Application – Microfluidic Cell Culture Devices (1)

Date: 2015/05/29

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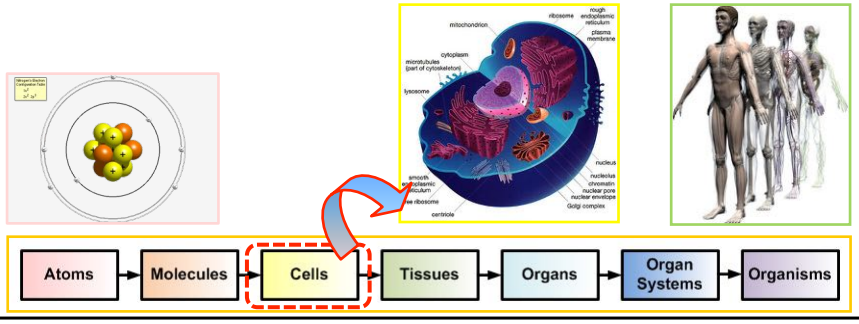
Cells

- 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.

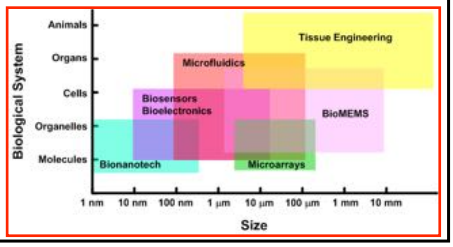


In vitro Cell Culture

- ***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**

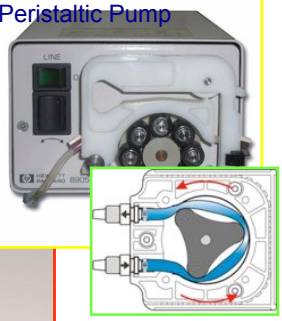


Microfluidic Actuation

- **Old ways…**

Vacuum Pump

Peristaltic Pump

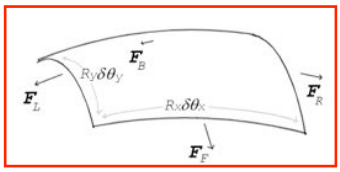


Syring Pump

Droplet-Based Passive Pumping

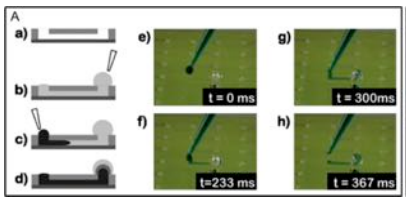
- **Surface Tension Driven Flow**
- Surface Tension (γ):

  Young-Lapalace Equation:



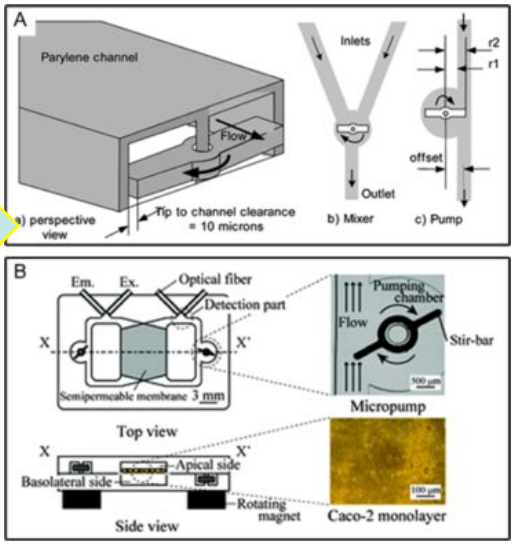
$$\Delta p = \gamma\left(\frac{1}{R_x}+\frac{1}{R_y}\right)$$

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, γ = 71.97 (dyn/cm)



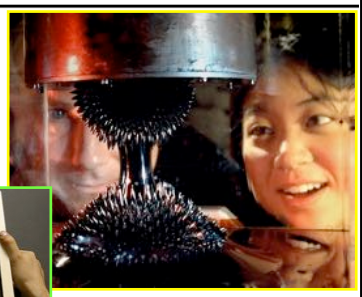
Actuation by External Magnetic Field

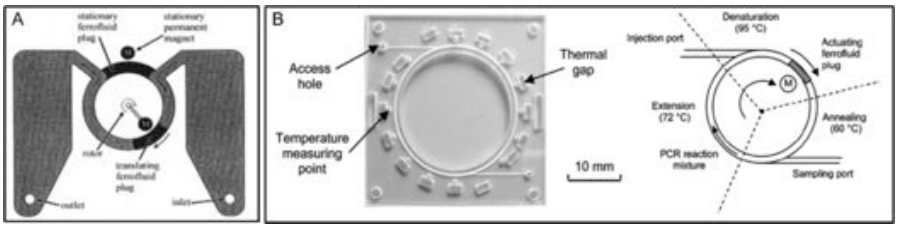
- **Micro-stirrer**.



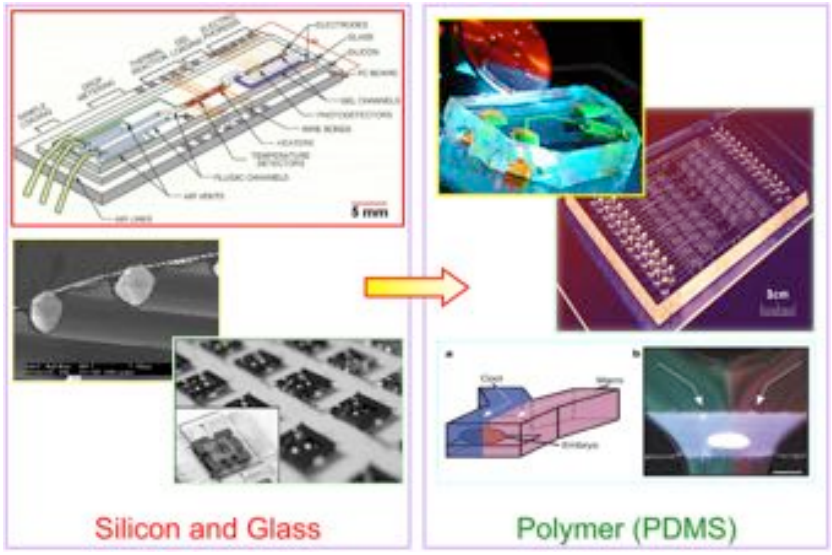
Actuation by External Magnetic Field

- **Ferrofluid -** a liquid which becomes strongly polarised in the presence of a magnetic field.

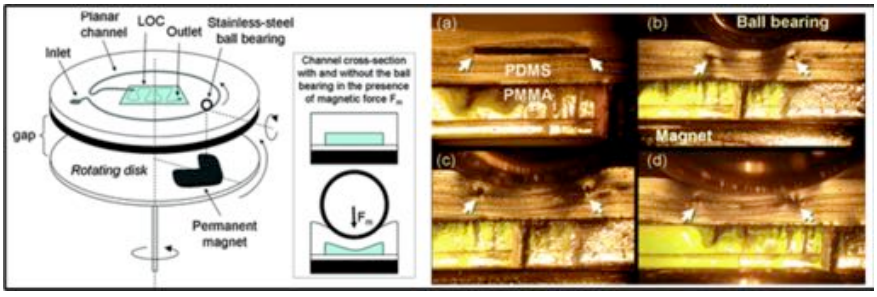




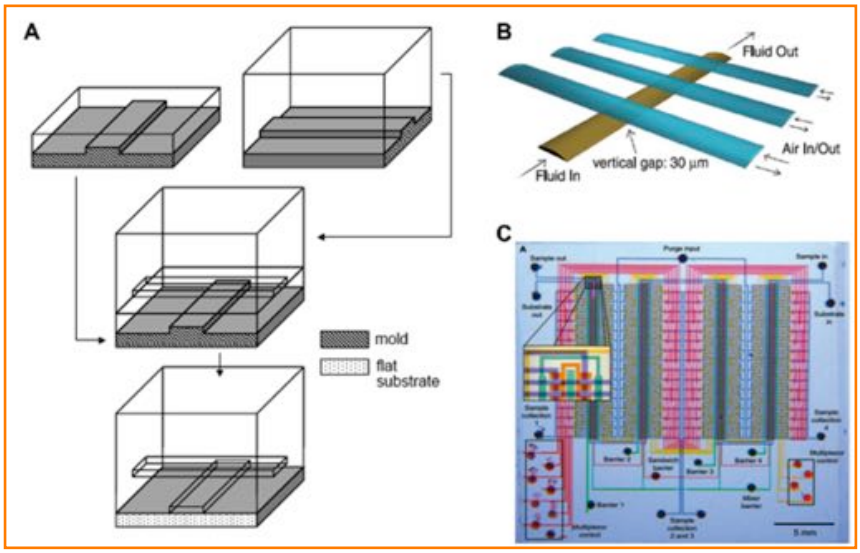
Polymer Microfluidic Devices



Deformation-Based Microfluidic Actuation

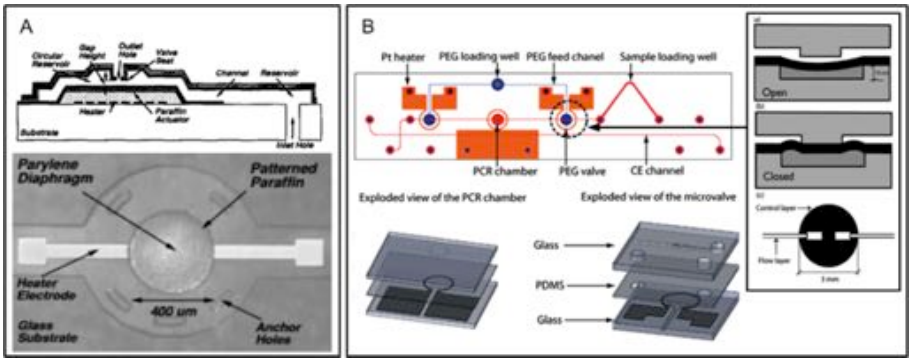


Multilayer Soft Lithography (MSL) with Pneumatic Actuation



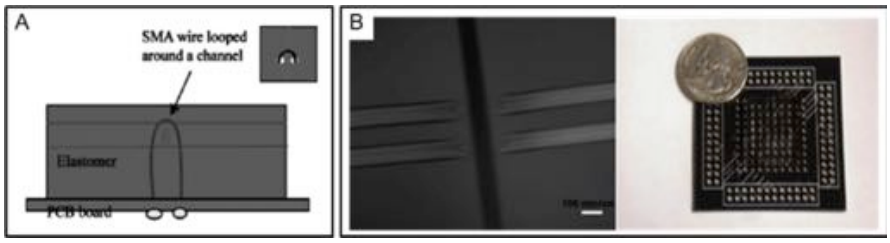
Thermally Actuated Phase-Change Microfluidic Components

- 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.

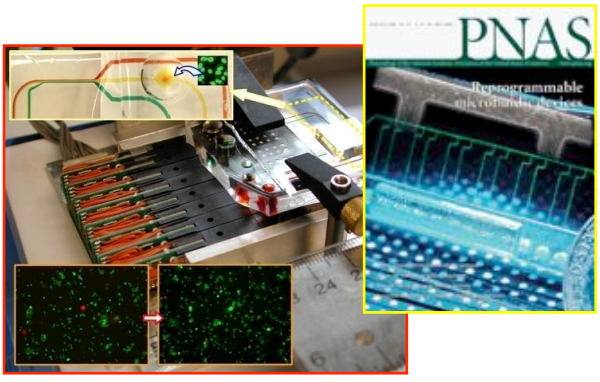


Shape Memory Alloy Actuators

- 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.



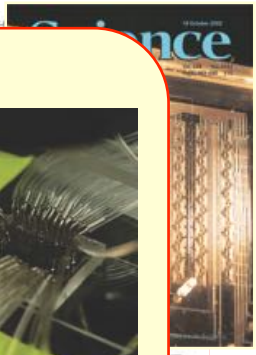
Computerized Microfluidic Device Using PDMS Channels and Braille Display



Introduction – PDMS-Based Devices

- Applications
  - ?

However…



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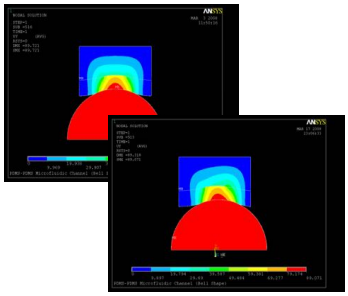
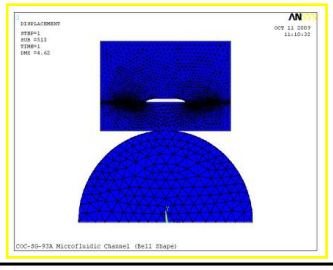
Braille Display-Based Microfluidic Systems

Braille Display

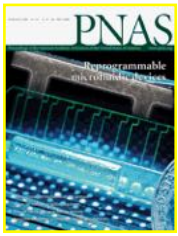
- Piezoelectric Actuation
- Computer Programmable (USB)
- Low Power Consumption
- Fast Response

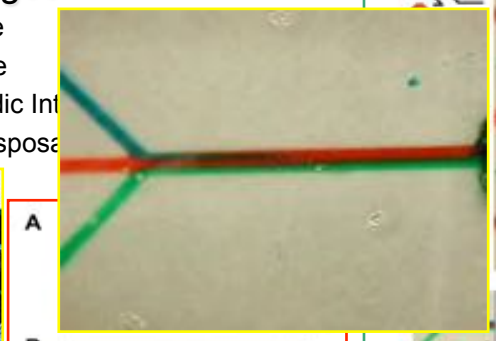


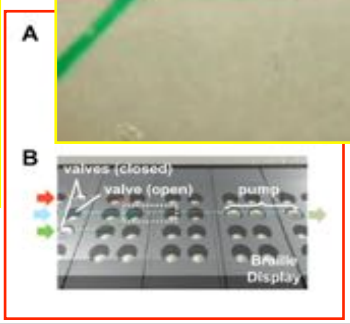


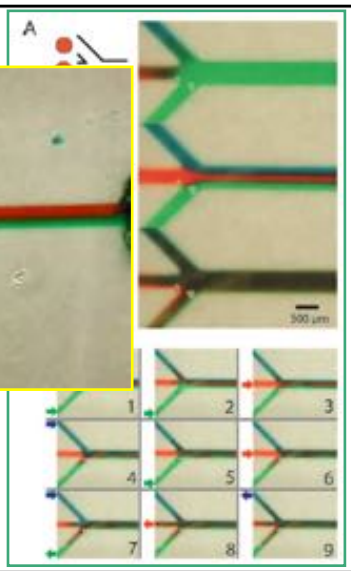
Braille Display-Based Microfluidic Systems

- Advantages
  - Portable
  - Versatile
  - No Fluidic Int
  - Fully Disposa



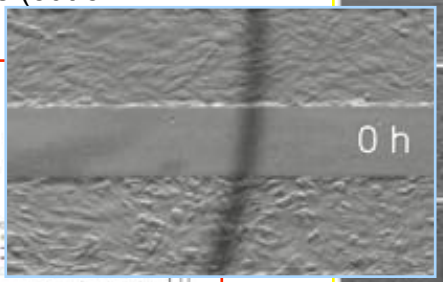


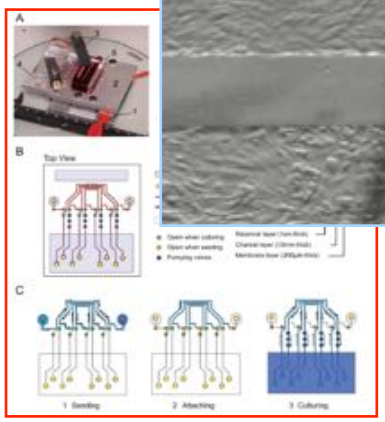


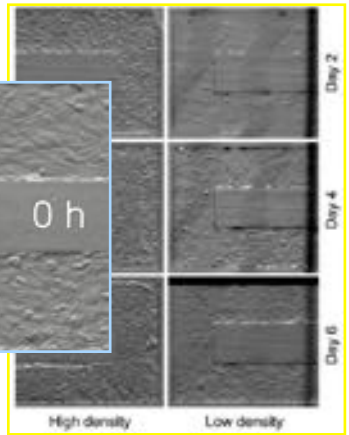


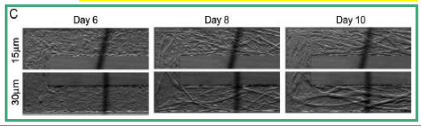
Braille Display-Based Cell Culture

- Long-term culture for C2C12 myoblast cells (out of incubators)



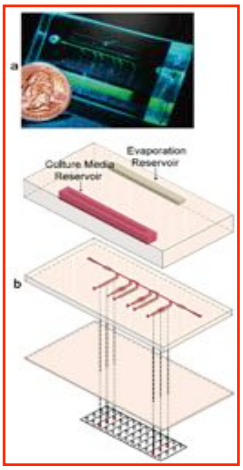


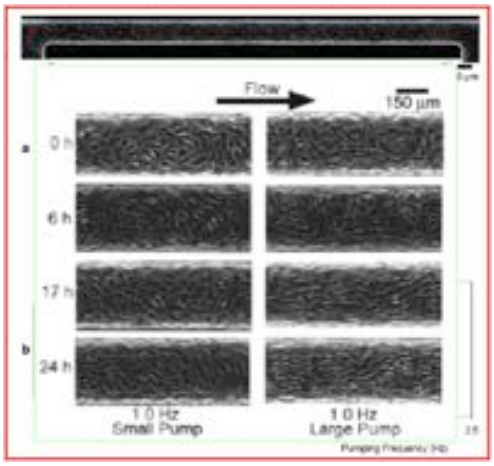




Cell Culture in Various Shear Stress Conditions

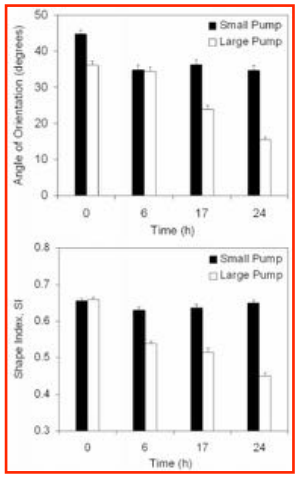
- Endothelial Cell (EC) Culture under Various Shear Stress Conditions – Temporal Patterns



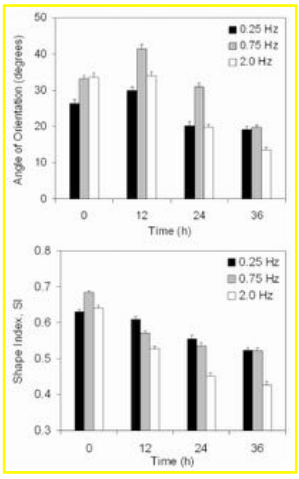


Cell Culture in Various Shear Stress Conditions

Shear Stress Magnitude:

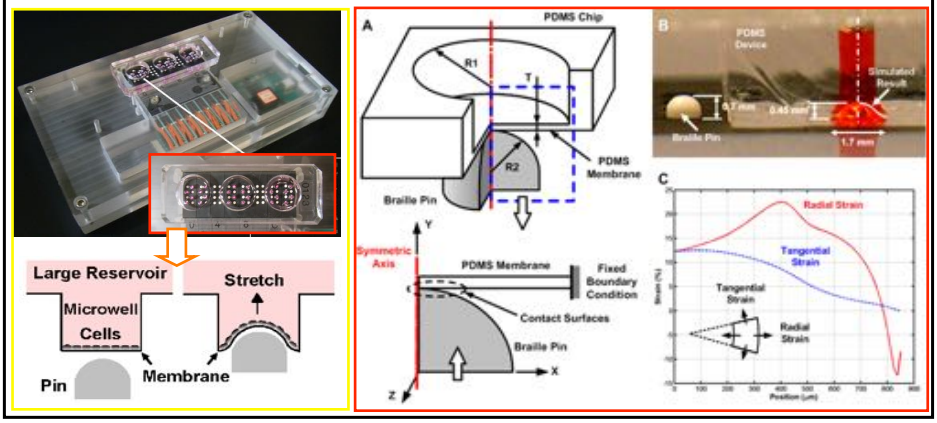


Shear Stress Frequency:

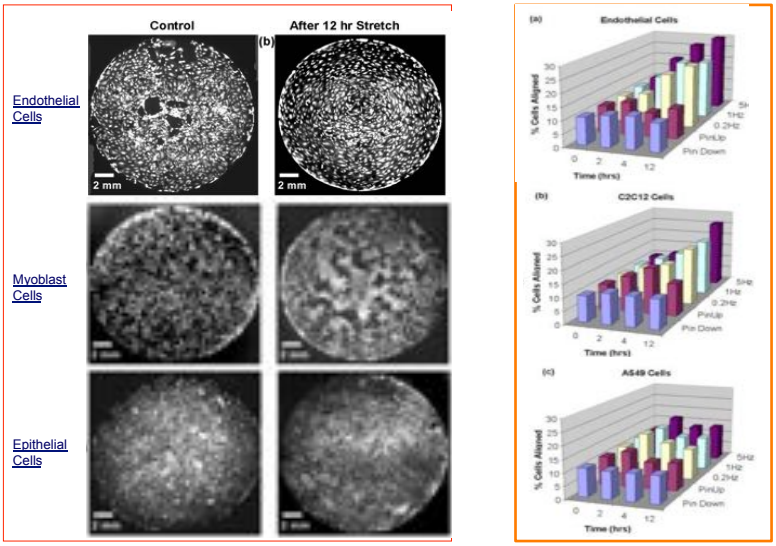


Individually Programmable Cell Stretching Microwell Arrays Actuated by a Braille Display

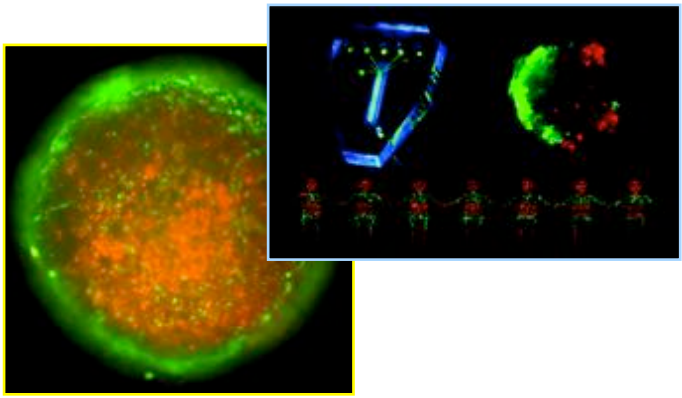
- Test various cell samples, culture medium, and stretching conditions simultaneously
- Small cell sample required



Individually Programmable Cell Stretching Microwell Arrays Actuated by a Braille Display

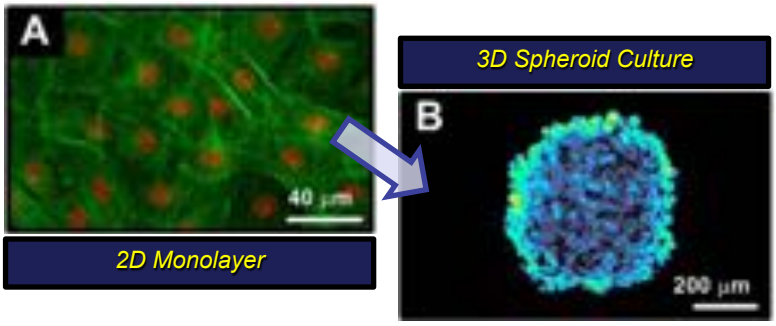


Three-Dimensional Cell Spheroid Culture in PDMS Microfluidic Device

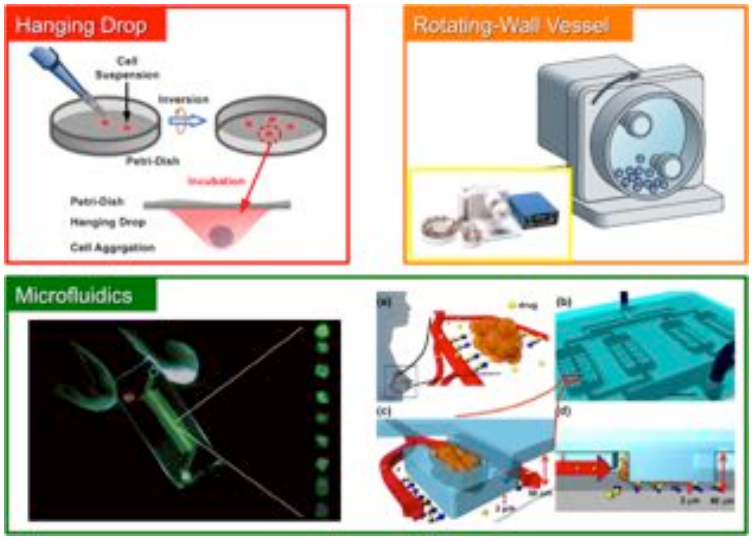


Three-Dimensional Cell Culture - Spheroid

- 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.

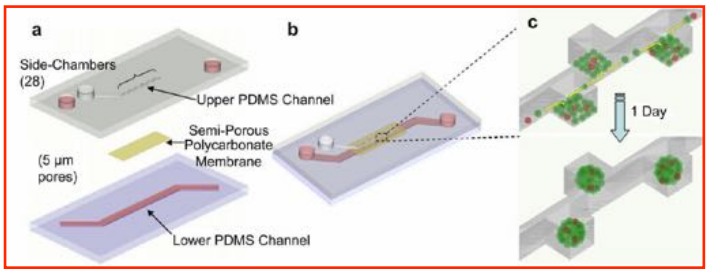


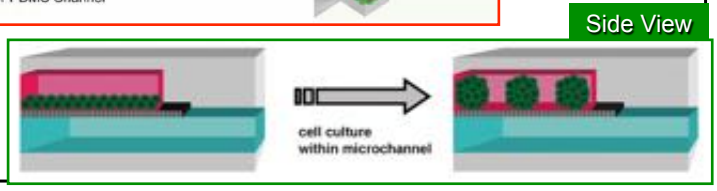
How to Form Spheroids ?



Spheroid Culture Microfluidic Device

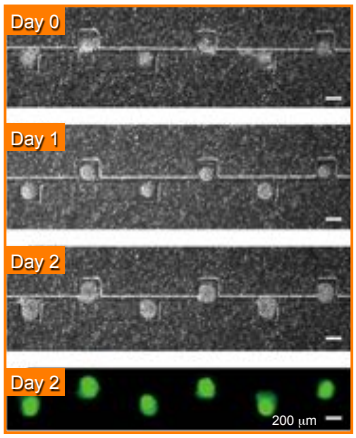
- Dual-Layer Compartmentalized PDMS microfluidic Device
  - Efficient formation of uniform-sized spheroids
  - Simple yet robust operation
  - Capable of scaling up for high throughput screening

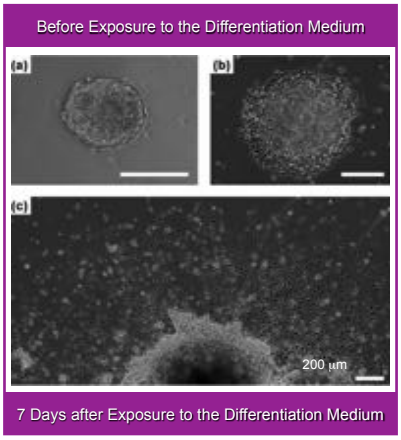




Application – Embryoid Body (EB) Culture

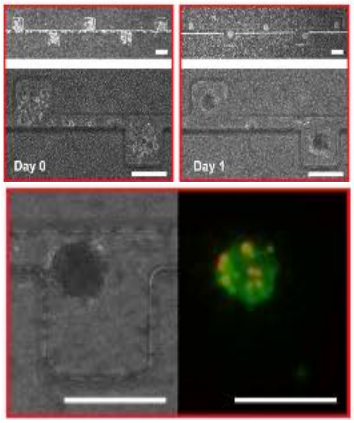
- Appropriate morphology and embryo size are critical for the sequential development stages of naturally conceived embryos.
- Mouse embryonic stem (ES) cells (ES-D3 cell line, ATCC)

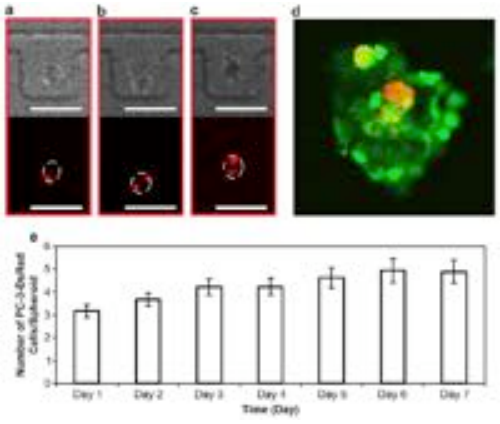




Application – Cancer Cell Co-Culture

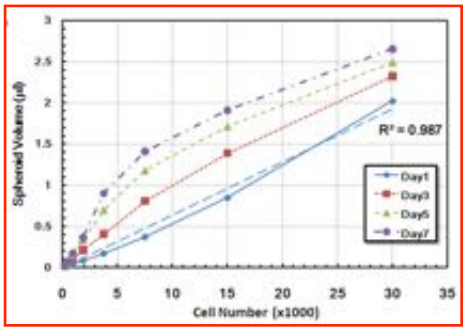
- Endothelial, osteoblast, and prostate cancer cell co-culture to mimic the *in-vivo* niche microenvironment
- Slower prostate cancer cell proliferation rate (doubling time: ~9 days)
- Endothelial cells proliferate, and osteoblast cells are quiescent.

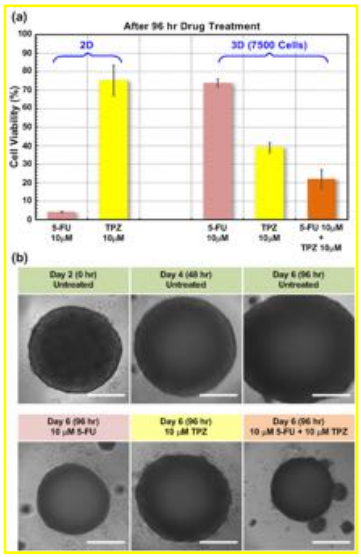




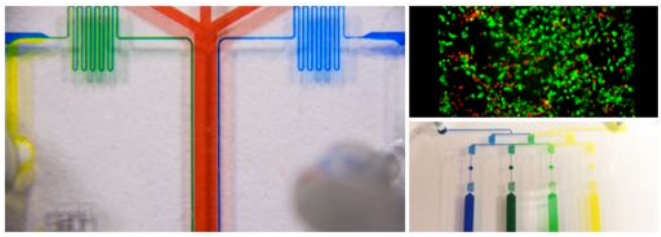
Application – Cancer Cell Drug Test

- 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*



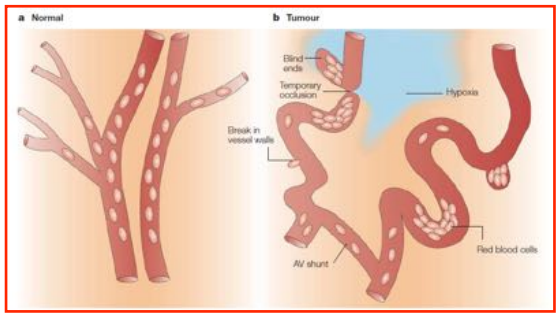


Generation of Oxygen Gradients in Microfluidic Devices for Cell Culture using Locally Confined Chemical Reactions



Introduction

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



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

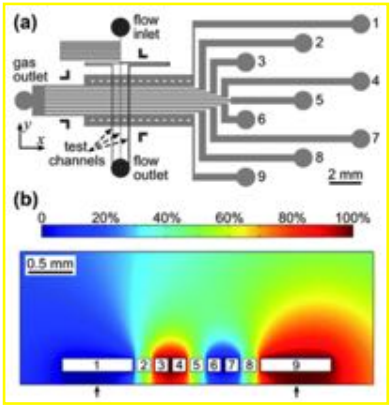
Tumours contain regions of hypoxia and necrosis because their vasculature can not supply oxygen and other vital nutrients to all the cells.

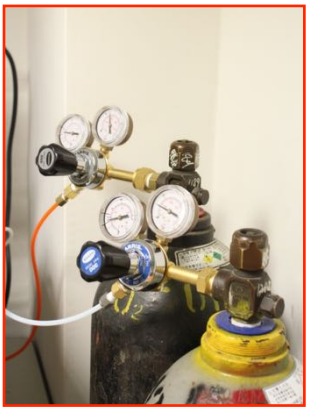
(a)Normal vasculature is hierarchically organized, with vessels that are sufficiently close to ensure adequate nutrient and oxygen supply to all cells.

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

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

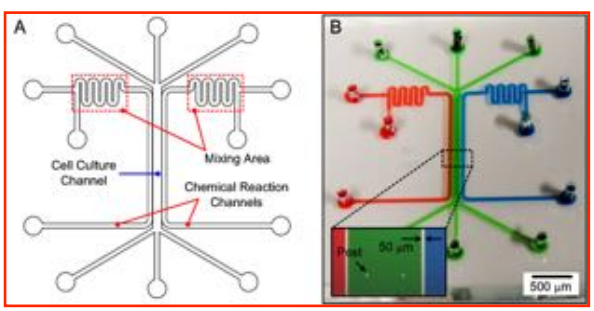
Excellent Cell Incubator Compatibility

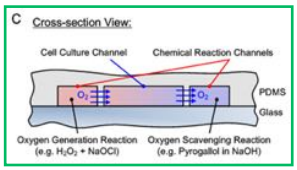
No Gas Cylinders and Interconnections

Minimal Chemicals Required

Microfluidic Device Design

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



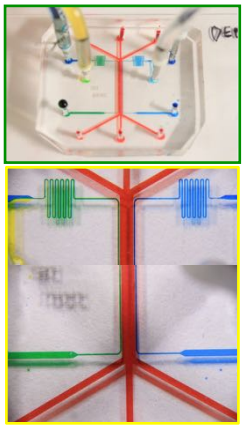


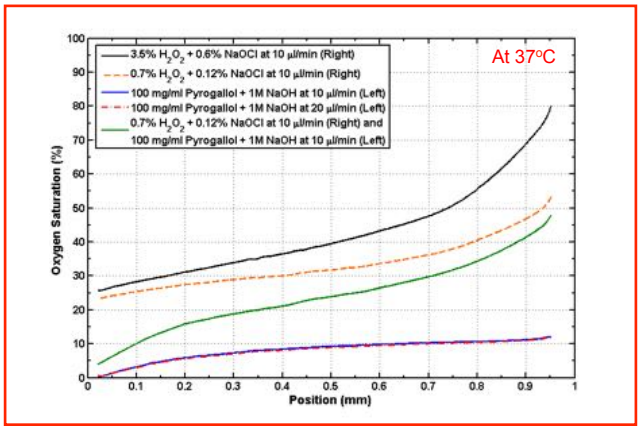
Oxygen Diffusion Coefficient, D (cm^2/s)

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

Oxygen Tension Profile Measurement

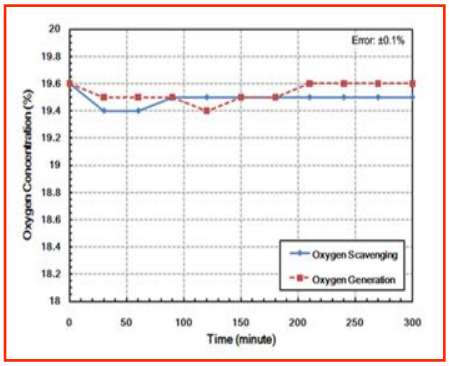
- Pure *Nitrogen* and *Oxygen* gases were flowed into the chemical reaction channels as *0%* and *100%* oxygen saturation for calibration.





Cell Incubator Compatibility

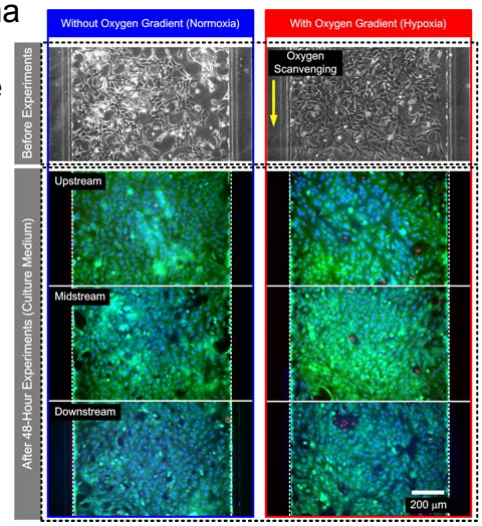
- The oxygen tension of the incubator (Thermo HERAcell 240i) is monitored while performing the oxygen generation and scavenging reactions within the device in the incubator.



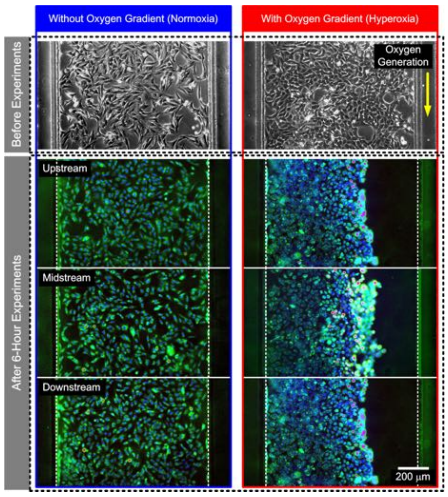
Oxygen Scavenging:
Pyrogallol (100 mg/ml) + 5M NaOH
Oxygen Generation:
35% H_2O_2 + 6% NaOCl

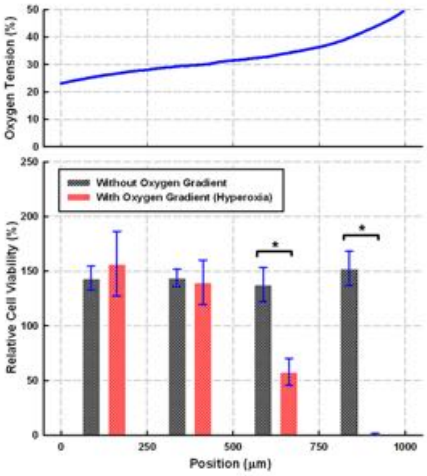
Cell Culture Compatibility

- 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 μg/ml), before seeding cells.



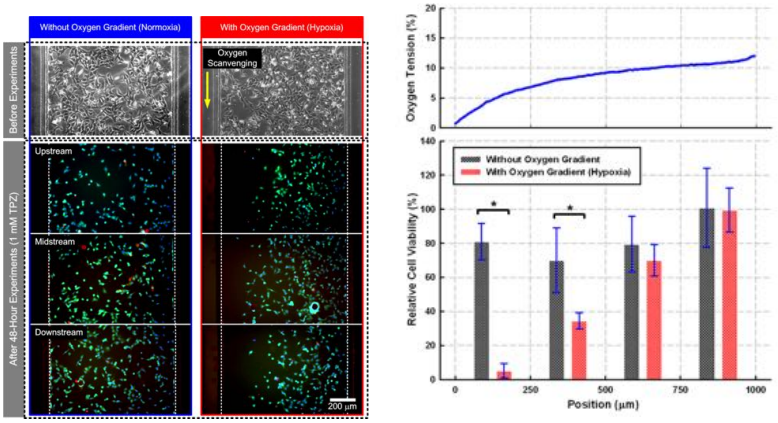
Hyperoxia-Induced Cell Death





Drug Testing on Cell Models

- Drug testing with/without oxygen gradients.
- Triapazamine (TPZ) 24-hour treatment with the flow rate of 10 μl/min in cell incubators.



Integrated Biomedical Microdevices

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