Laser Cut and Assembly of Microphysiological Systems for the Gut-Brain-Axis

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Abstract

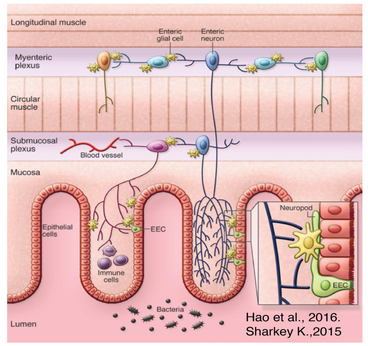
Microphysiological cell culture systems or “organs-on-chips” have garnered interest from both academia and industry. Facile, rapid, economic, and reliable fabrication of organs-on-chips would promote interdisciplinary adoption and technological development. The prevalent microfabrication of organs-on-chips via poly(dimethylsiloxane) (PDMS) via soft lithography requires microfabrication training and infrastructure. Because the initial design and prototyping phase may require multiple iterations, lithographic mold fabrication can be prohibitively expensive. While traditional lithography has benefits such as resolution, PDMS has several intrinsic material properties may limit the use of PDMS organs-on-chips such as hydrophobic molecule adsorption, cyclosilane leaching, and high gas permeability. To overcome these limitations, we developed a “laser cut and assemble” process for manufacturing thermoplastic, membrane-integrated, multi-layer, organs-on-chips. ICC revealed biocompatibility with human Caco-2 cells with tight junctions and F-actin expression comparable to Transwell controls. Alkaline phosphatase (AP) assay demonstrated a 2.2x increase in AP expression compared to controls, with more mucus via Alcian Blue and Muc2+ ICC. Human patient derived small intestinal monolayers and organoids remain viable on chip for up to 10 days. Primary monolayers exhibited 3D morphology spanning 100-200 μm, expressed tight junctions and F-actin similar to Transwell controls, and organoids expressed the proliferation marker Ki-67. Enteric neurons isolated from h9 stem cells or primary human resections exhibit neural processes and sub type diversity. ENS from Wnt-1:GCaMP5 mouse small intestine were seeded in 3D on an AIM chip with enteroendocrine cells (EEC), interfacing the EEC and ENS cultures for 48 hours. Calcium imaging with 300 mM sucrose and Na/Ca blocking implicates direct ENS synapsing to EEC cells are necessary and sufficient to activate ENS under sucrose stimulation *suggesting that EECs act as sensory transducers of dietary stimuli in vitro*. Ongoing work is investigating stromal cells and instrumentation for real-time monitoring towards a better understanding of the brain-gut-axis.

Motivation

What is the role of the enteric nervous system in regulating gut homeostasis?

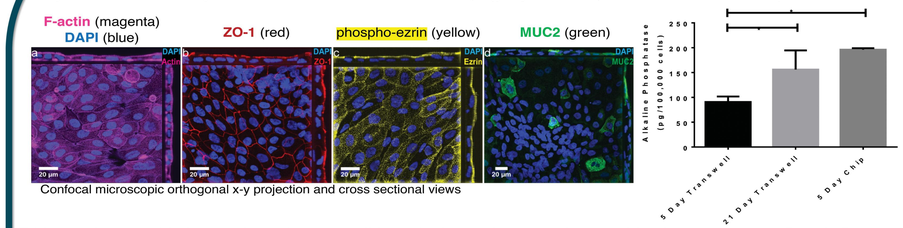
What is the role of the enteric nervous system in the brain-gut-connection?

Need for models to simplify and study



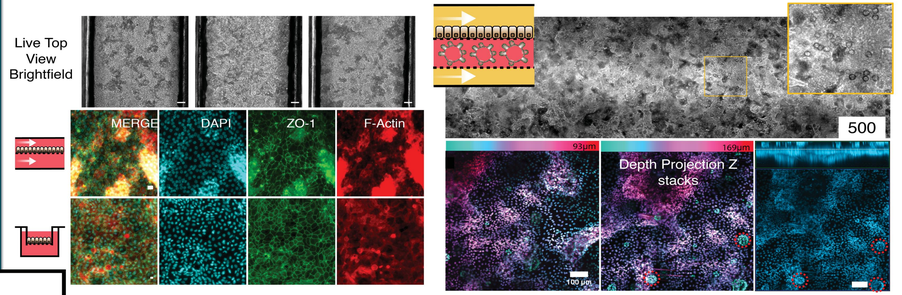
Results

Epithelium exhibits polarized brush border formation, tight junctions, increased ALP on chip to controls



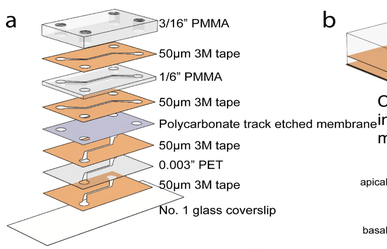
Confocal microscopic orthogonal x-y projection and cross sectional views

Tri-layered organ chip integrating primary small intestinal monolayers and organoid culture, supports 2D and 3D growth

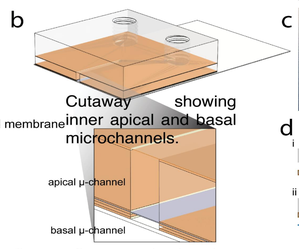


Methods

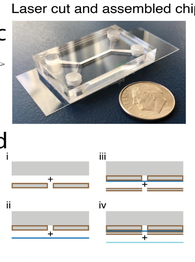
Laser cut & Assembled MPS



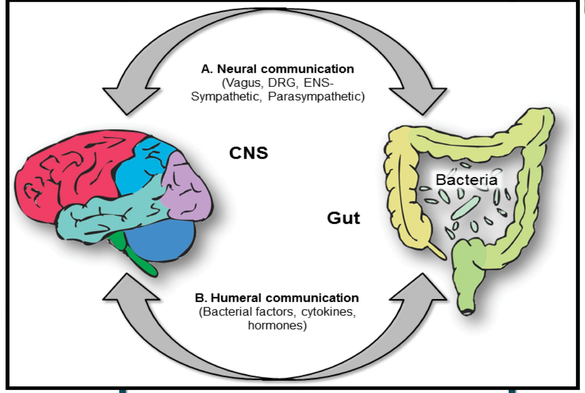
9 layer bi-layered chip composed of PMMA, acrylic adhesive, polycarbonate track etched membrane, and glass coverslip.



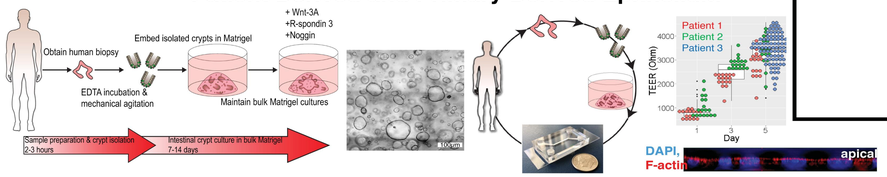
Cutaway showing inner apical and basal microchannels



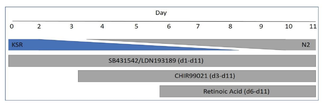
5 layers are aligned and irreversibly bonded in 4 steps.



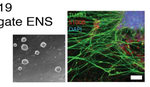
Patient Derived and Primary Derived Epithelium



Enteric Neural Cell Sourcing

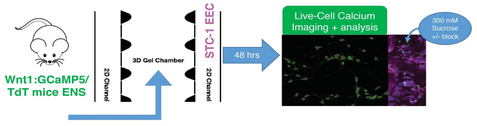


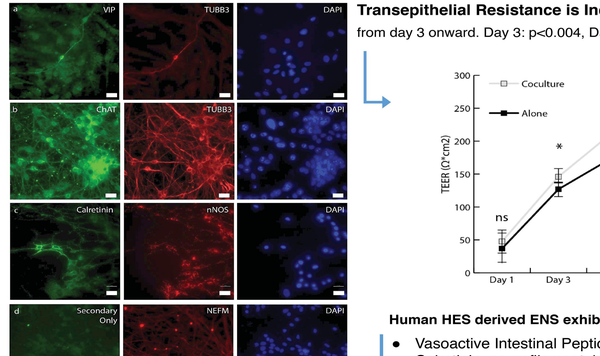
- Human resected small intestine, or h9 HES
- Fattahi method 2016, 2019
- D11 CD49d sort + propagate ENS neurospheres



Epithelial EEC to ENS Relay

- ENS in Matrigel + EEC on microfluidics for 48 hours.
- Ca^{2+} Imaging with 300 mM sucrose (Bohórquez 2019) and analyzed in MATLAB
- Na channel blocker (tetrodotoxin, 5µM) or Ca blockers (diltiazem, nitrendipine, 1 mM)





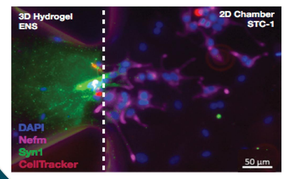
Transepithelial Resistance is Increased in ENS Cocultures

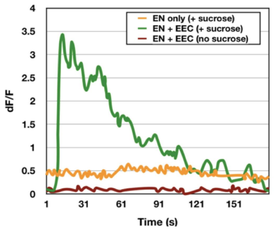
from day 3 onward. Day 3: p<0.004, Day 5: p<6.9e-5, Day 7: p<3.5e-5.

Human HES derived ENS exhibit diverse subpopulation

- Vasoactive Intestinal Peptide, Acetylcholine, Nitric Oxide, Calretinin, neurofilament, beta-III-tubulin identified
- Nestin+, glia (s100, GFAP) not shown

Primary enteric neurons directly interface and receive functional dietary signals from EEC on chip





- EEC STC-1 express NF and have ‘neuropod’ projections.
- ENS + EEC Ca2+ increased activity
- ENS only do not respond to sucrose, suggesting EECs are necessary for chemosensory response
- Blocking with TTX, and Ca = no EEC to ENS relay (not shown)

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