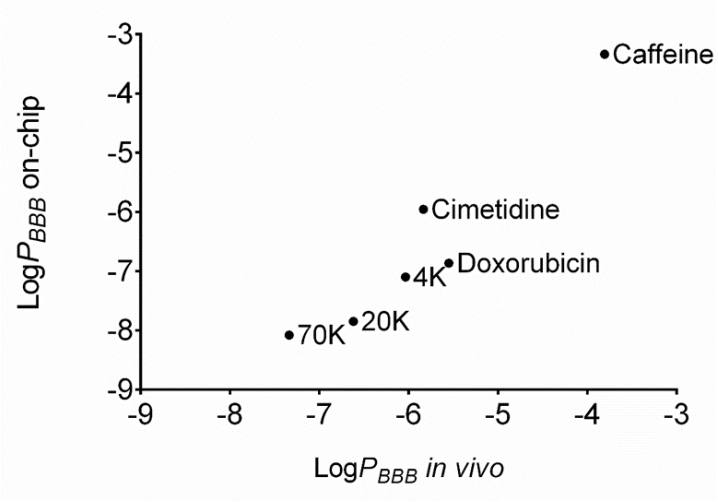
Primary Human Colon Epithelial Cells, by knock-in of telomerase reverse transcriptase (TERT), co-cultured with myofibroblasts and 5 nM GSK-3beta inhibitor

Tight junction staining in 2D and 3D





Compound Permeability Comparison



What COULD efficacy mean in terms of regulatory evaluations?

- Allow pre-clinical results to steer and/or limit clinical trial construction
- Reduce the number of people necessary for clinical trials, especially for Phase III, by creating human variants to represent uncommon genome profiles
- Allow in vitro clinical trials for rare diseases
- Better evaluations for children
- Better evaluation for aged individuals
- The ultimate precision medicine → individualized disease chips for each human