體內體外大不同 – 微流體細胞培養

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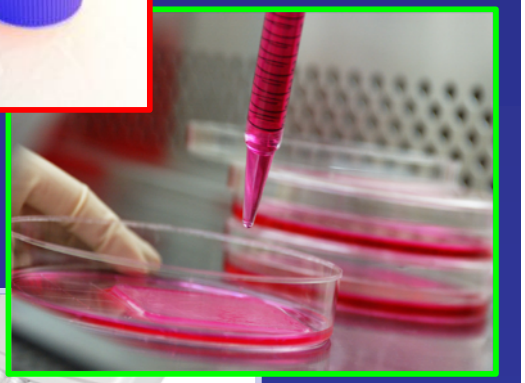
Outline

- Introduction
  - Why Microfluidic Cell Culture?
- Microfluidic Cell Culture Devices
- Integration
- Conclusion

細胞培養… WHY？？Problem ？？

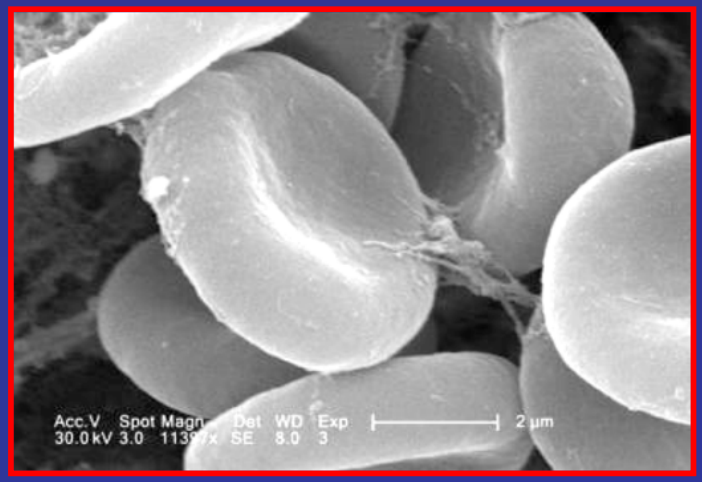
細胞培養 *(Cell Culture)*

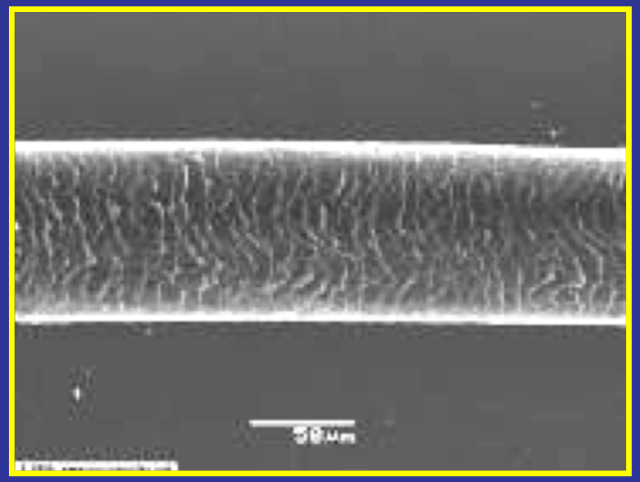
in vivo vs. *in vitro*

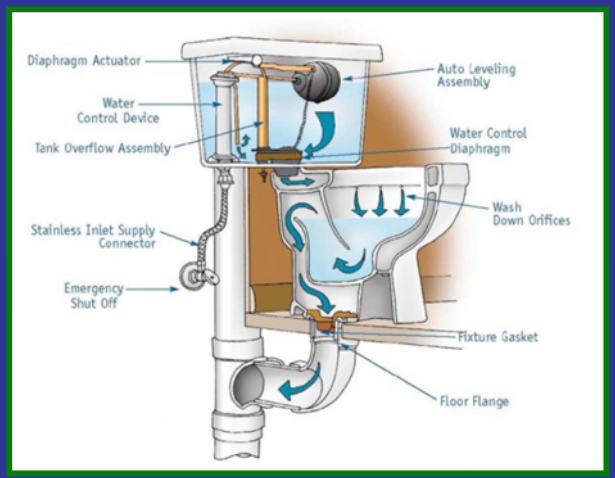


微流體 (Microfluidics)… WHAT？？

- ***Microfluidics*** = Micro + Fluidics
- ***Micro/Nano:*** 10^{-6} , 10^{-9}
  - Small size (sub-mm)
  - Small volumes (μl, nl, pl)
- ***Fluidics:*** handling of liquids and/or gases

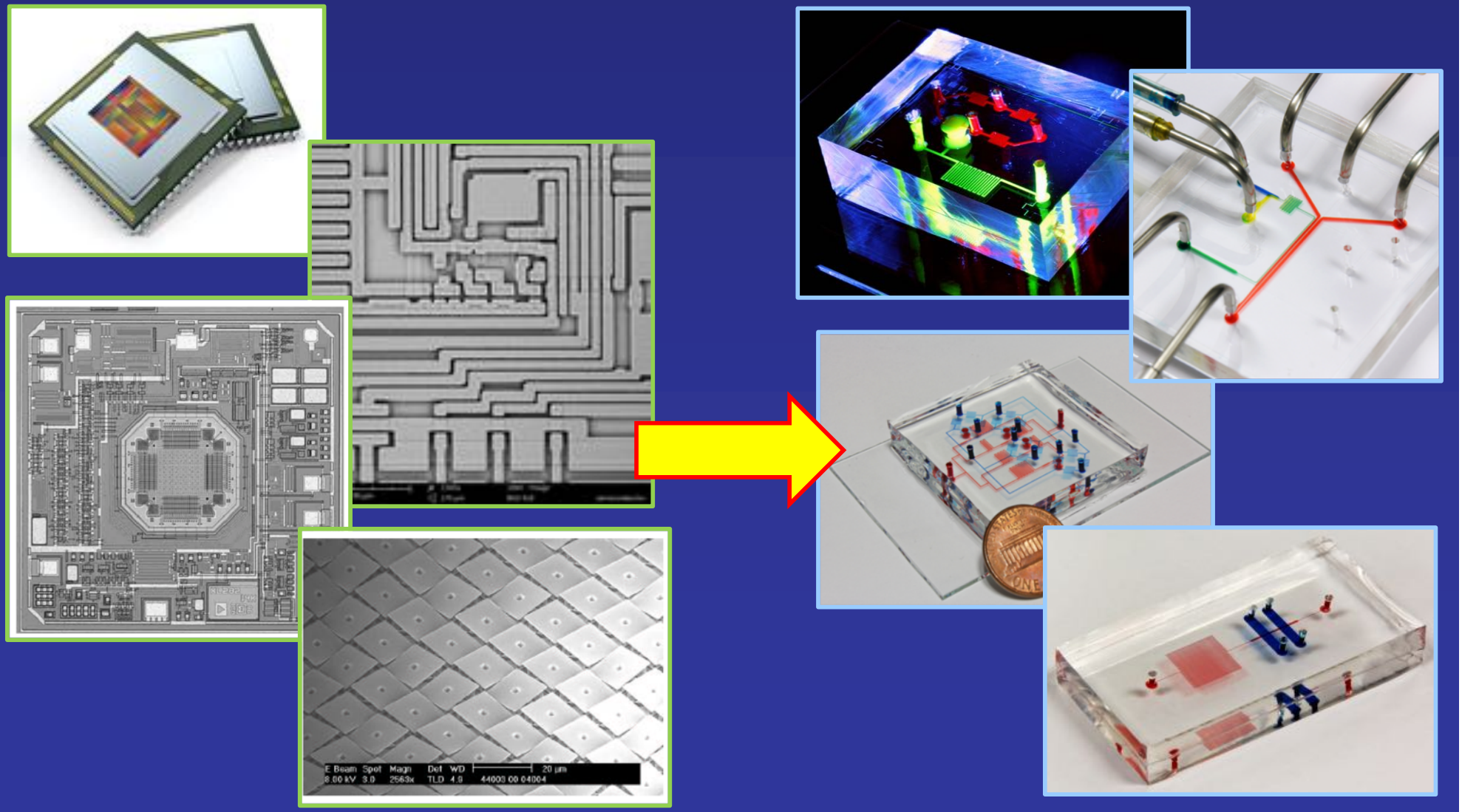






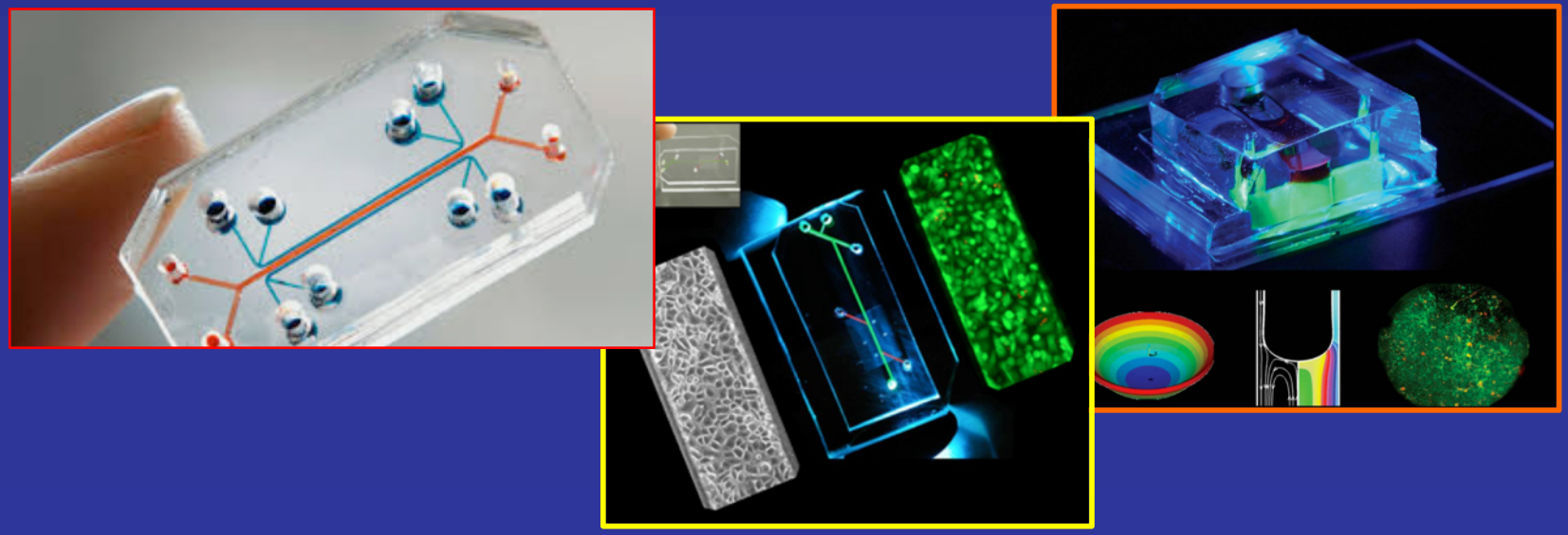
晶片 (Chips)… WHAT？？

- 積體電路 (IC), 微機電 (MEMS) 及 微流體晶片



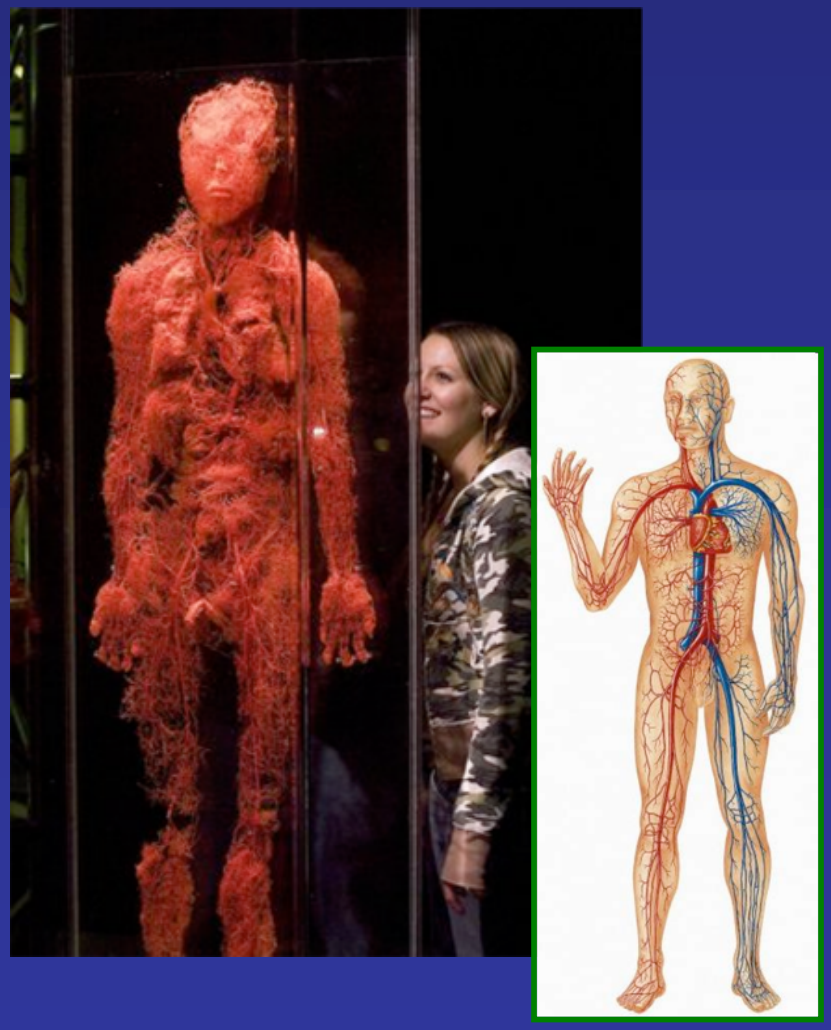
Introduction – Microfluidic Devices

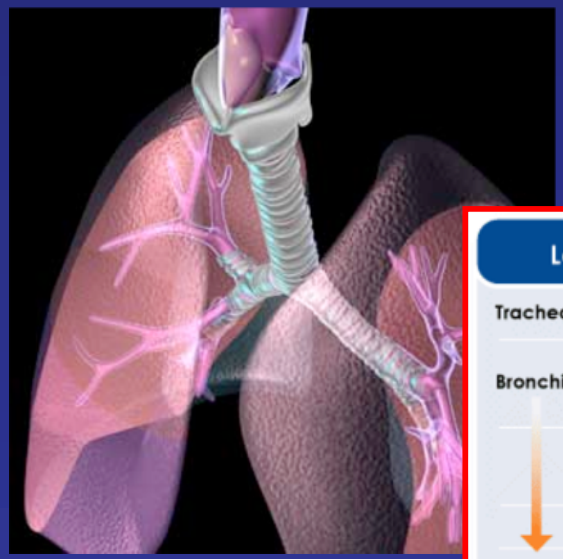
- **Why Microfluidic Devices?**
  - Unique properties (laminar flow, surface tension…)
  - Small sample volume and easy to scale up
  - Well-controlled microenvironments
  - Precise spatial and temporal control
  - Able to mimic the rich biochemical and biophysical complexity of the cellular microenvironment

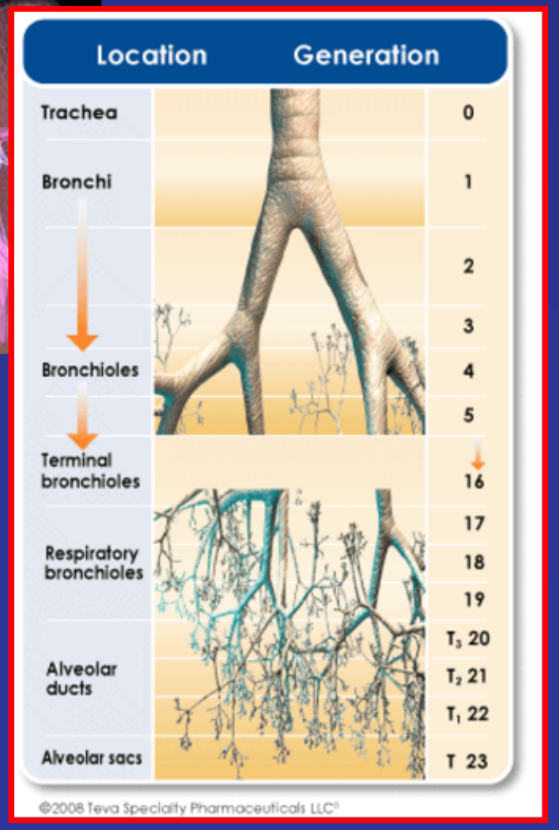


身體內的微流體系統 Microfluidics *in vivo*

- 循環系統 及 呼吸系統

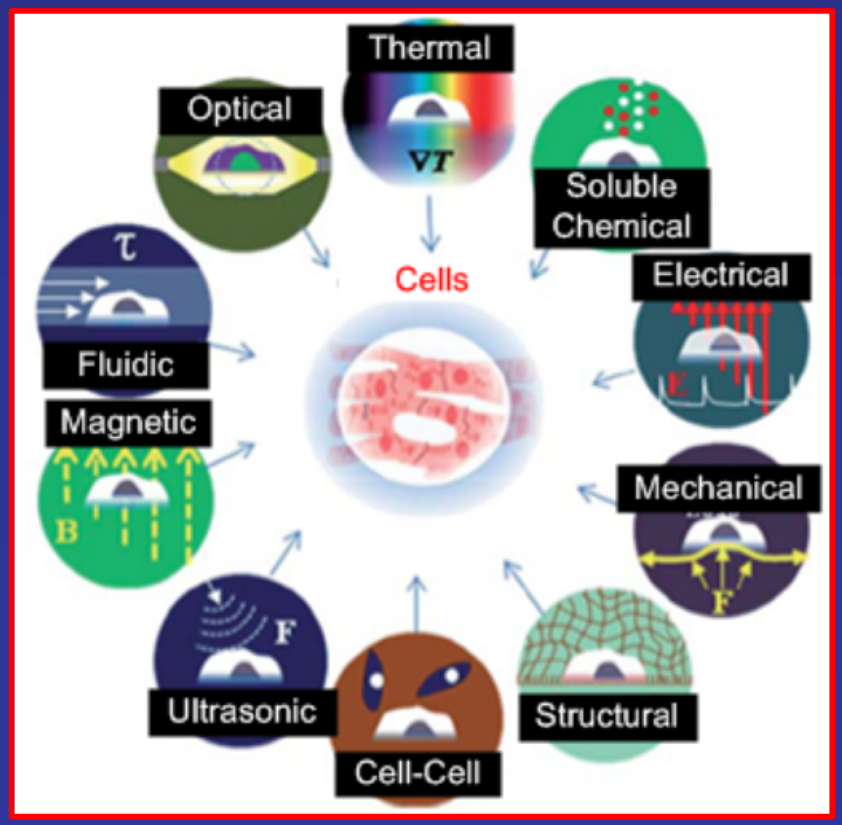






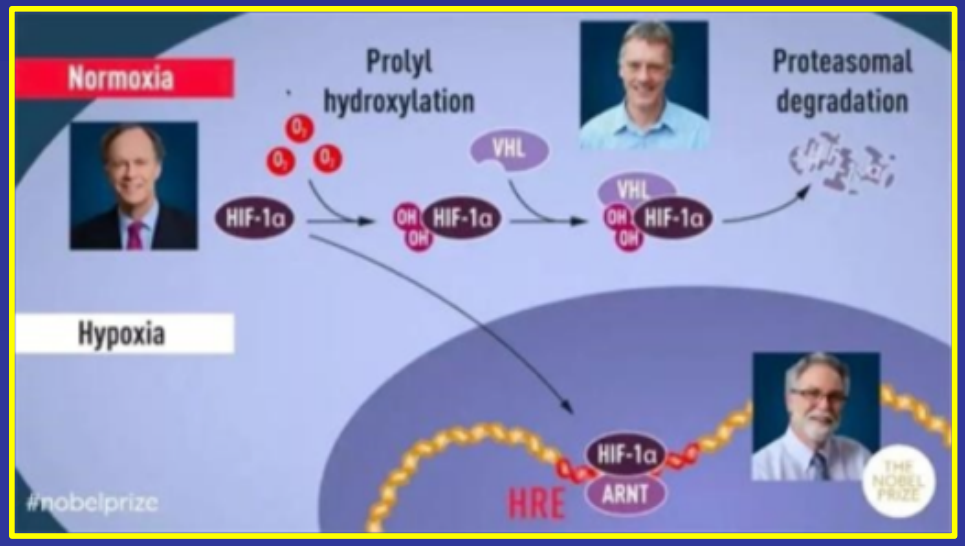
Introduction – Gaseous Microenvironments

- Why Gaseous Microenvironments?
  - Regulate Important Biological Functions: Breathing, Blood Vessel Dilation etc.
  - Essential Biological Gases: Oxygen, Carbon Dioxide, Nitric Oxide (EDRF) etc.
  - *Important* but *Difficult* to Control.
- Diffusivity in water at 20°C:
  - O_2: 1.97×10^{-5} cm^2/s
  - CO_2: 1.76×10^{-5} cm^2/s
  - NO: 2.21×10^{-5} cm^2/s
  - Ribonuclease (14 kDa): 0.12×10^{-5} cm^2/s
  - Albumin (65 kDa): 0.060×10^{-5} cm^2/s



Introduction – Oxygen Tension

- Oxygen tension plays an essential role in biological systems. For examples:
  - Tumor Malignant Progression and Angiogenesis
  - Cancer Treatment
  - Stem Cell Differentiation



The Nobel Prize in Physiology or Medicine 2019

Ill. Niklas Elmedhed. © Nobel Media.
William G. Kaelin Jr
Prize share: 1/3

Ill. Niklas Elmedhed. © Nobel Media.
Sir Peter J. Ratcliffe
Prize share: 1/3

Ill. Niklas Elmedhed. © Nobel Media.
Gregg L. Semenza
Prize share: 1/3

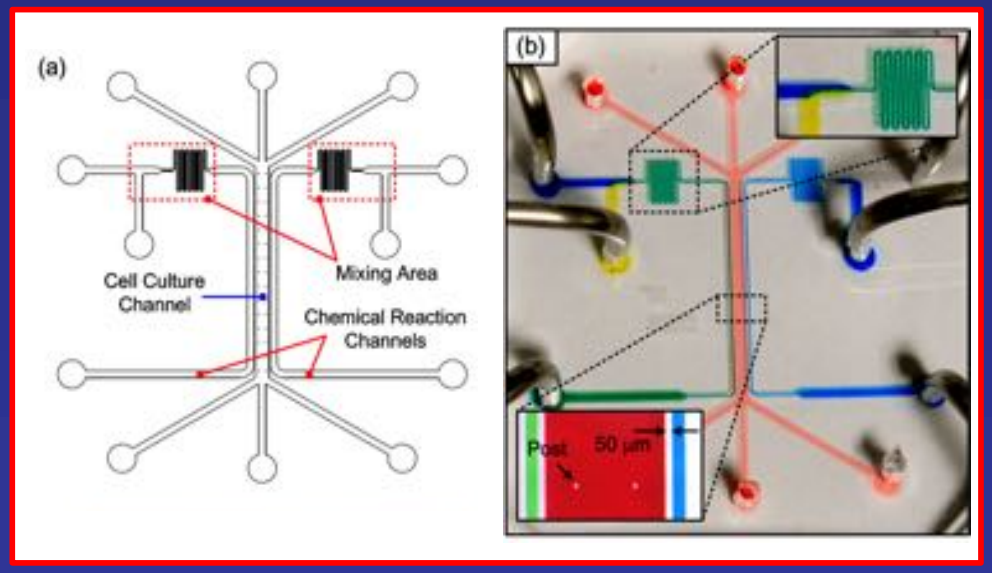
The Nobel Prize in Physiology or Medicine 2019 was awarded jointly to William G. Kaelin Jr, Sir Peter J. Ratcliffe and Gregg L. Semenza "for their discoveries of how cells sense and adapt to oxygen availability."

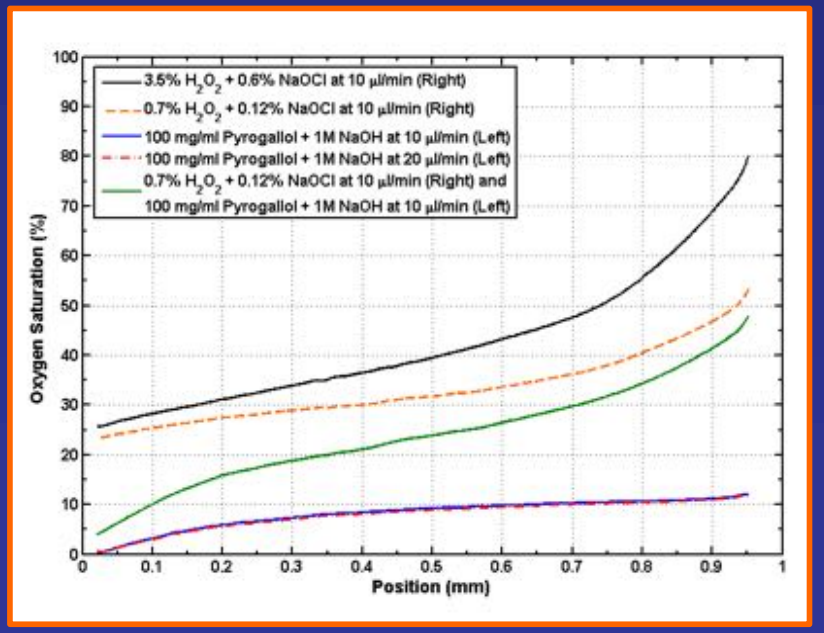
Introduction – Conventional Methods

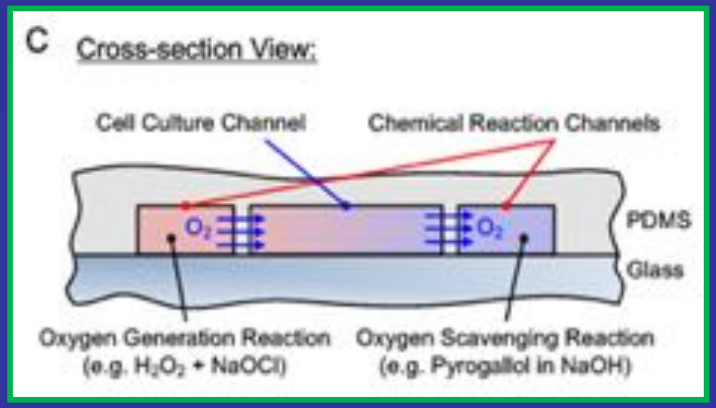
- Existing methods to control gaseous compositions for cell culture are often complicated, require gas cylinders, tedious interconnections, and sophisticated flow control systems.
- Direct chemical additions that may alter cell behaviors.
- WITHOUT Spatial Control.

New Microfluidic Device Design

- Spatially-Confined Chemical Reaction Method
- The device is constructed using polydimethylsiloxane (PDMS).







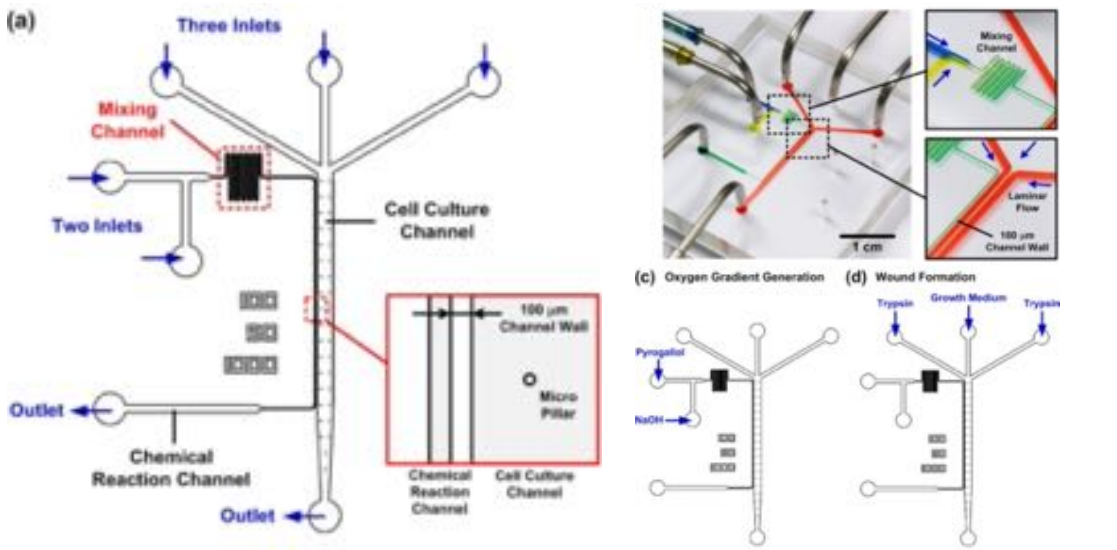
Oxygen Diffusion Coefficient, D (cm^2/s)

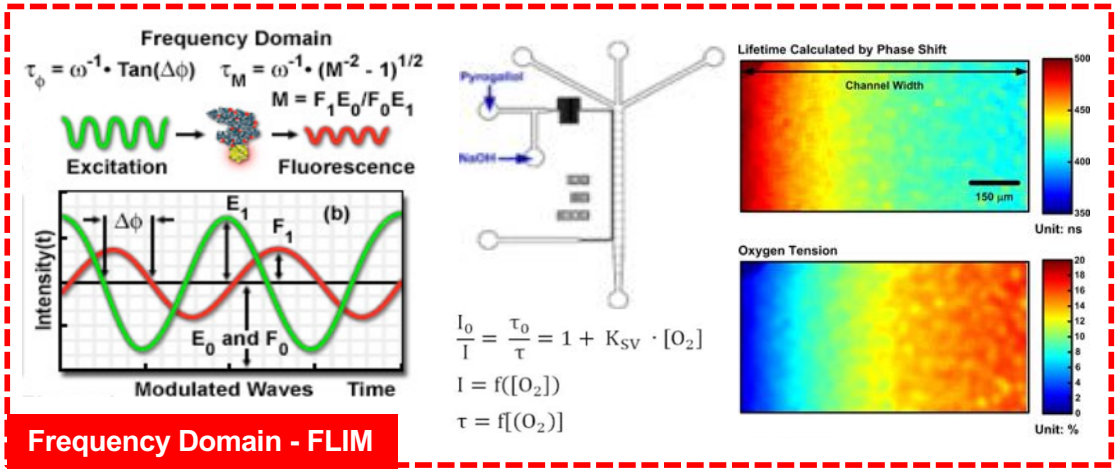
25°C in PDMS, D = 3.55×10^{-5}
20°C in Water, D = 1.97×10^{-5}
40°C in Water, D = 3.24×10^{-5}

Lab Chip, 2011, 11, 3626

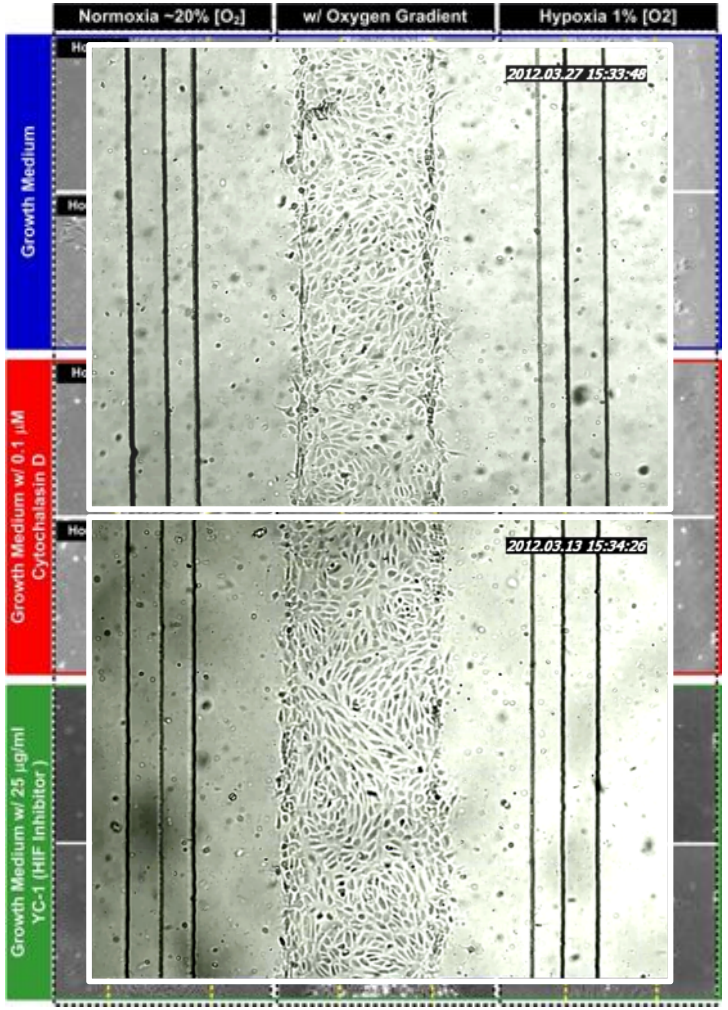
Microfluidic Wound Healing Assay w/ O_2 Gradients

Study Endothelial Cell Proliferation and Migration under Combinations of Oxygen Tensions, Gradients, and Drug Treatments





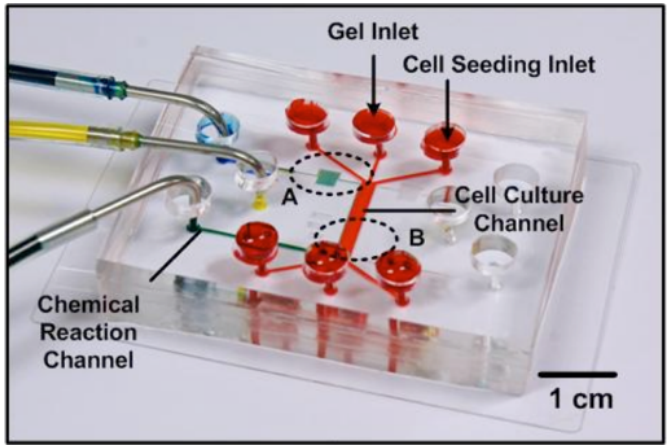
Frequency Domain - FLIM

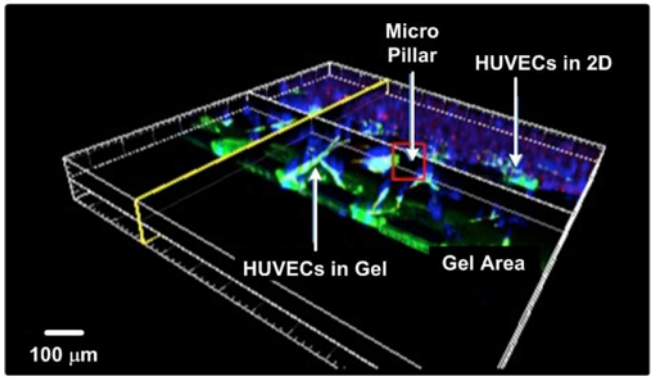


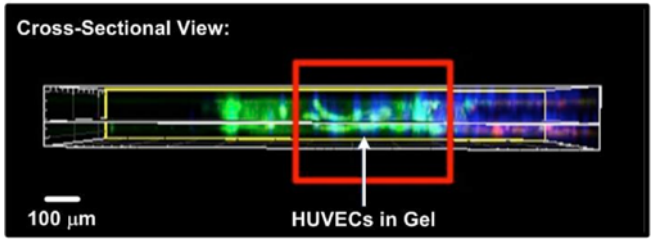
Scientific Reports 2019

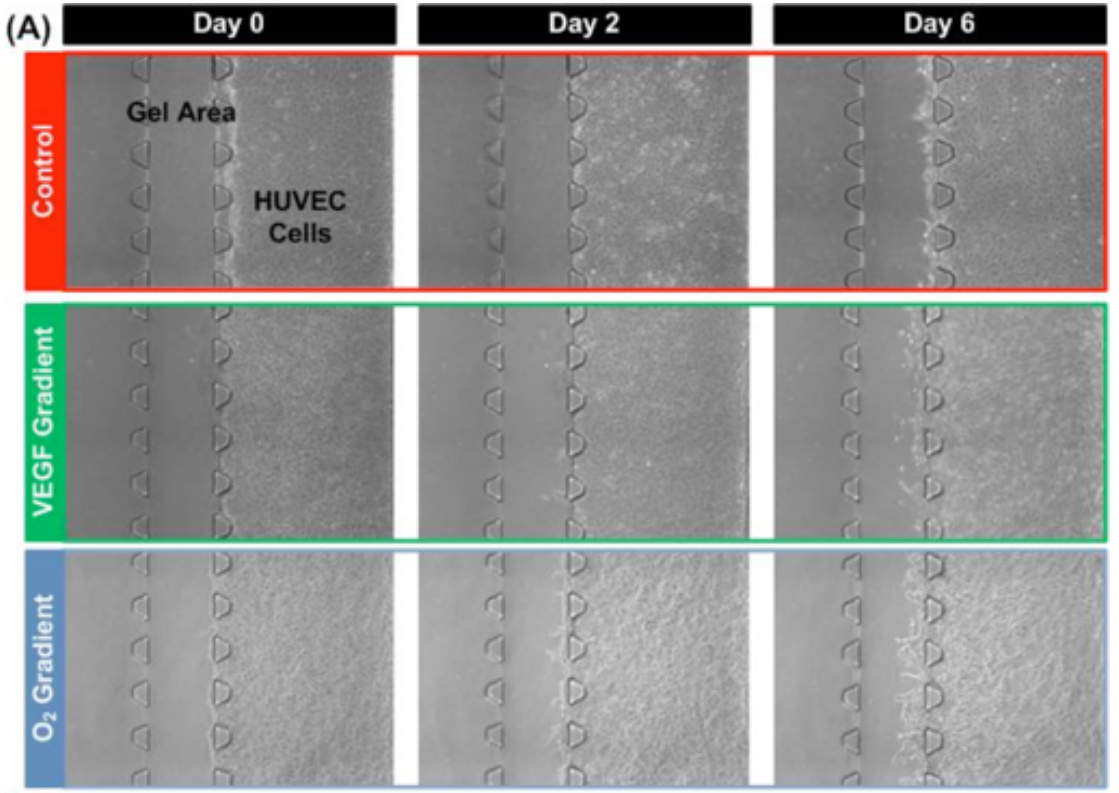
Cell Sprouting in 3D under O_2 Gradients

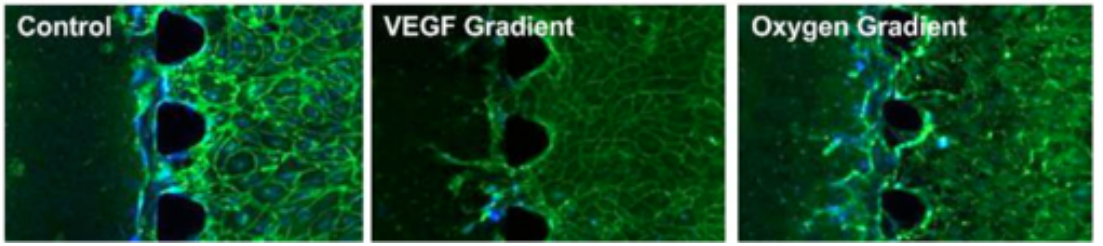
Study Endothelial Cell Sprouting in Three-Dimensional Matrix under Oxygen Gradients







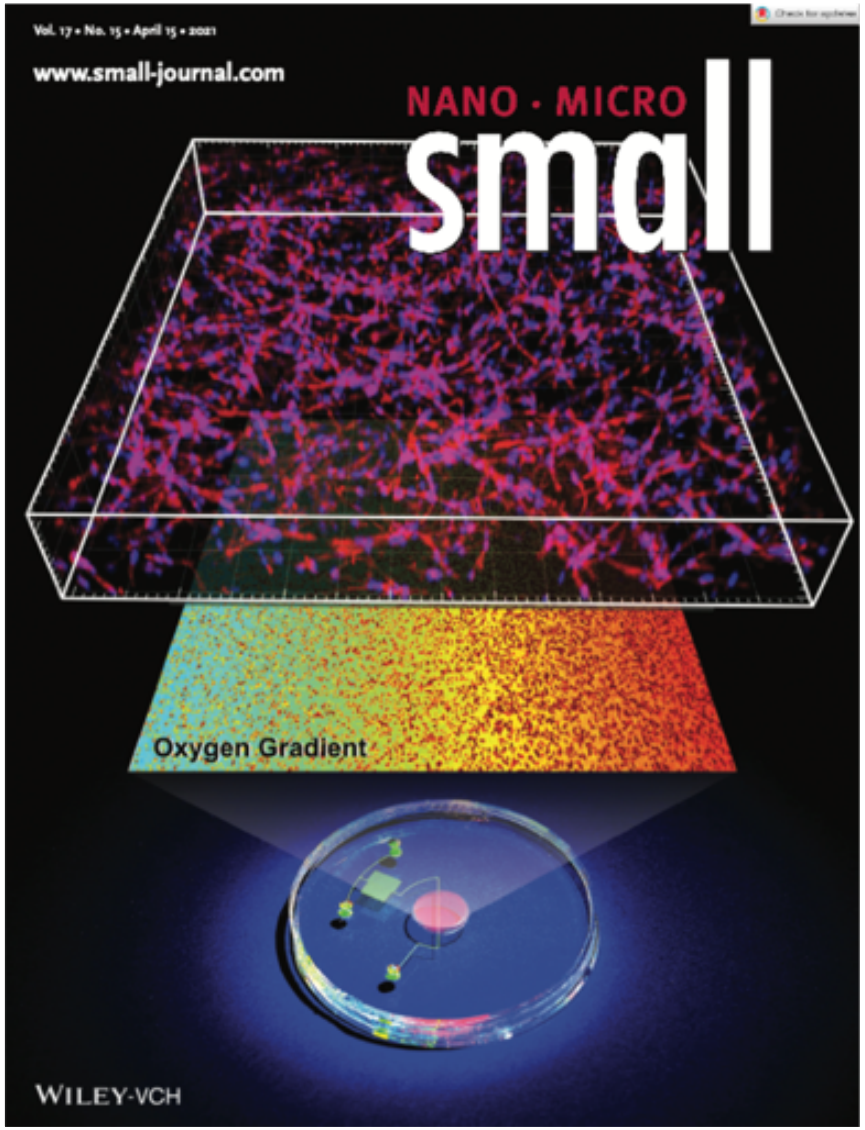


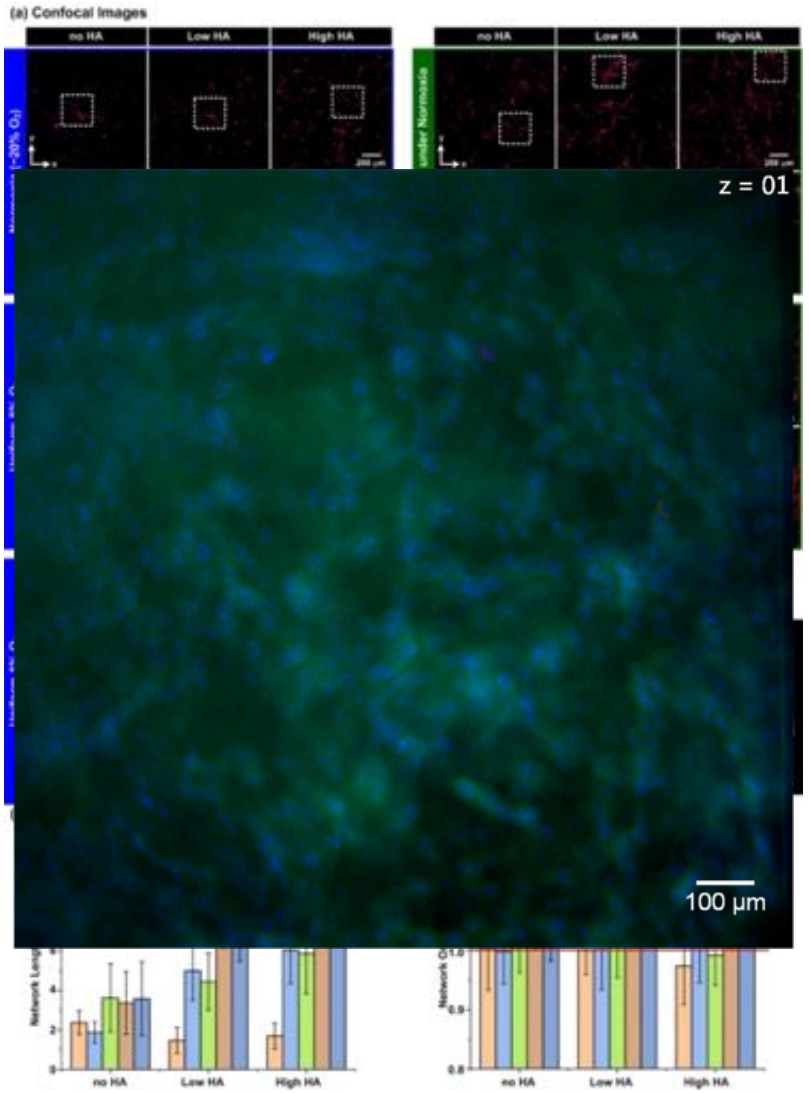


Presented in microTAS 2017, Manuscript in Preparation.

Network Formation in 3D under O_2 Gradients

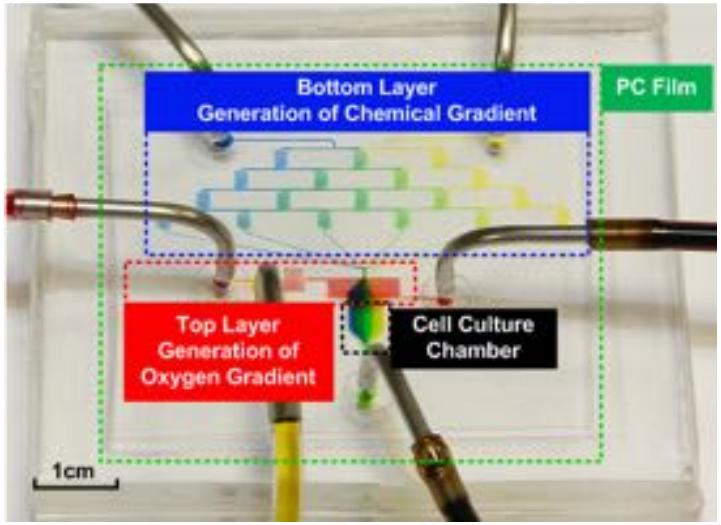
Study 3D Network Formation of Endothelial Cells under Oxygen Gradients

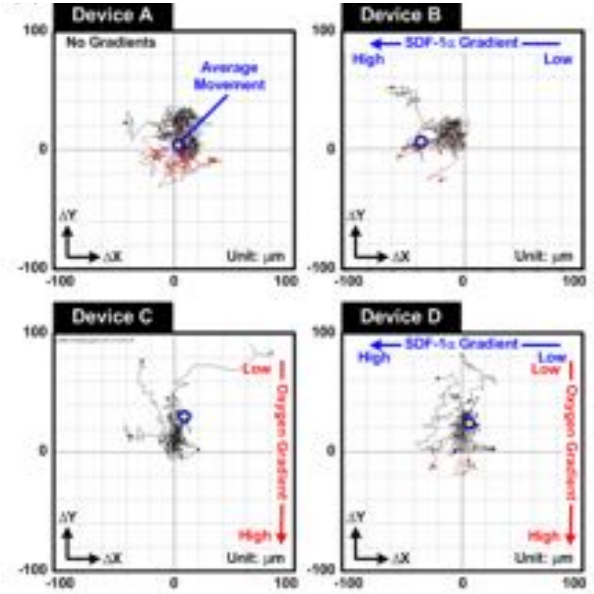




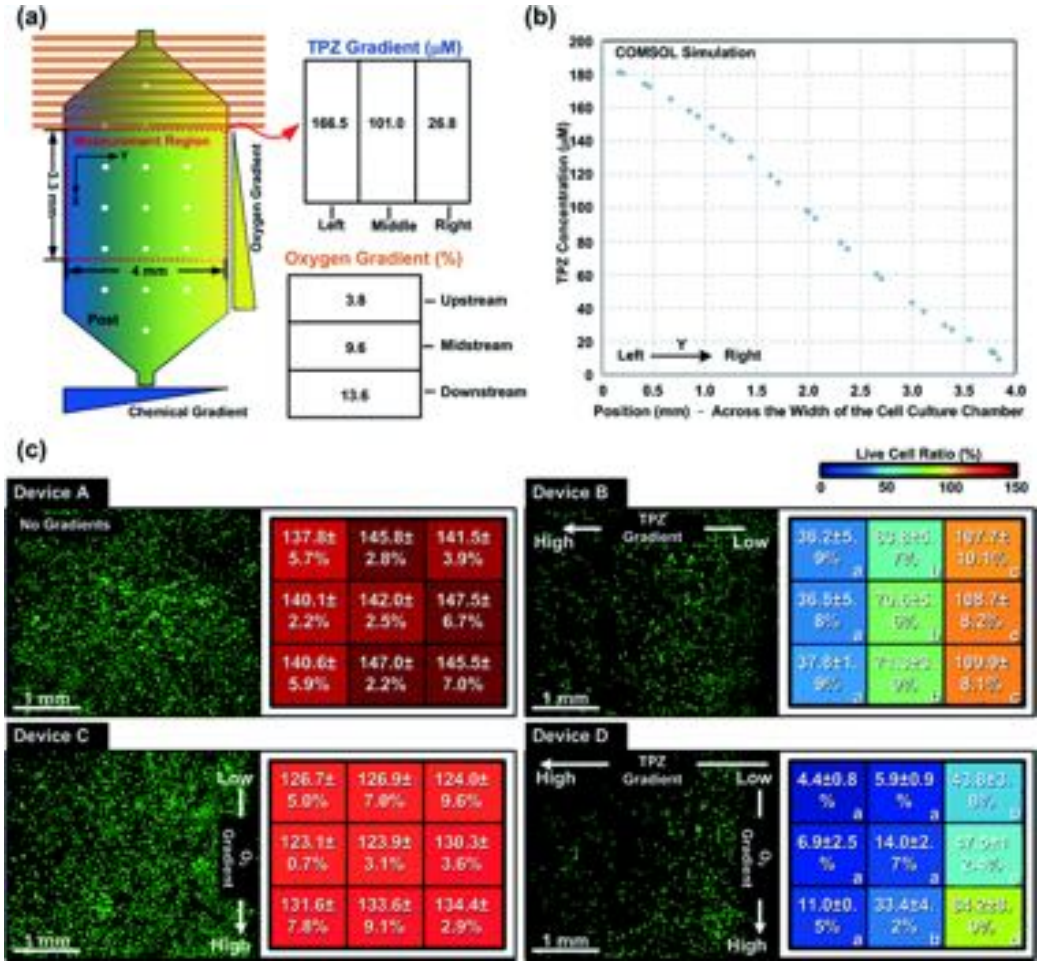
Accepted in *Small*.

Microfluidic Devices for w/ O_2 and Chemical Gradients





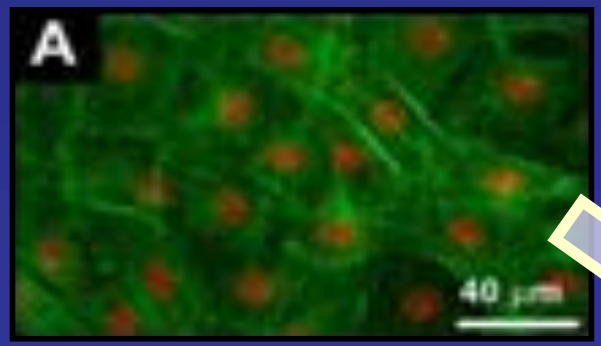
Cell Culture Device w/ Chemical & O_2 Gradients (1 + 1 = 2 ?)



Lab Chip, 2014, 14, 3762 (Most Read Article in August 2014, Top 10% of Highly Cited Authors)

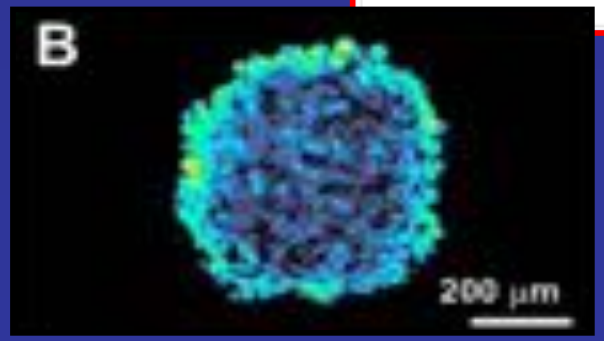
Introduction – Additional Dimension

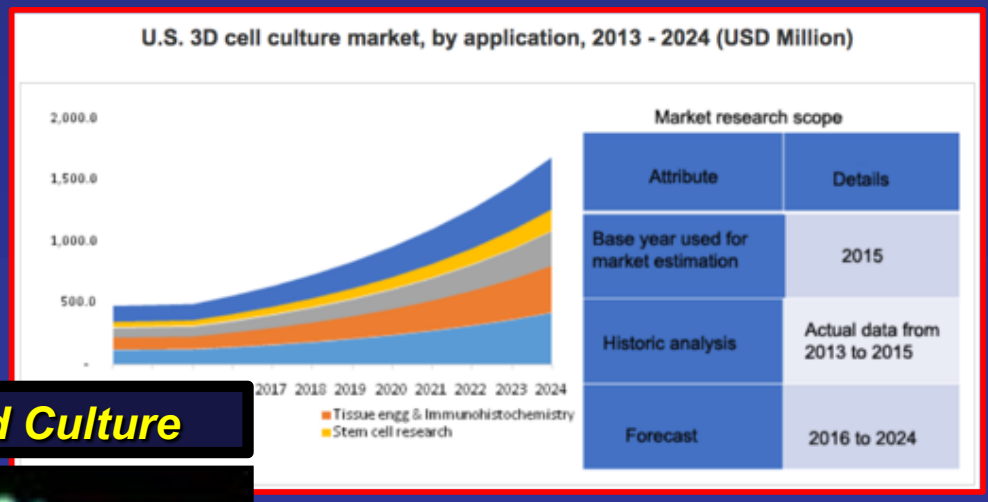
- Moving from cell monolayers to three-dimensional (3D) cultures is motivated by the need to work with cellular models that better mimic the environment of living tissues.
- For example, tumor spheroids have been widely used as an *in vitro* 3D model to simulate the multicellular microenvironment when investigating tumor cell physiology and responses to therapeutic agents.



2D Monolayer

3D Spheroid Culture

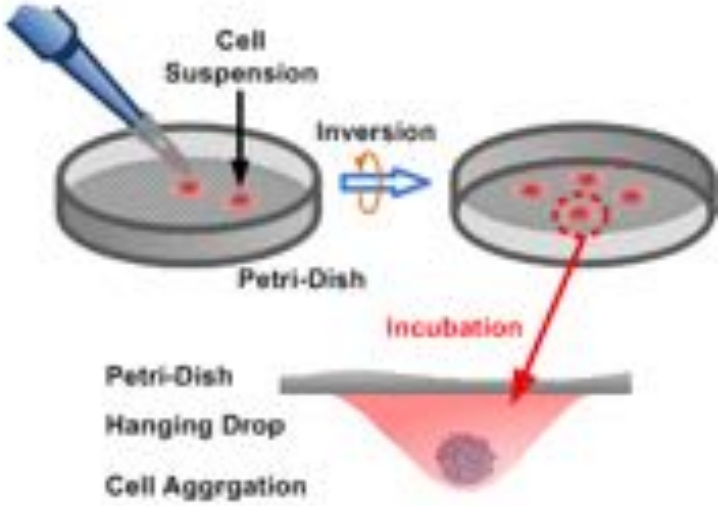




Scaffold free 3D cell culture is expected to witness the fastest growth through to 2024.

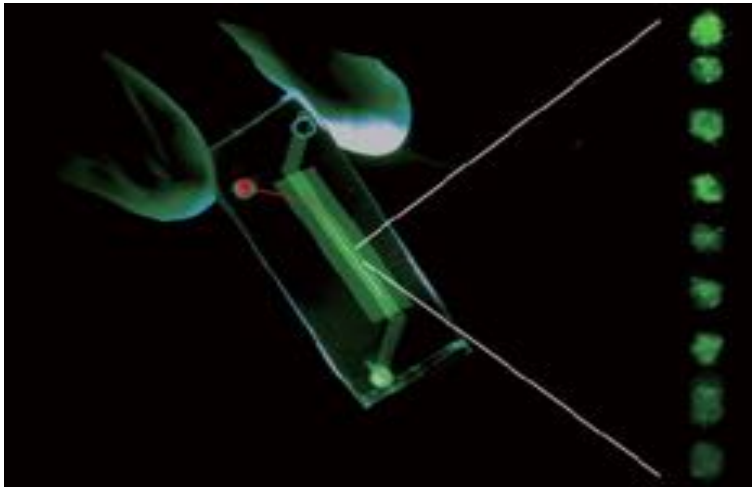
Introduction - How to form Spheroids?

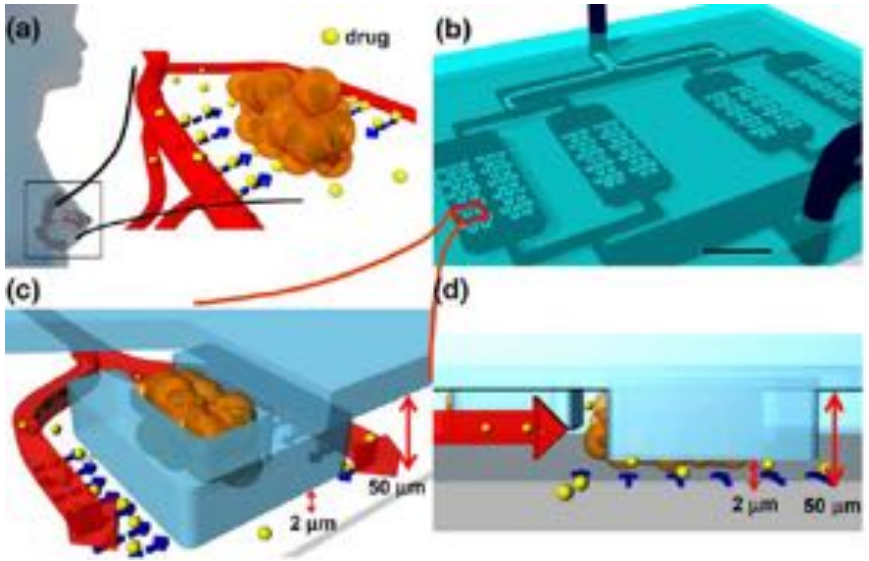
Hanging Drop



Rotating-Wall Vessel

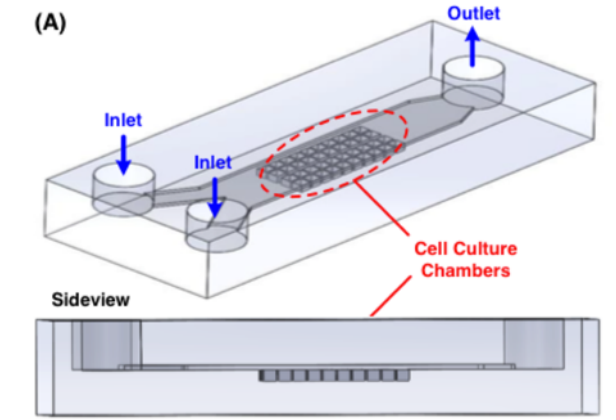
Microfluidics

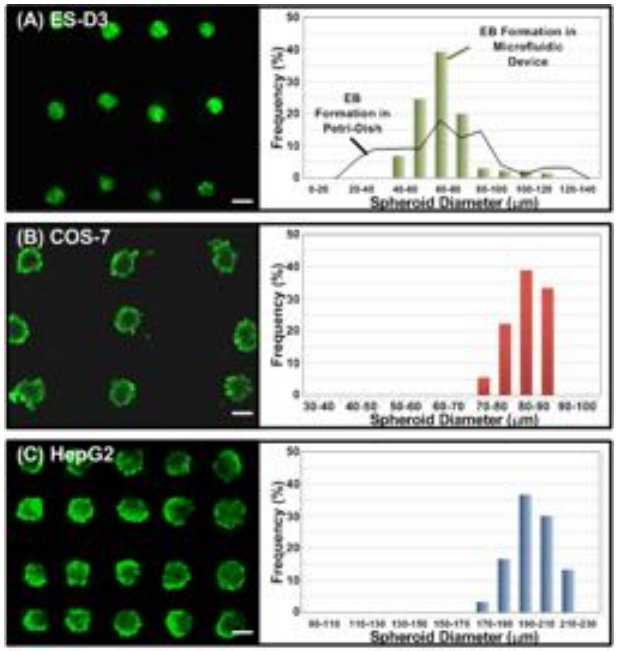




Device for Spheroid Formation & Culture

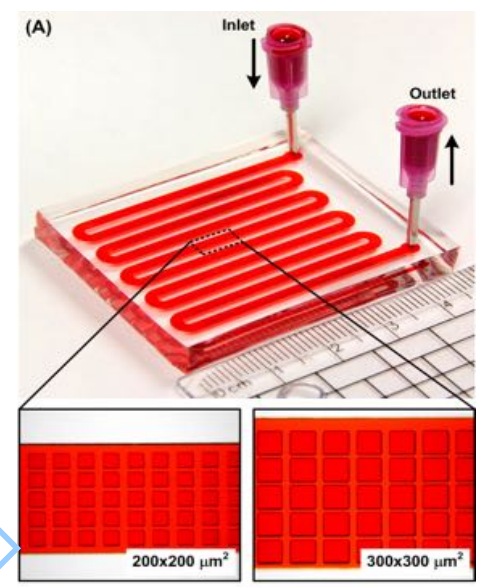
Uniform-Sized Spheroid Formation, Culture & Harvest

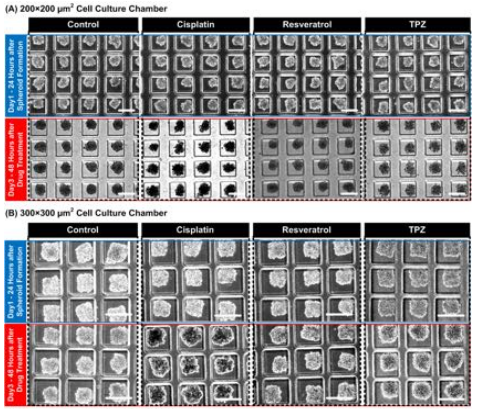


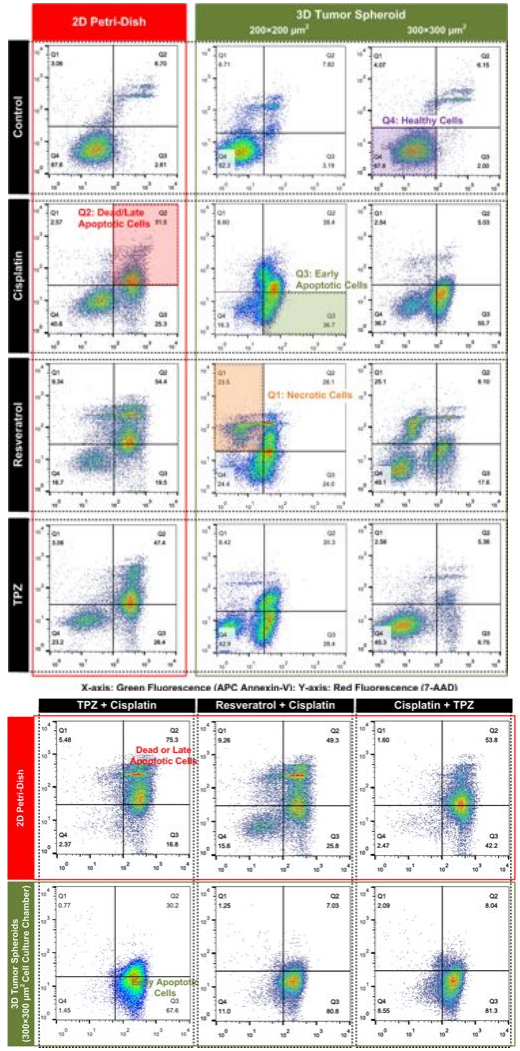


Biomicrofluidics, 2013, 7, 054114

Scale up (5000 Spheroids) for Flow Cytometry Analysis on Tumor Spheroids



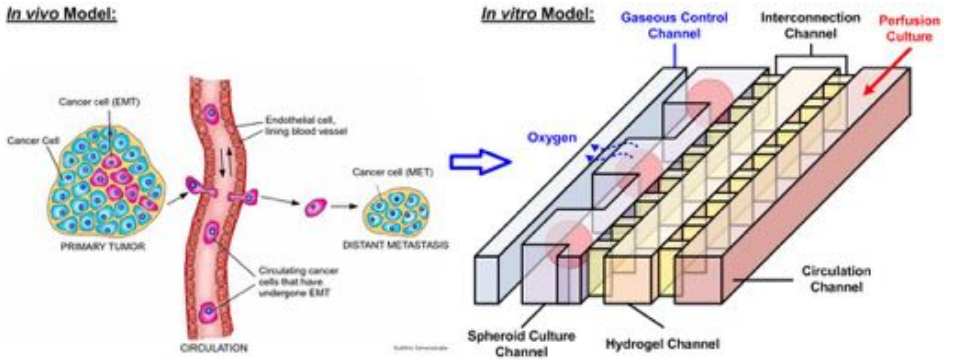


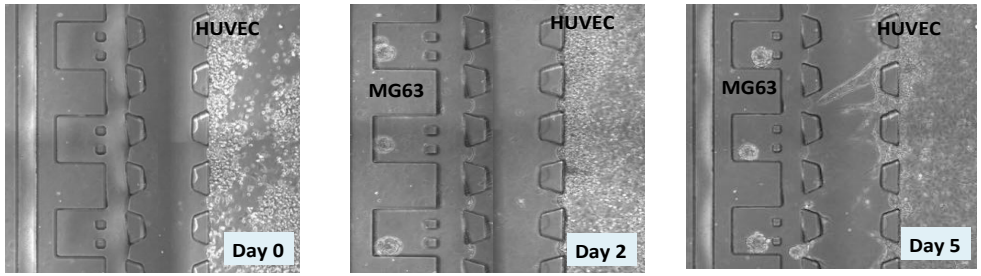


Sci. Rep., 2016, 6, 21061

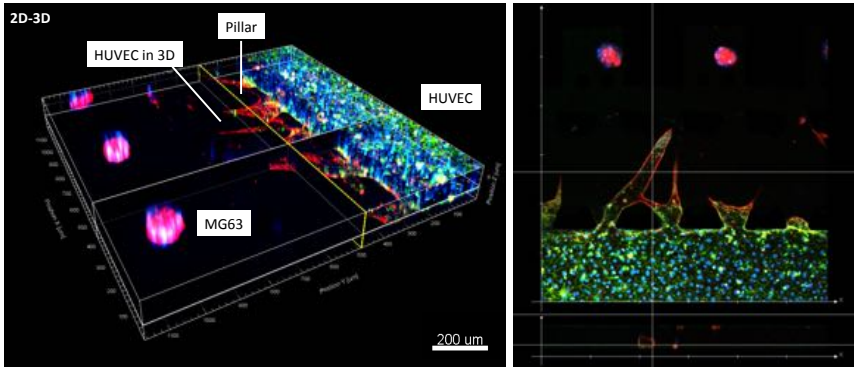
Integrate Multiple Dimensions in a Platform

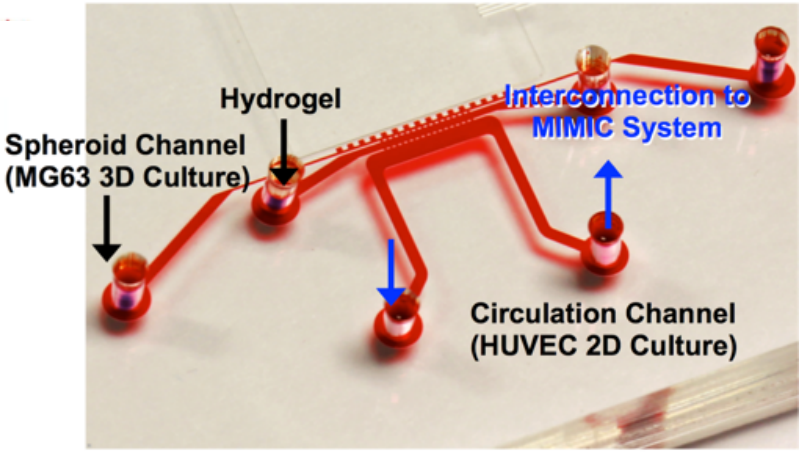
3D Osteosarcoma Cell (MG63) Spheroid and 2D Monolayer HUVEC Co-Culture to Study Endothelial Cell Sprouting into 3D Hydrogel Affected by Tumor Spheroids

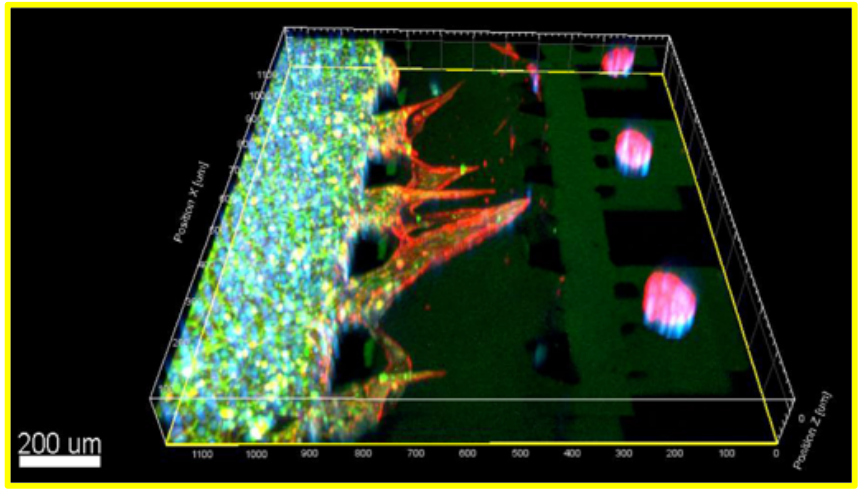




Blue: DAPI Red: F-Actin Green: VE-Cadherin







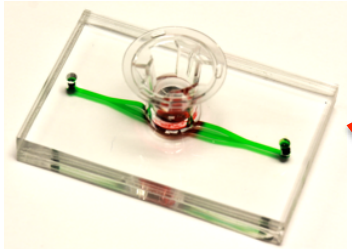
Presented in microTAS *2017*, Manuscript in Preparation.

Microfluidic *in vitro* Circulatory System

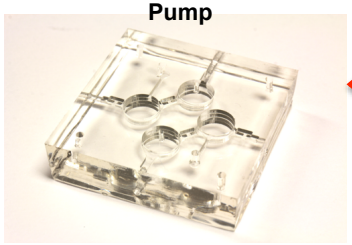
Modularized Integrated Microfluidic (MIMIC) System to Mimic Circulatory Systems for *in vitro* Cell Studies

MIMIC System – *in vitro* Circulatory System

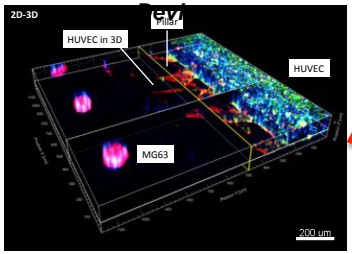
***In vitro* Bloor-Air Barrier**



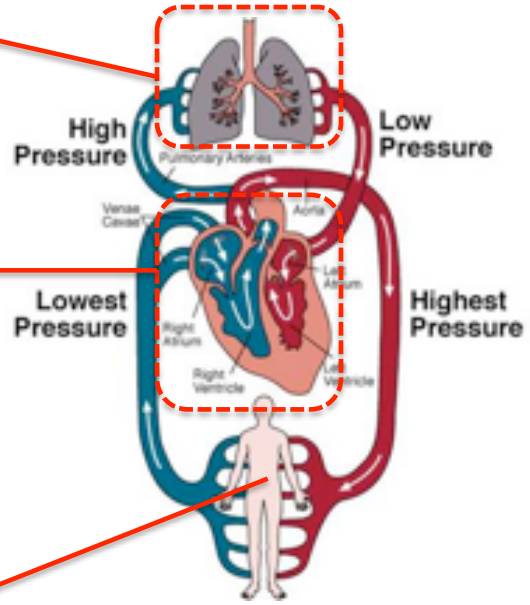
Heart-Mimicking Microfluidic Pump



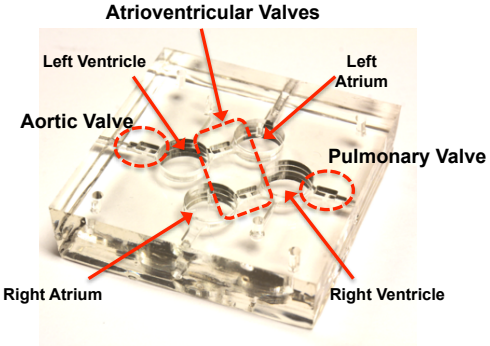
Cell Co-Culture Microfluidic



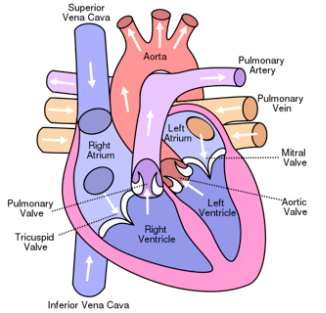
***In vivo* Circulatory System**



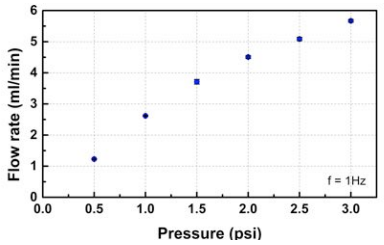
Heart-Mimicking Microfluidic Pump



Human Heart



(a)



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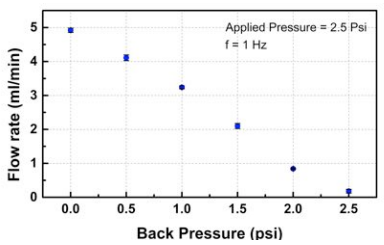

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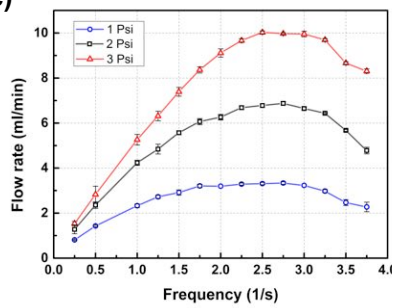
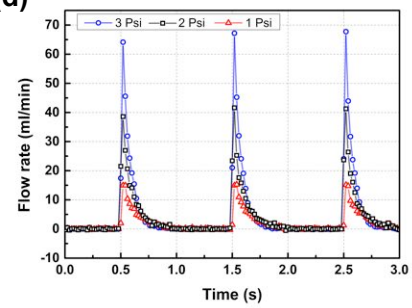
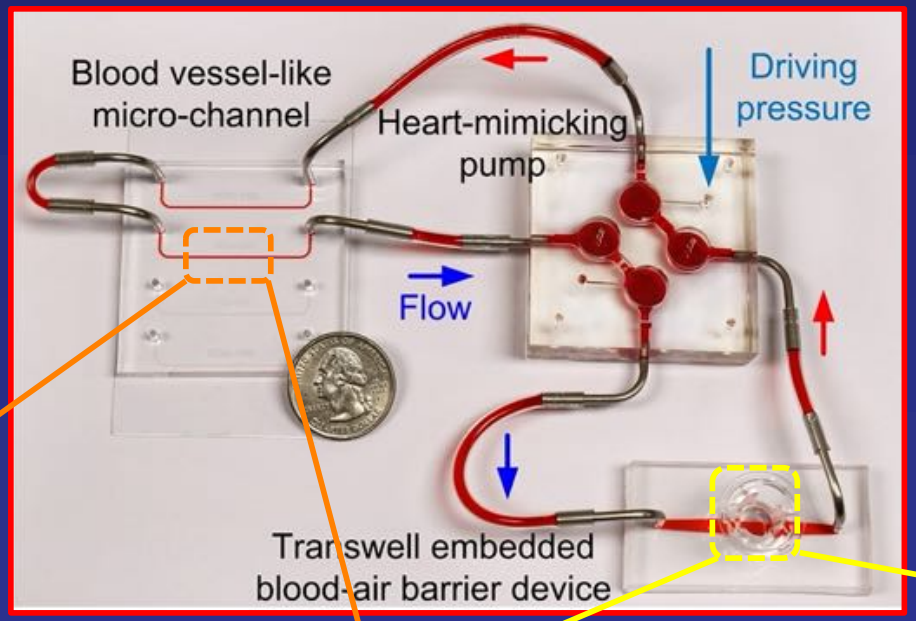


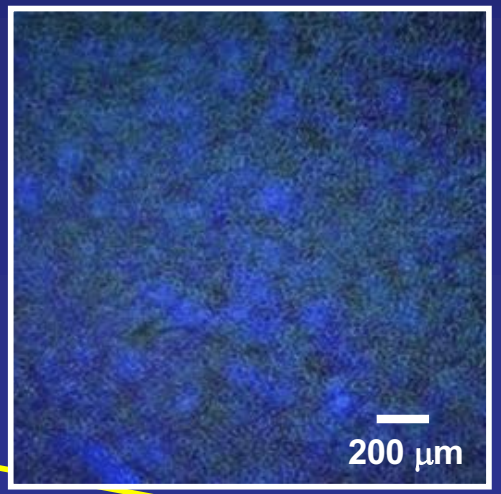
(d)

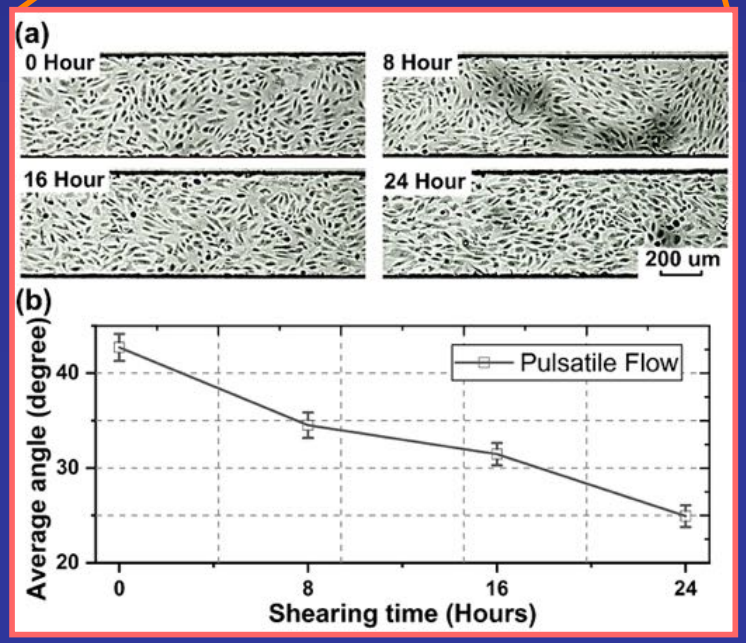


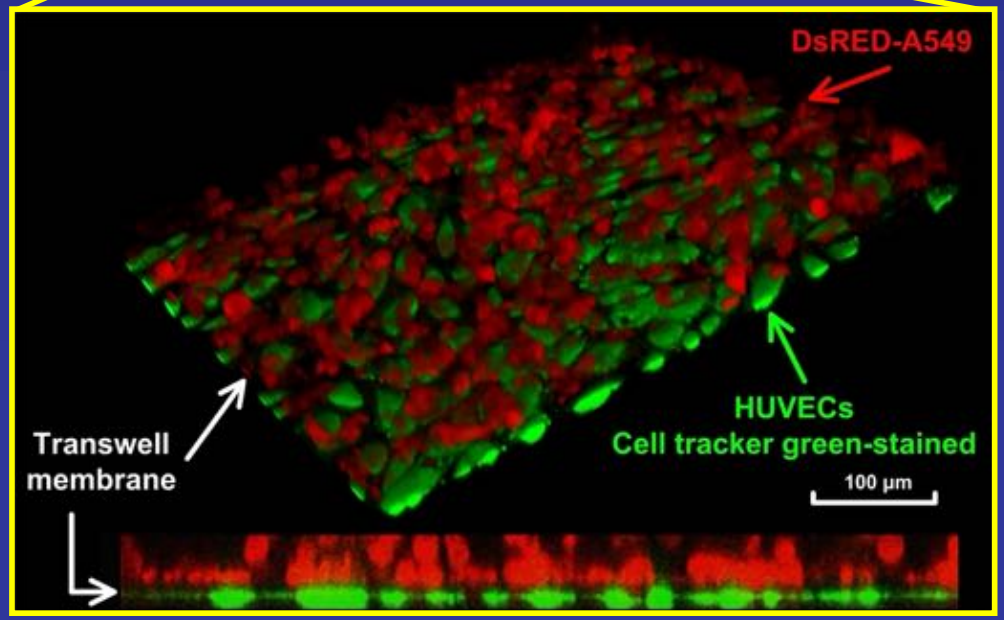
Toward Systemic Model – Integration



HUVEC/A549
(Live/Dead Stain)







Introduction – Engineering a Robot?

- Different Thought about Robot?

Conclusion

- Development of various microfluidic cell culture devices capable of controlling and analyzing gaseous and physical microenvironments.
- Multi-dimensional cell culture to mimic physiological arrangements of the cells *in vivo*.
- The new systemic *in vitro* model will provide biomedical scientists next generation cell culture models with better prediction power, and the results can be more easily to be translated into animal experiments and clinical tests.

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