



Application – Microfluidic Cell Culture Devices (1)

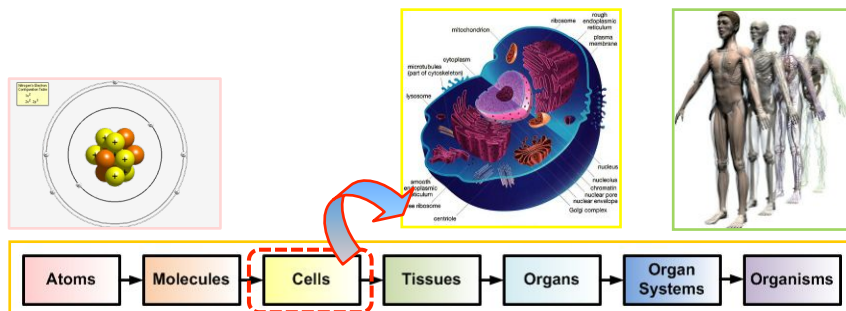
Date: 2013/05/10

Dr. Yi-Chung Tung



Cells

- Cells are the basic functional units of most living organisms.
- Cells sense and response to changes in their environment, and communicate with neighboring cells by releasing chemicals or by generating electrical signals.





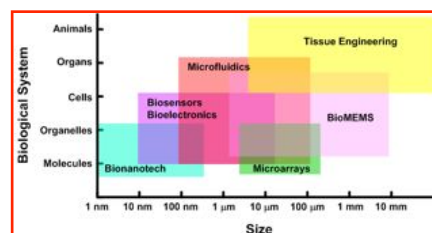
In vitro Cell Culture

- ***in vivo* Cell Study**
 - Lack of fully understanding microenvironments
 - Difficult to control all biological parameters
 - Clinical operation required
- ***in vitro* Cell Culture**
 - Well-defined microenvironments
 - Large number of samples available
 - Time-consuming for taking care of cells
 - Changing Media, Passaging etc.
 - Different from *in-vivo* environment



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





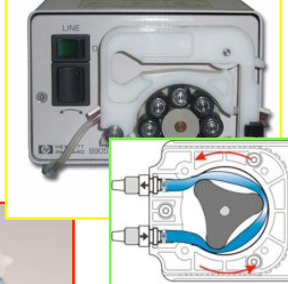
Microfluidic Actuation

- Old ways...

Vacuum Pump



Peristaltic Pump



Syringe Pump



Droplet-Based Passive Pumping

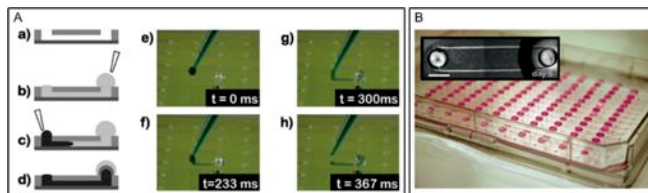
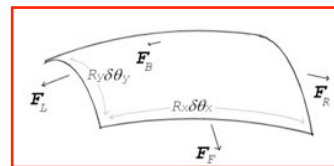
- Surface Tension Driven Flow

- Surface Tension (γ):

Young-Laplace Equation:

$$\Delta p = \gamma \left(\frac{1}{R_x} + \frac{1}{R_y} \right)$$

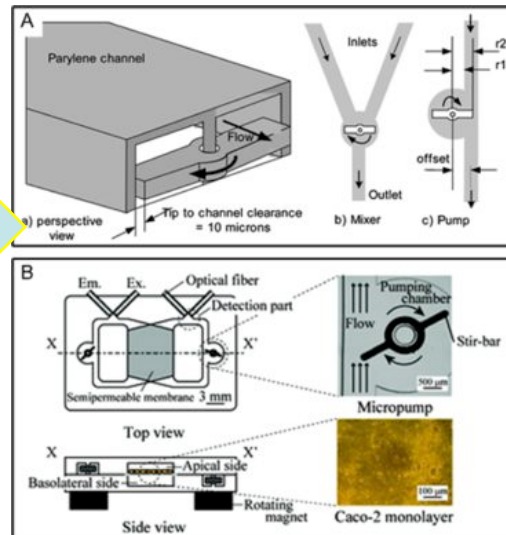
Where Δp is the pressure difference, γ is surface tension, R_x and R_y are radii of curvature in each of the axes that are parallel to the surface. Water at 25°C, $\gamma = 71.97$ (dyn/cm)





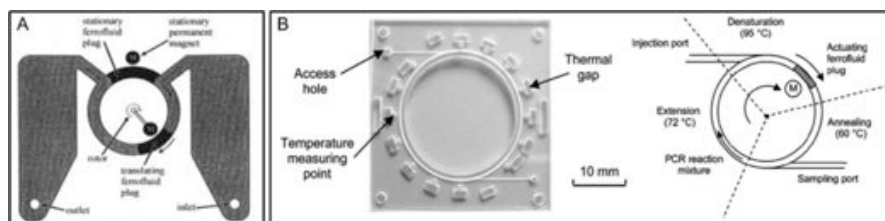
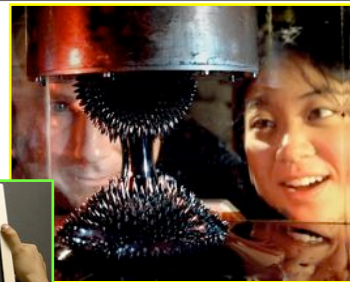
Actuation by External Magnetic Field

- **Micro-stirrer.**



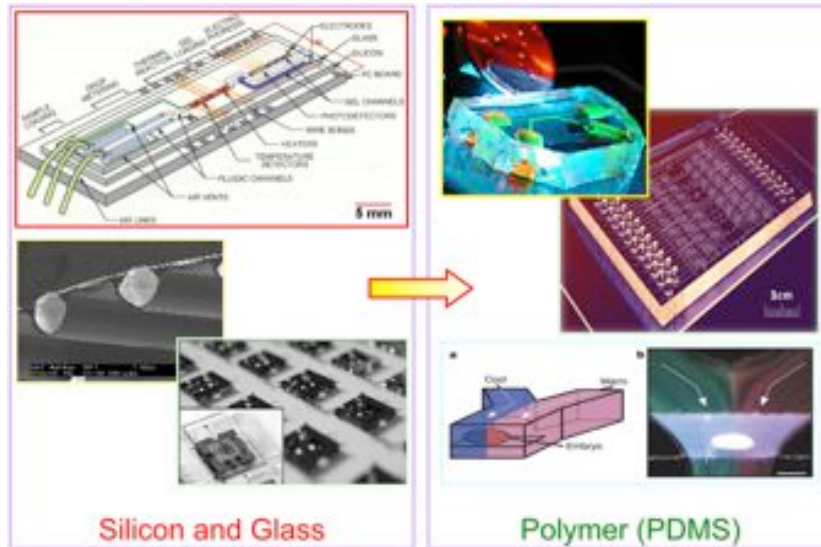
Actuation by External Magnetic Field

- **Ferrofluid** - a liquid which becomes strongly polarised in the presence of a [magnetic field](#).

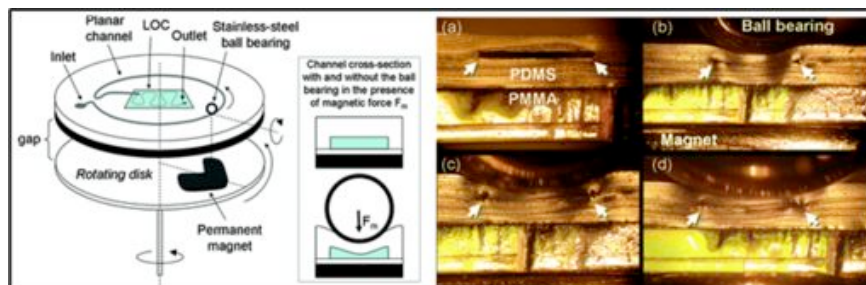




Polymer Microfluidic Devices

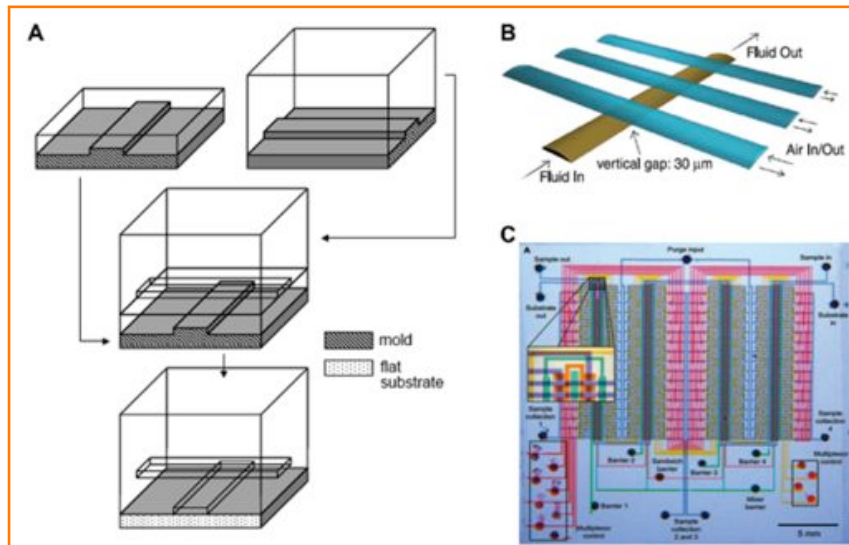


Deformation-Based Microfluidic Actuation



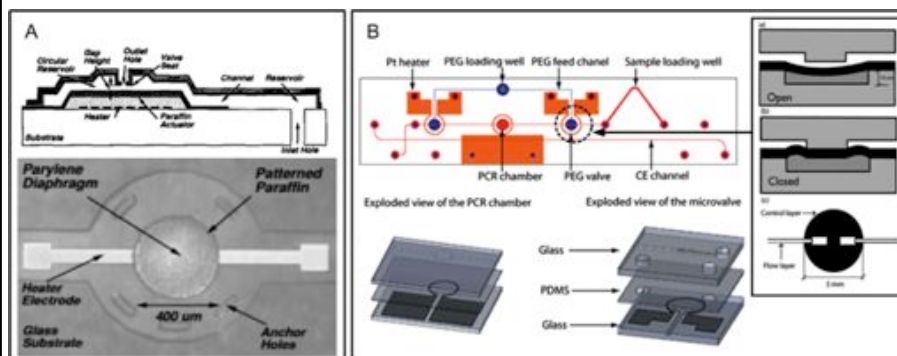


Multilayer Soft Lithography (MSL) with Pneumatic Actuation



Thermally Actuated Phase-Change Microfluidic Components

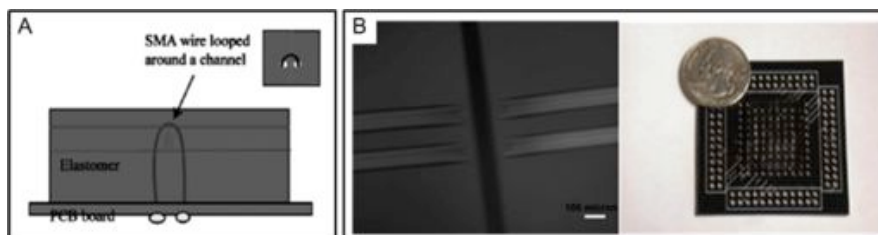
- Taking advantage of materials that exhibit large volumetric change during phase change (e.g. polyethylene glycol (PEG), and paraffin), temperature regulated active microfluidic components have been developed.



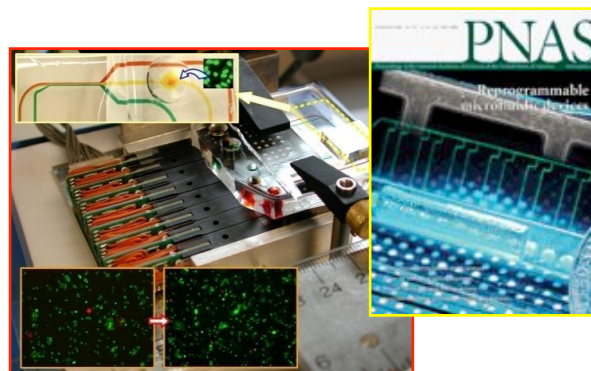


Shape Memory Alloy Actuators

- A shape memory alloy (SMA) is an alloy that “remembers” its shape, and can be returned to that shape after being deformed, by applying heat to the alloy.



Computerized Microfluidic Device Using PDMS Channels and Braille Display



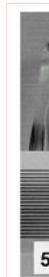


Introduction – PDMS-Based Devices

- Applications

-

However...



Schuelke

Fluid in

Fluid out

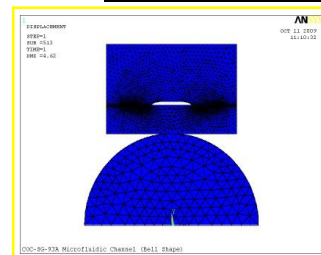
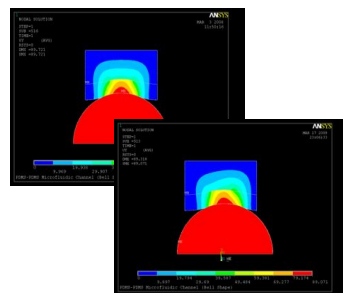
Pressure in/out



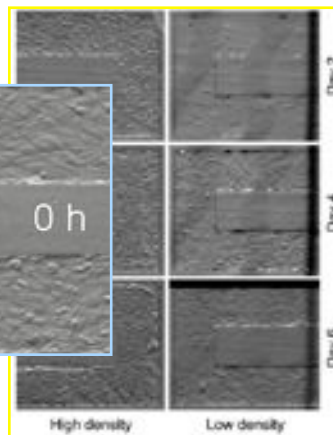
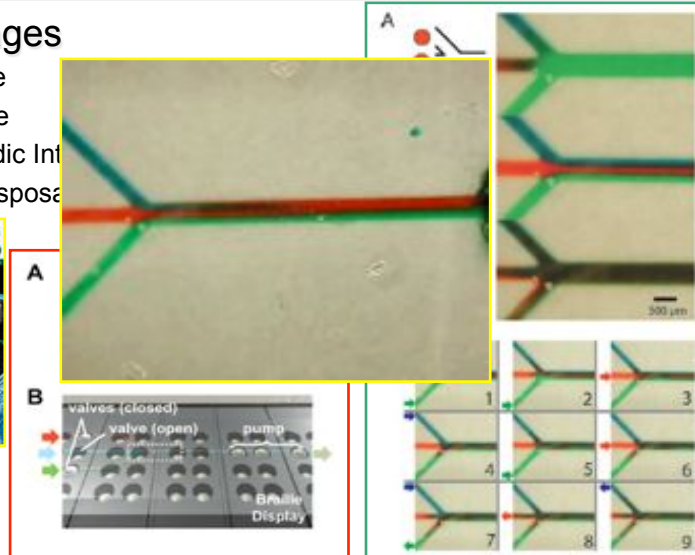
Braille Display-Based Microfluidic Systems

Braille Display

- Piezoelectric Actuation
- Computer Programmable (USB)
- Low Power Consumption
- Fast Response



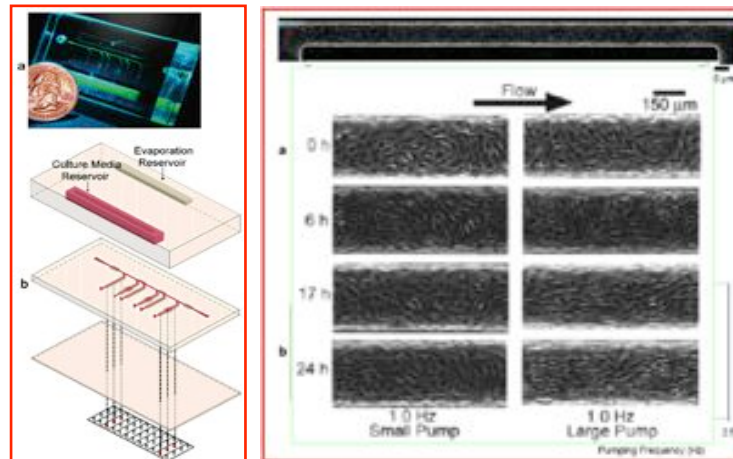
- Portable
- Versatile
- No Fluidic Int
- Fully Dispos





Cell Culture in Various Shear Stress Conditions

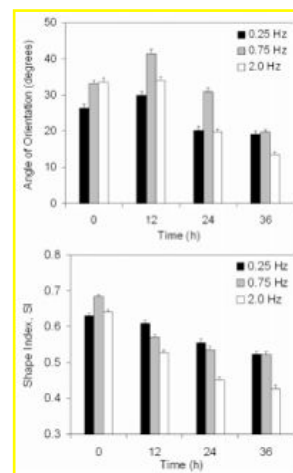
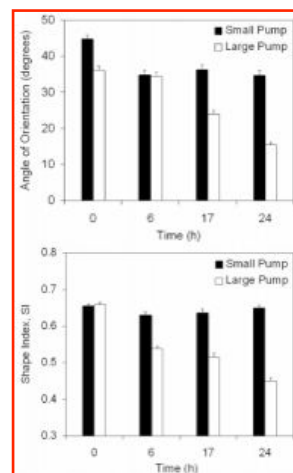
- Endothelial Cell (EC) Culture under Various Shear Stress Conditions – Temporal Patterns



Cell Culture in Various Shear Stress Conditions

Shear Stress Magnitude:

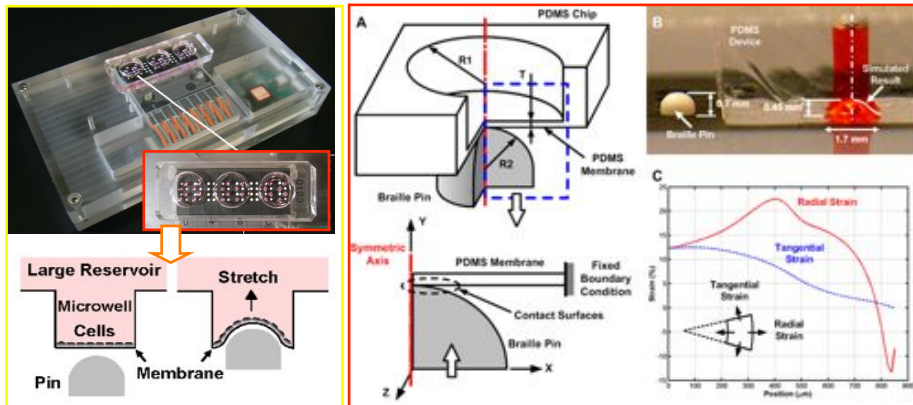
Shear Stress Frequency:



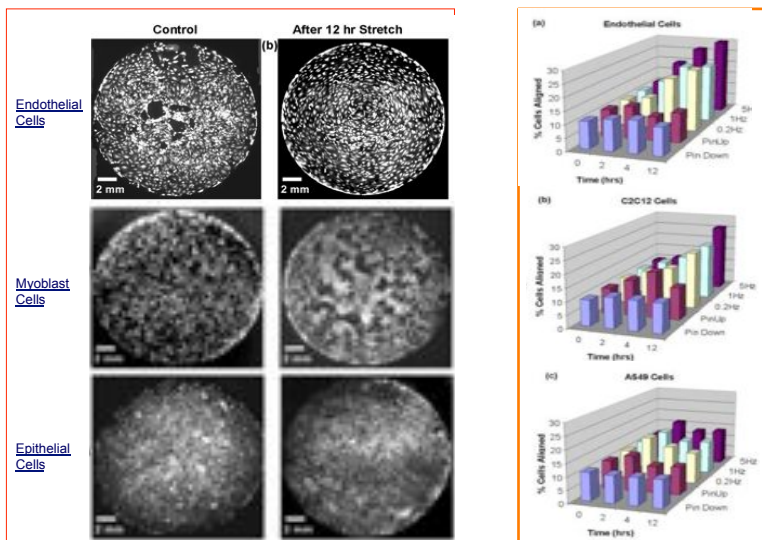


Individually Programmable Cell Stretching Microwell Arrays Actuated by a Braille Display

- Test various cell samples, culture medium, and stretching conditions simultaneously
- Small cell sample required

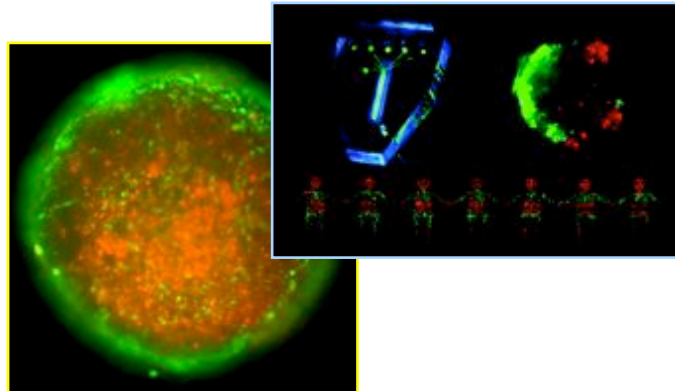


Individually Programmable Cell Stretching Microwell Arrays Actuated by a Braille Display



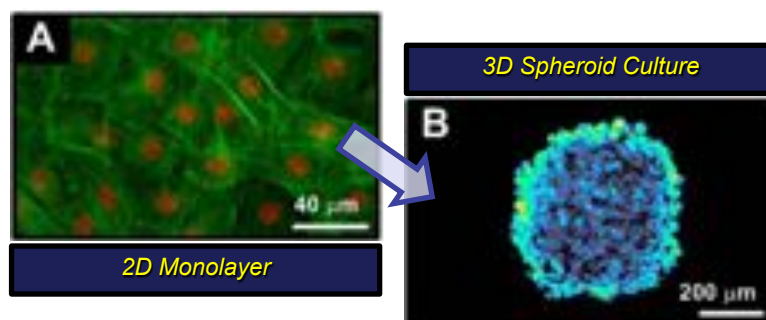


Three-Dimensional Cell Spheroid Culture in PDMS Microfluidic Device



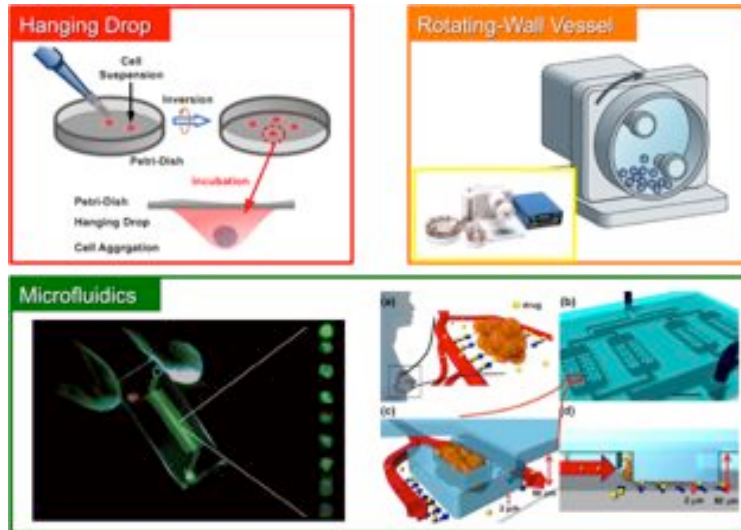
Three-Dimensional Cell Culture - Spheroid

- 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.



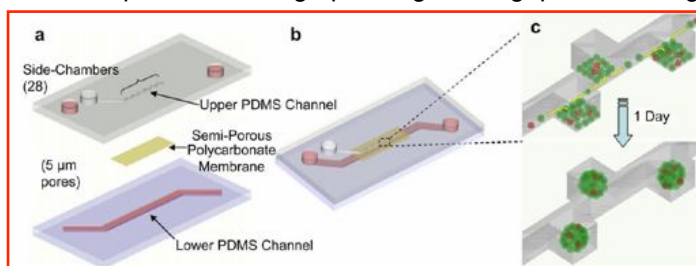


How to Form Spheroids ?

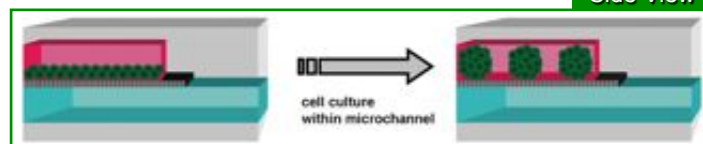


Spheroid Culture Microfluidic Device

- Dual-Layer Compartmentalized PDMS microfluidic Device
 - Efficient formation of uniform-sized spheroids
 - Simple yet robust operation
 - Capable of scaling up for high throughput screening



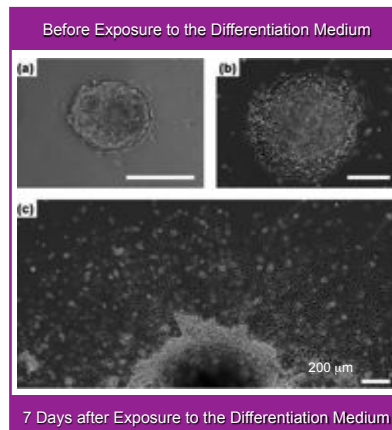
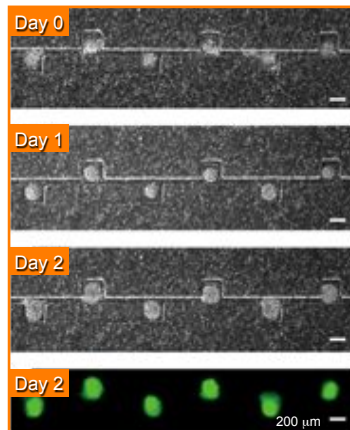
Side View





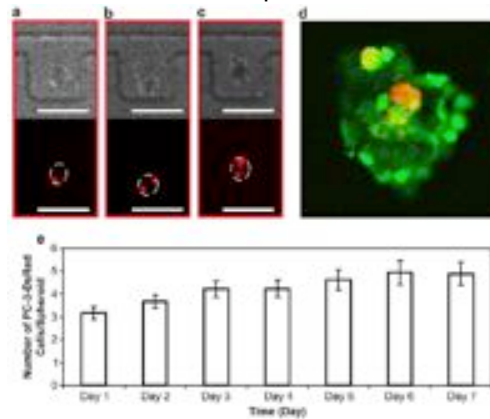
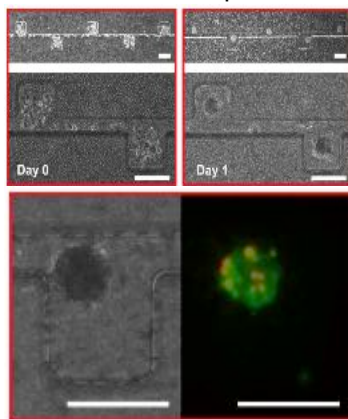
Application – Embryoid Body (EB) Culture

- Appropriate morphology and embryo size are critical for the sequential development stages of naturally conceived embryos.
- Mouse embryonic stem (ES) cells (ES-D3 cell line, ATCC)



Application – Cancer Cell Co-Culture

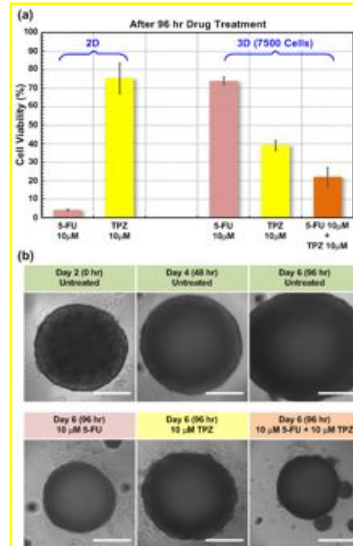
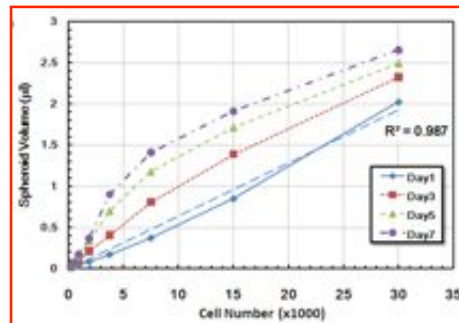
- Endothelial, osteoblast, and prostate cancer cell co-culture to mimic the *in-vivo* niche microenvironment
- Slower prostate cancer cell proliferation rate (doubling time: ~9 days)
- Endothelial cells proliferate, and osteoblast cells are quiescent.



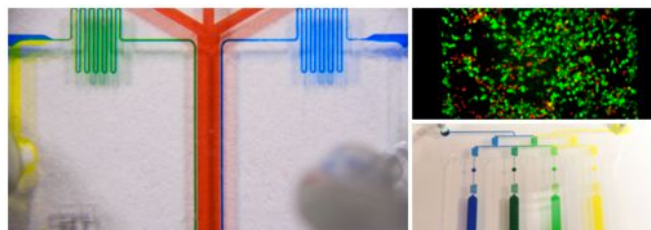


Application – Cancer Cell Drug Test

- Human carcinoma cell that stably express mesothelin (A431.H9) cell
- Drug test for commonly used chemotherapy agent: Fluorouracil (5-Fu) and Tirapazamine (TPZ)
- Cell viability test using *Alamar blue*



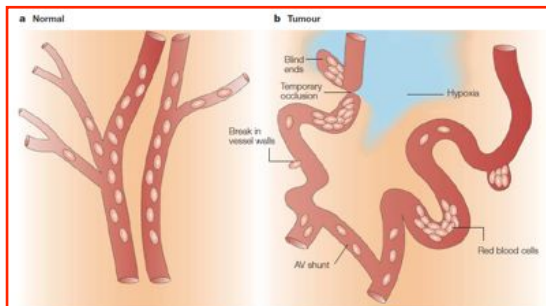
Generation of Oxygen Gradients in Microfluidic Devices for Cell Culture using Locally Confined Chemical Reactions





Introduction

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



Tumours contain regions of hypoxia and necrosis because their vasculature can not supply oxygen and other vital nutrients to all the cells.

(a) Normal vasculature is hierarchically organized, with vessels that are sufficiently close to ensure adequate nutrient and oxygen supply to all cells.

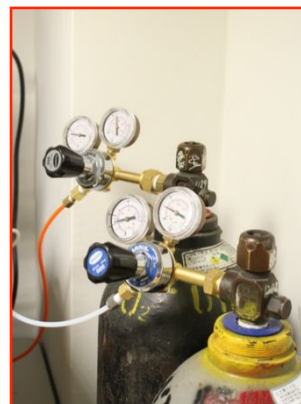
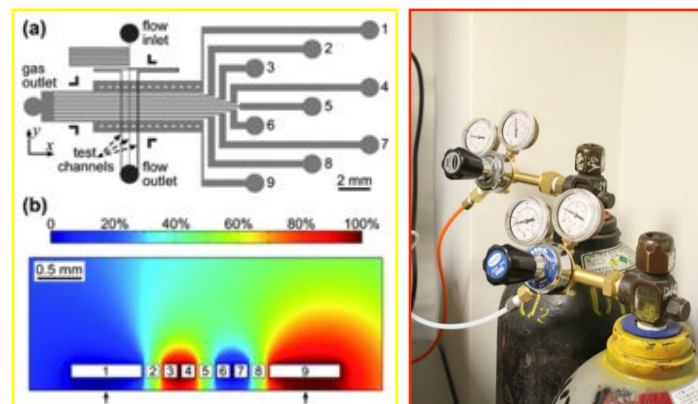
(b) Tumor vessels are chaotic, dilated, tortuous and are often far apart and have sluggish blood flow.

EXPLOITING TUMOUR HYPOXIA IN CANCER TREATMENT (Nature Reviews Cancer 2004, 4: 437)



Motivation

- Existing methods to generate oxygen gradients for cell culture are often complicated, require gas cylinders and interconnections, are not compatible with cell incubators.





Objective

- Develop a new single-layer microfluidic device capable of stably generating oxygen gradients within a confined area.
- Using chemical reactions instead of gas cylinders and interconnections.
- Fully compatible with incubators, and suitable for long term cell culture studies.

Simple Fabrication –
Single Layer Construction

Excellent Cell
Incubator Compatibility

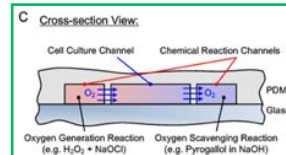
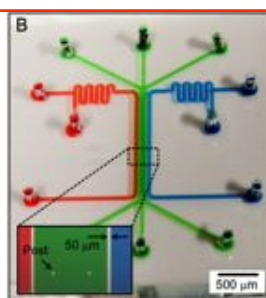
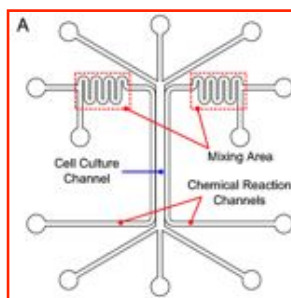
No Gas Cylinders and
Interconnections

Minimal Chemicals Required



Microfluidic Device Design

- The device is constructed by a single-layer polydimethylsiloxane (PDMS) microfluidic channel sealed with a PDMS-coated glass slide.
- The microfluidic actuation is achieved by syringe pumps.



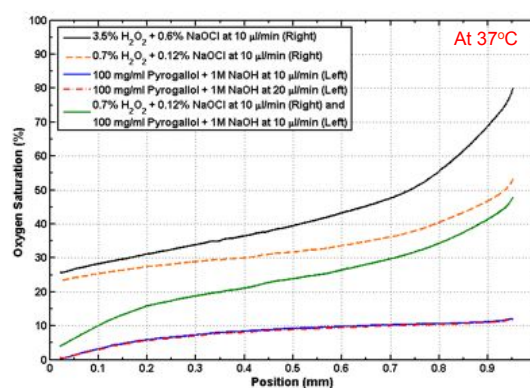
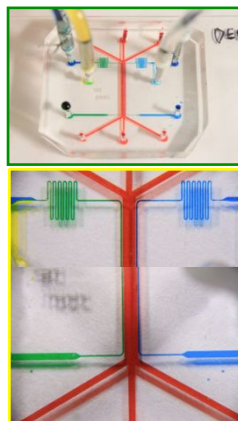
Oxygen Diffusion Coefficient, D (cm^2/s)

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



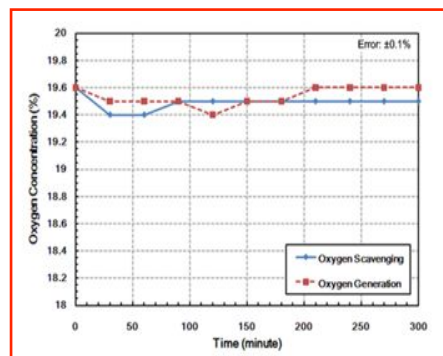
Oxygen Tension Profile Measurement

- Pure *Nitrogen* and *Oxygen* gases were flowed into the chemical reaction channels as 0% and 100% oxygen saturation for calibration.



Cell Incubator Compatibility

- The oxygen tension of the incubator (Thermo HERAcell 240i) is monitored while performing the oxygen generation and scavenging reactions within the device in the incubator.

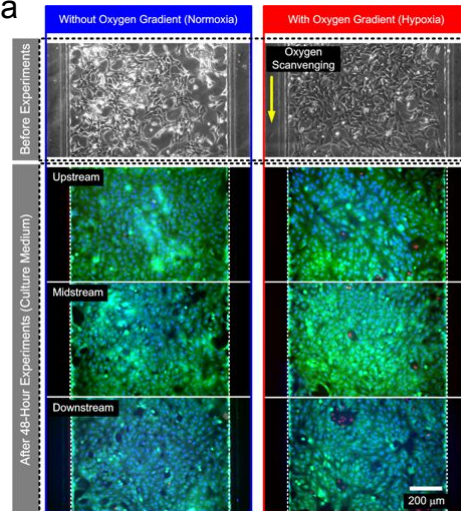


Oxygen Scavenging:
Pyrogallol (100 mg/ml) + 5M NaOH
Oxygen Generation:
35% H_2O_2 + 6% NaOCl

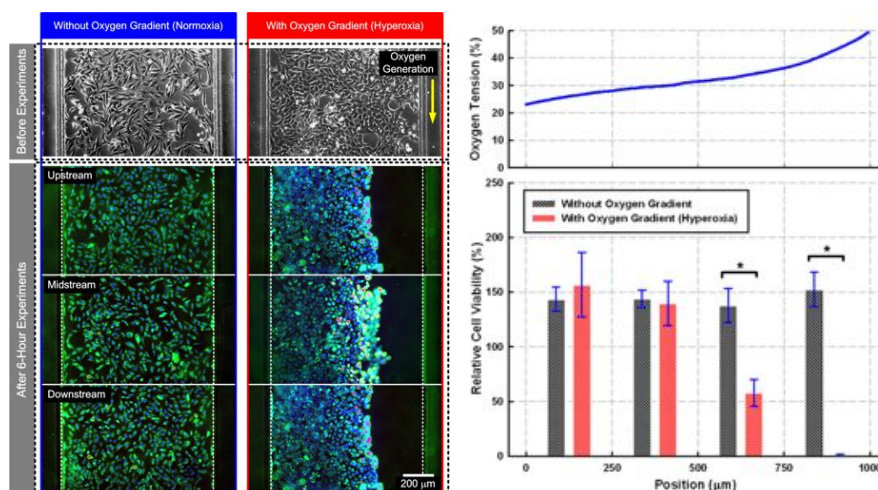


Cell Culture Compatibility

- A549 (lung adenocarcinoma epithelial cell line) cells are successfully cultured in the device. The device is treated with extra cellular matrix (ECM) protein, fibronectin (100 $\mu\text{g/ml}$), before seeding cells.



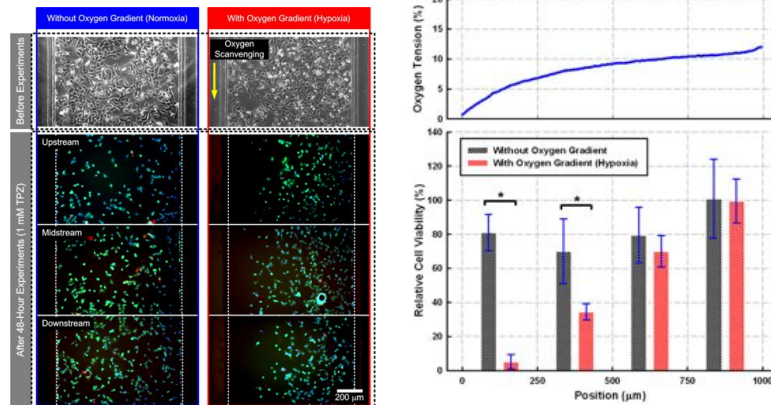
Hyperoxia-Induced Cell Death





Drug Testing on Cell Models

- Drug testing with/without oxygen gradients.
- Triapazamine (TPZ) 24-hour treatment with the flow rate of 10 $\mu\text{l}/\text{min}$ in cell incubators.



Integrated Biomedical Microdevices

- Precisely Control or Mimic the Rich Biochemical and Biophysical Complexity of the Cellular Microenvironments.
- Cell Activity Monitoring and Observation.

