THE INFLUENCES OF FLUID-INDUCED SHEAR STRESS IN OSTEOBLASTS CULTURED IN MICROCHANNELS

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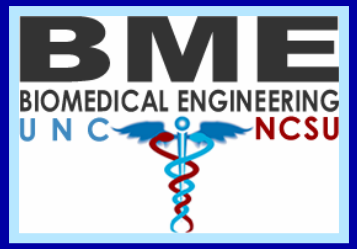
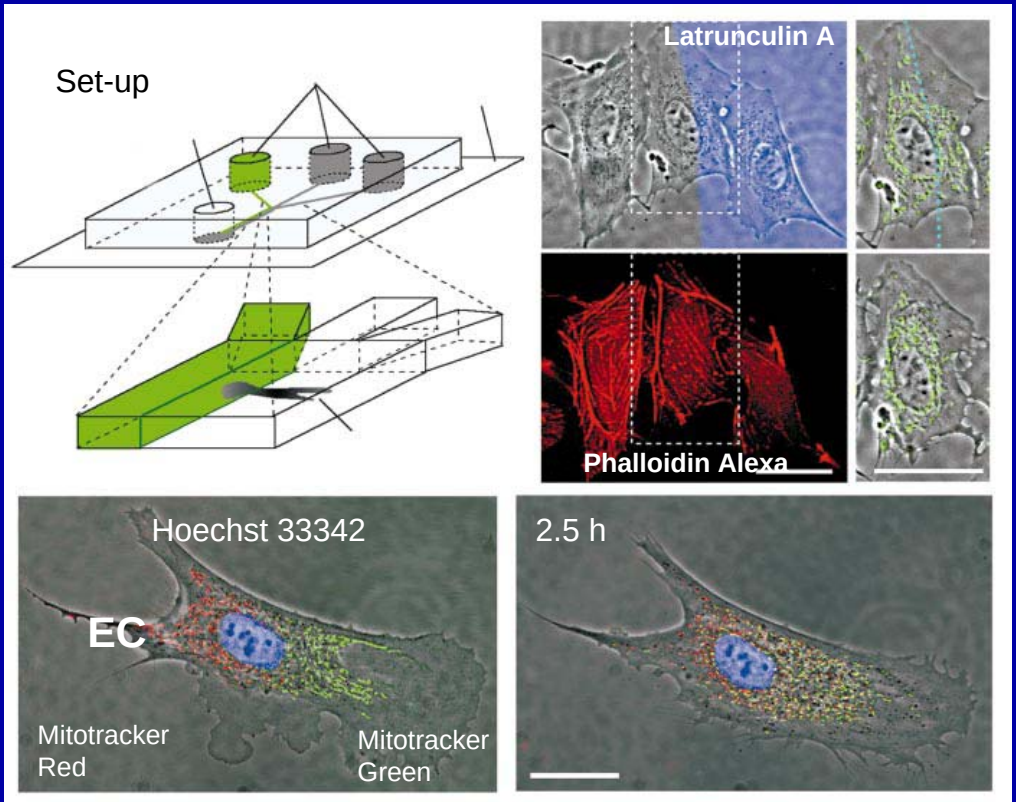


Why and how stimulate selected cell(s) in the microchannels?



Reference:
Takayama, Whitesides et al. *Nature*, 2001

Background:

- Traditional assays utilize cell groups
  - Data may be averaged
  - Confounding effects of cell A on cell B
- Our current studies address these challenges
  - Sample individual cells
  - Sample segments of a cell
  - Prevent downstream mediator effects

Microfluidic technology and laminar flow

Hypothesis

Selected cell stimulation technologies will:

- permit testing of cell segments.
- conserve reagents.
- increase assay sensitivity (?)

Objectives

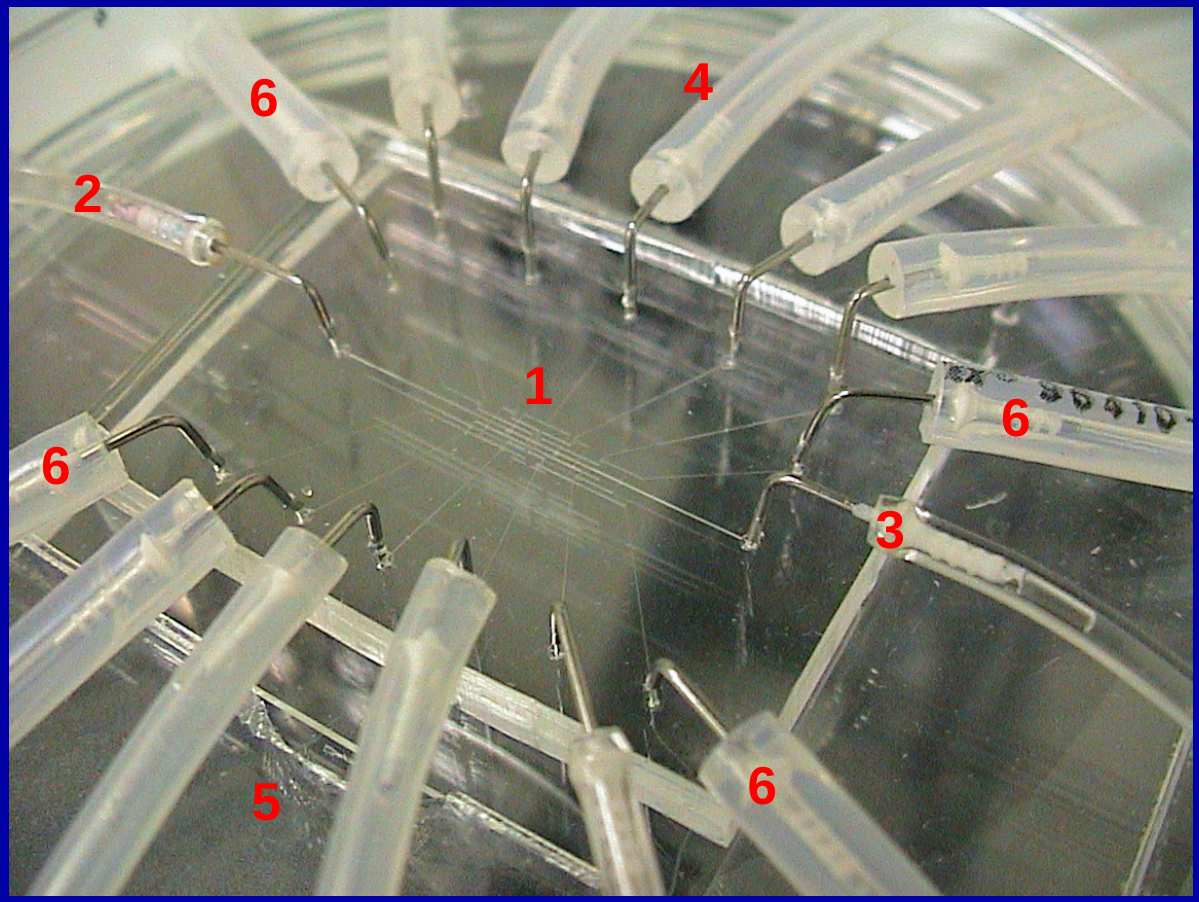
1. Fabricate micro flow chambers with optimal channel dimensions (h, w, l).
2. Optimize cell growth surface.
3. Test flow characteristics.
4. Test cell responses in micro flow chamber with different channel dimensions in laminar flow: cell morphology, nucleus position, actin alignment and c-Fos expression.

Methods

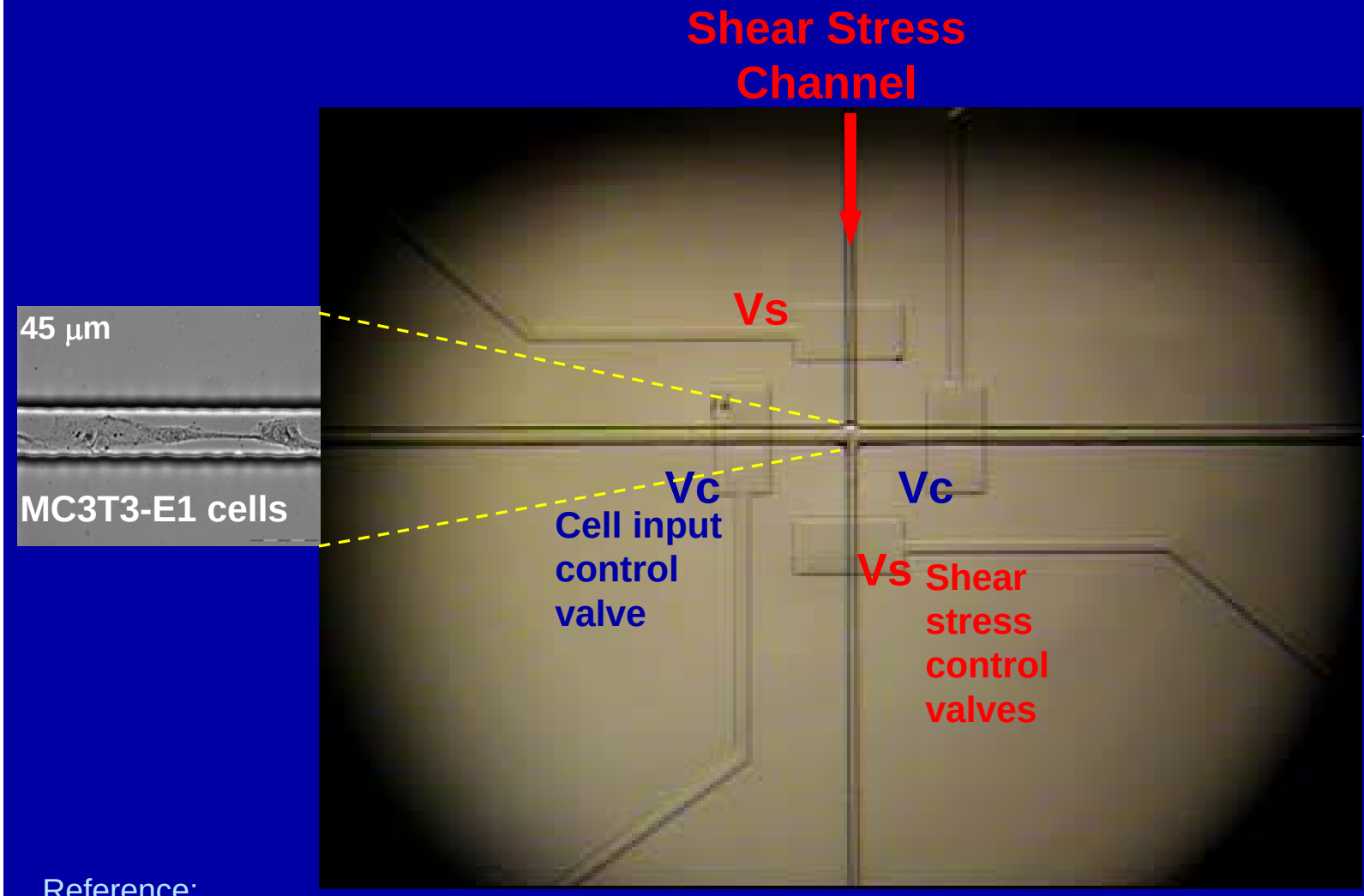
1. Microfluidic device fabrication.
2. Cell culture surface treatment and cell culture conditions.
3. Cell seeding and positioning (MC3T3-E1 osteoblasts, mouse tenocytes, human tenocytes and human endothelial cells).
4. Immunocytochemistry “on-chip”.

Microfluidic Device and Connections

1. Microchip
2. Cell culture inlet
3. Cell culture outlet
4. Shear stress inlets
5. Shear stress outlets
6. Flow control connectors



Micro-Valve System to Control Cell Position and Fluid Flow



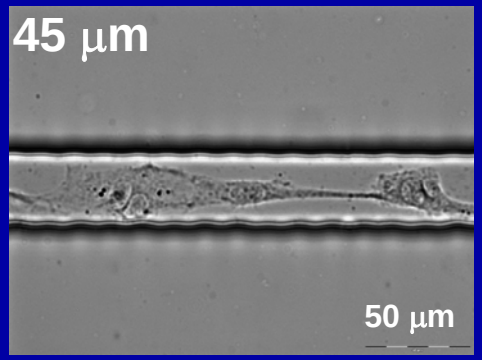
Step I:
Block SS channel for seeding cells.

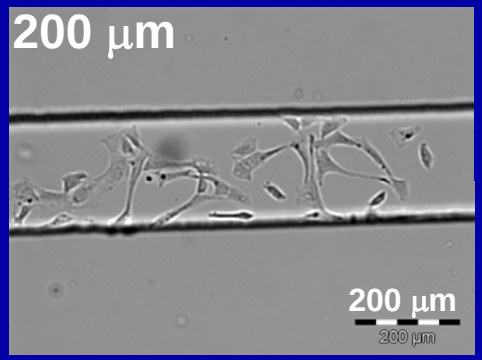
Step II:
Block cell channel for applying shear Stress.

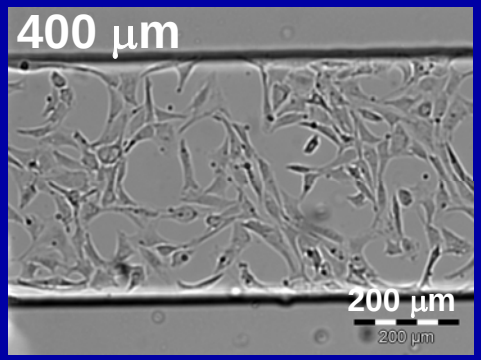
Step III:
Block all channels for cell seeding.

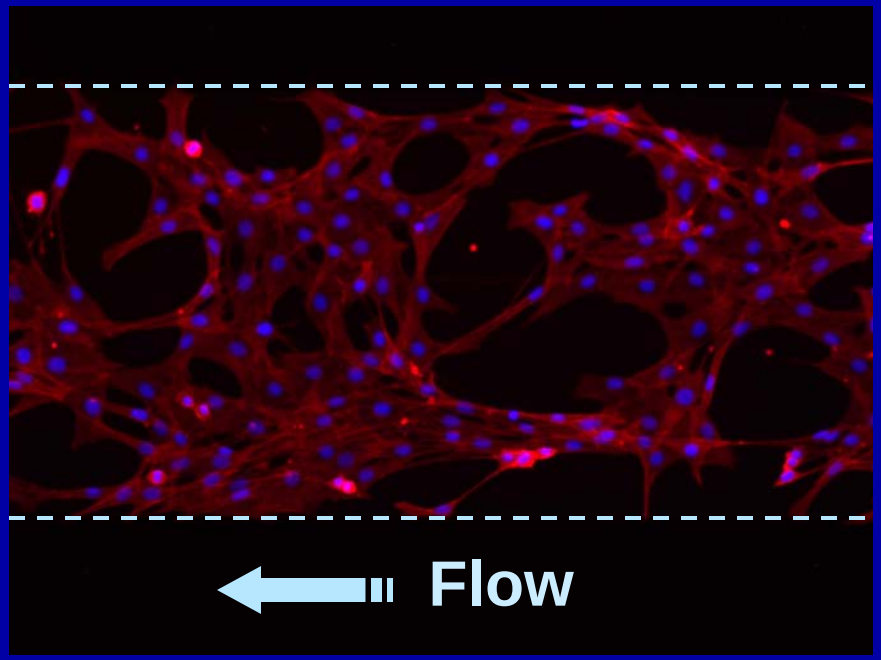
Reference:
Quake et al. *Science*, 2000

Cell Morphologies in Channels of Decreasing Dimensions









- 400 μm channels were used for shear stress microfluidic device.
- MC3T3-E1 Osteoblasts were labeled with Rhodamine Phalloidin (red) and Dapi (blue). Cells had normal morphologies comparing to cells in Petri dish .

We now have:

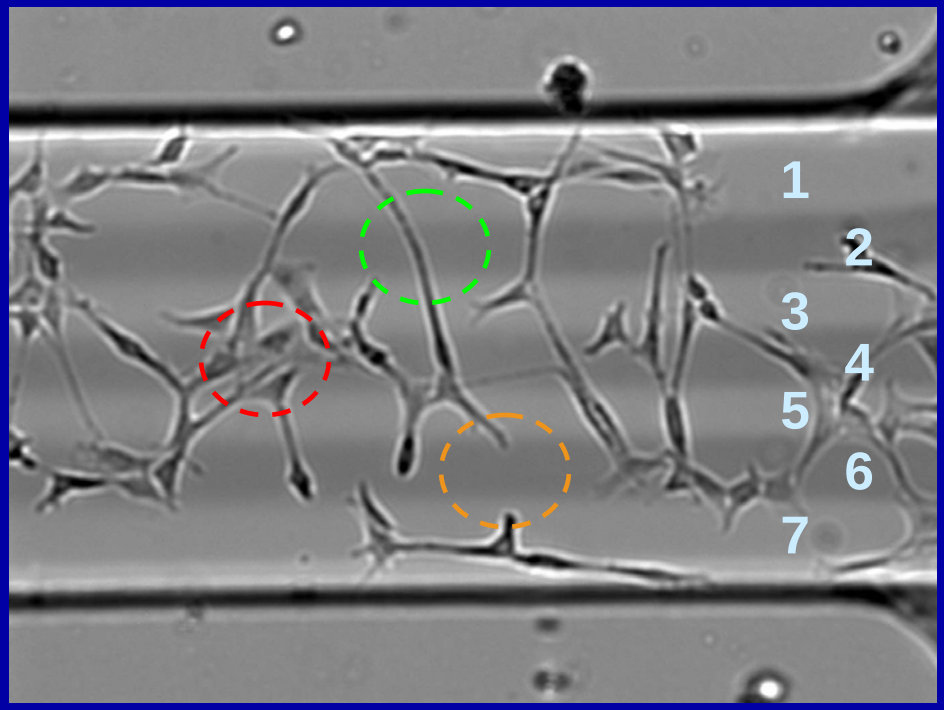
- A microfluidic shear stress device.
- Defined shear stress with a given flow rate.
- Seed target cells in specific positions.

For shear stress stimulation, if we can..

- Apply laminar flow to selected cells precisely, for instance, targeting two cells simultaneously and inhibit one cell only.
- Then we can generate results that traditional methods couldn’t collect before.

Controlling Fluid Flow Stream to Selected Cells or Targeted Cell Portion

Chemical in flow stream 2, 4 and 6

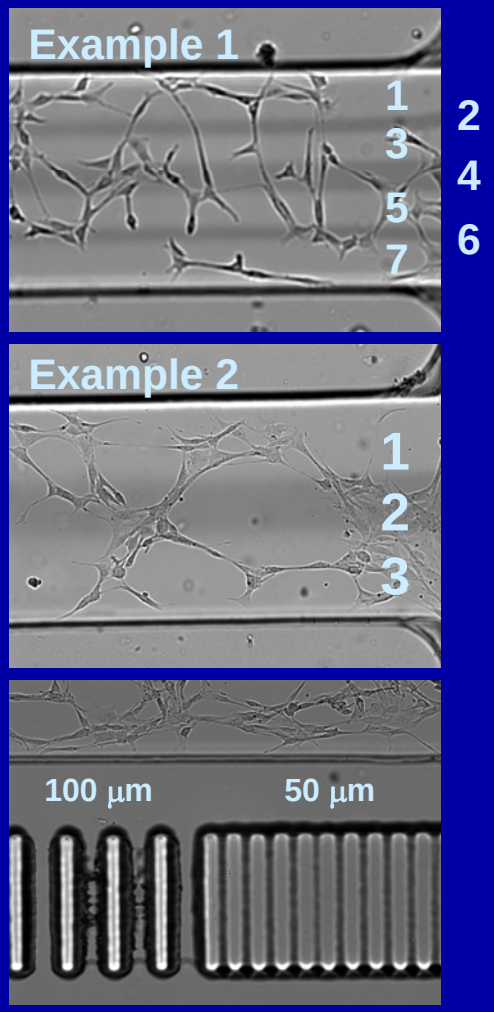


Target cell and nucleus

Target cell-cell junctions

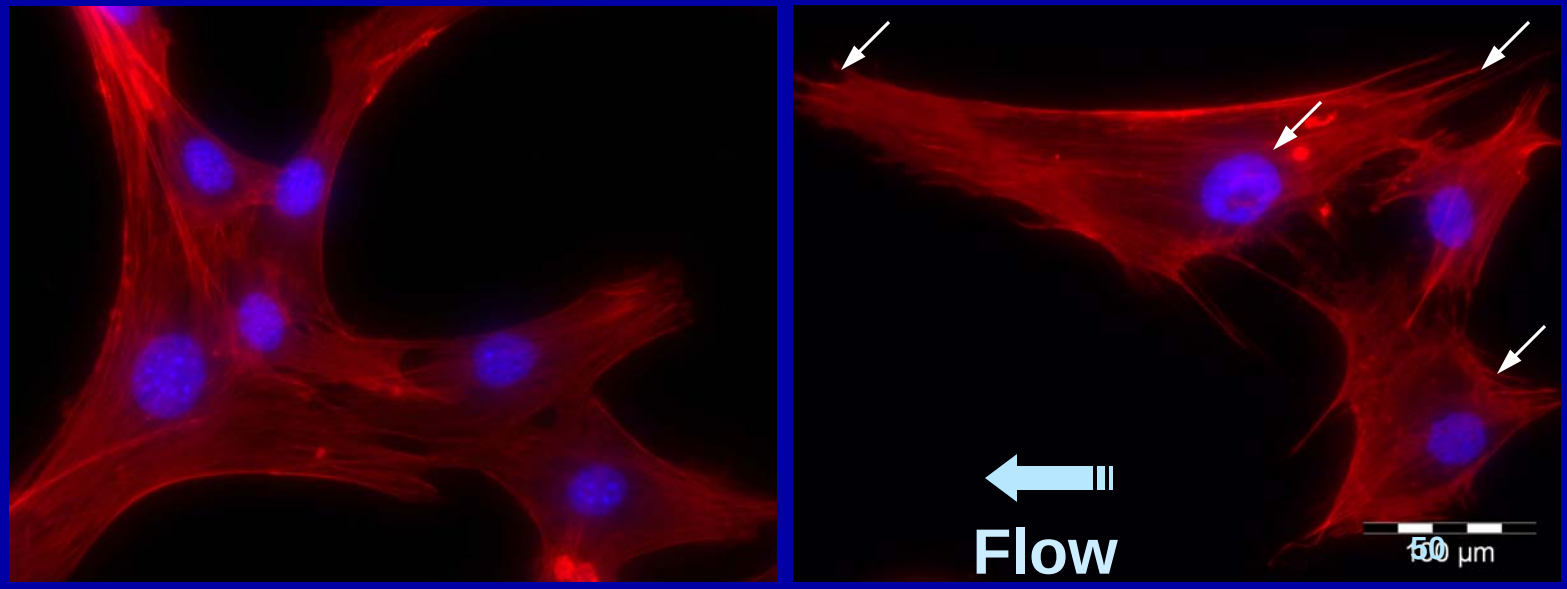
For cell migration, communication studies

Flow



On-chip scale

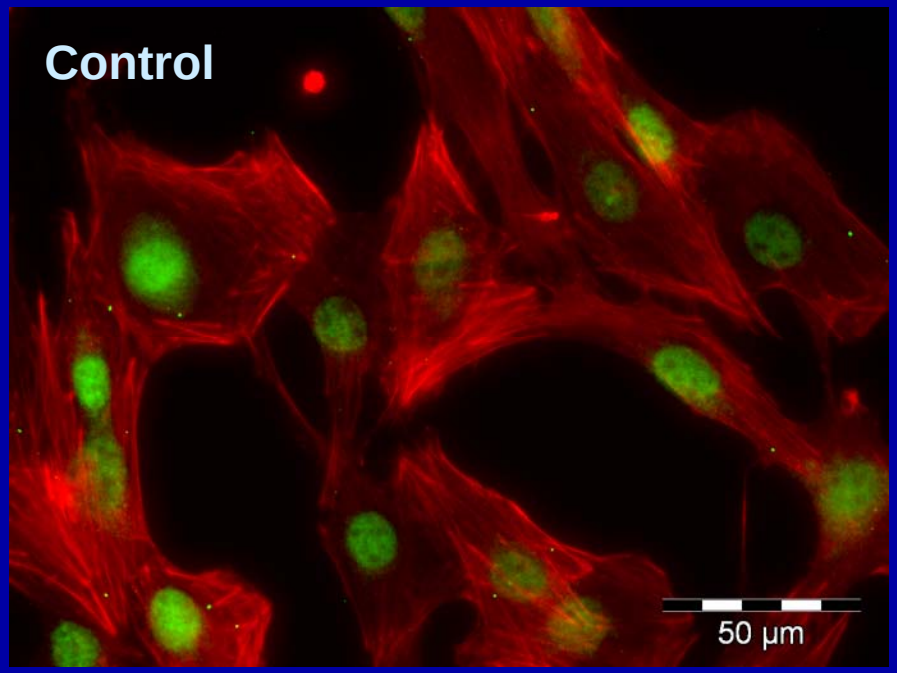
Actin Filaments Reorganized After Fluid Flow

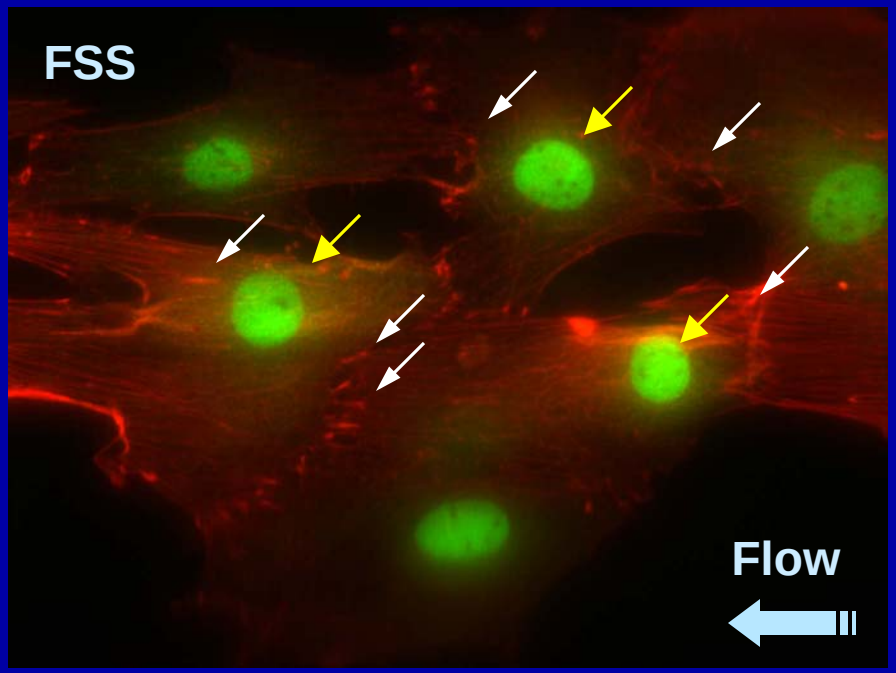


Static culture **4 h at 10 dynes/cm^2**

References:
Fredrick M et al, *Am J Physiol Cell Physiol*, 1998
Malone A et al, *Am J Physiol Cell Physiol*, 2007

c-Fos Increases Expression after Fluid Shear Stress (FSS)

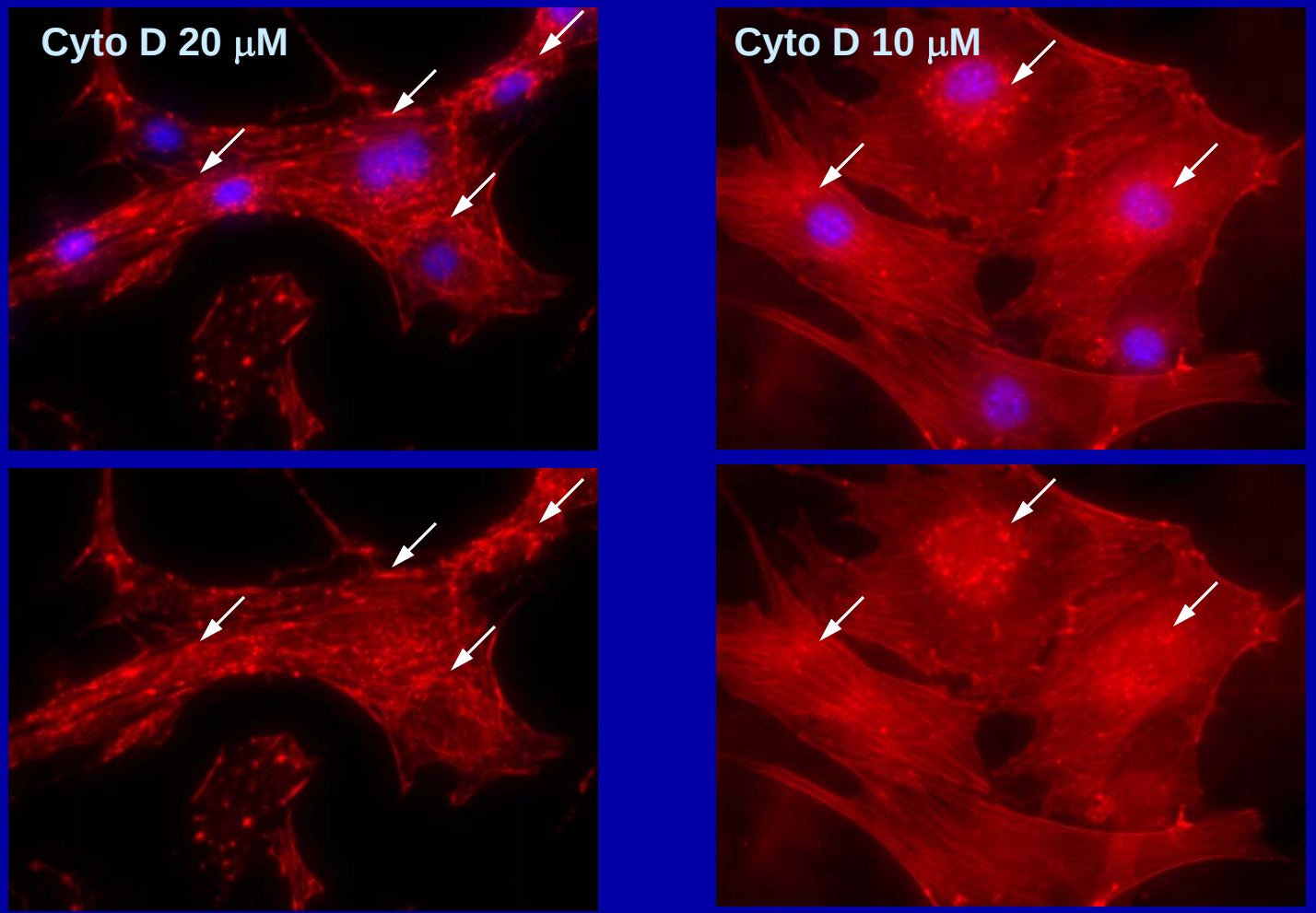




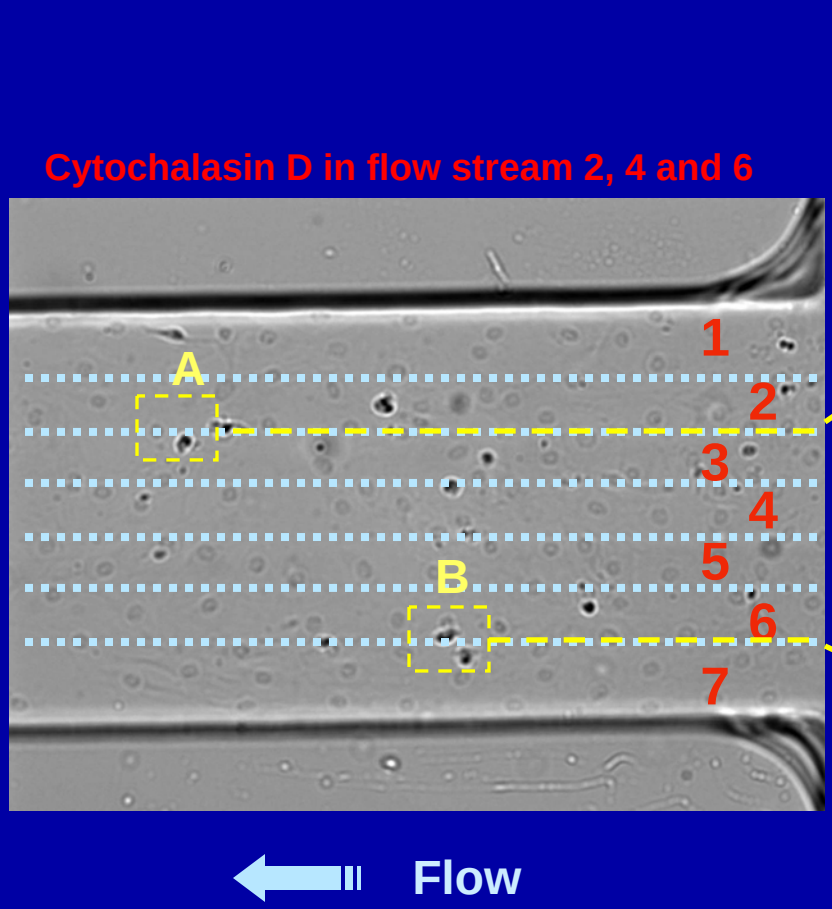
c-Fos in green. Actin in red.

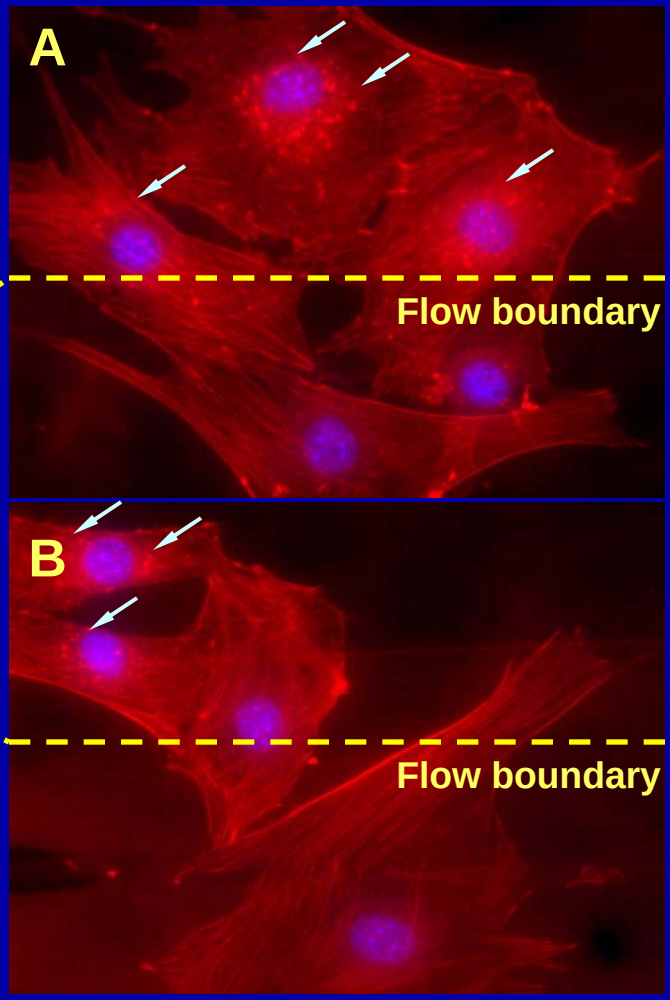
* Images were taken with the same camera setting.

Different Dose of Cytochalasin D Disrupted f-Actin Filaments in Microchannels



Laminar Flow for Selected Disruptin f-Actin Filaments in Microchannels





Summary

- Microfluidic device for targeted cell shear stress challenge
- Method to control flow to specific parts of a cell
- Real-time imaging and on-chip immunocytochemistry
- Potential for high throughput assay

Acknowledgement

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(Microfluidics patent pending)