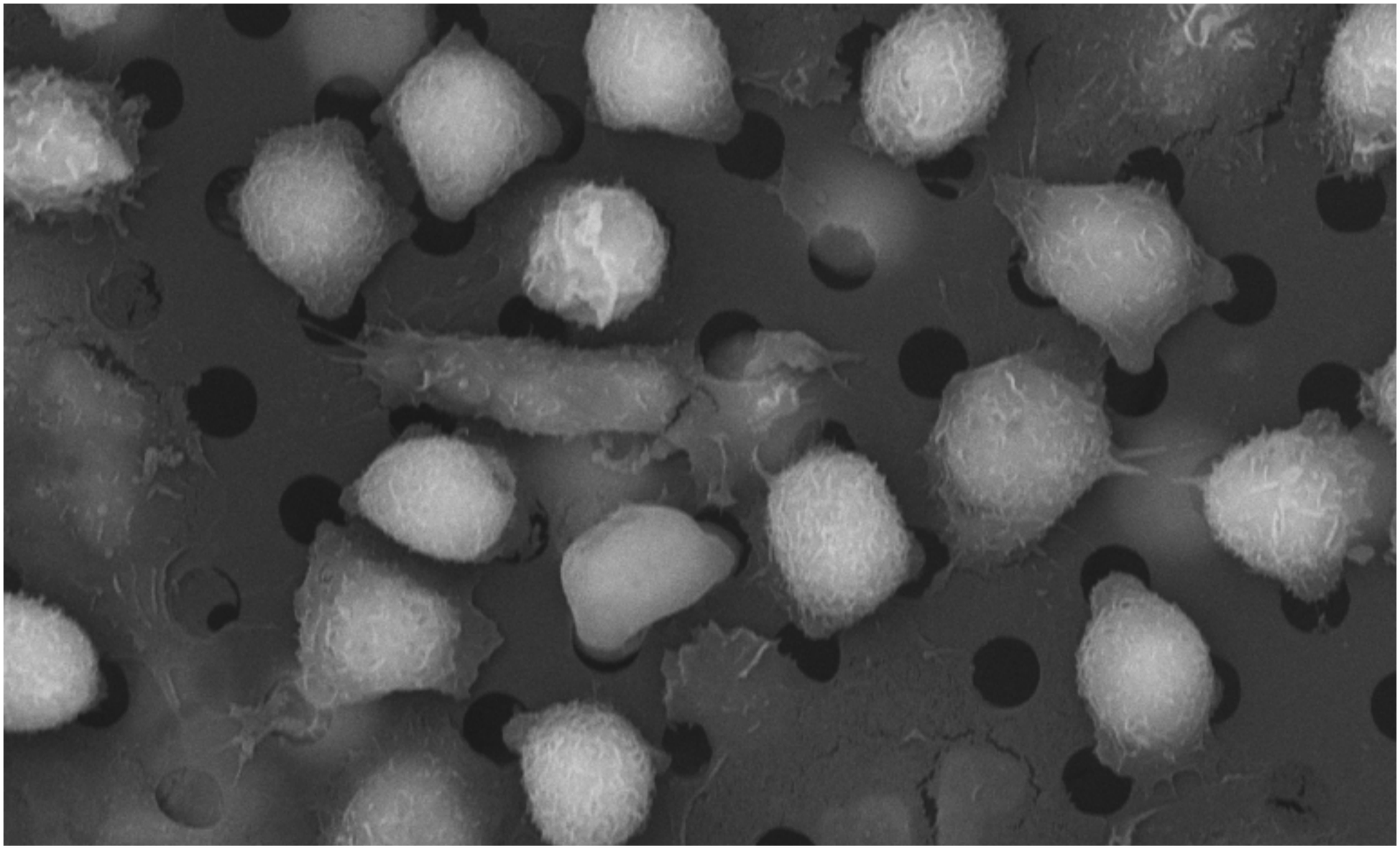

Ultrathin Transparent Membranes for Cellular Barrier and Co-Culture Models

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Biomedical Engineering
Rochester Institute of Technology

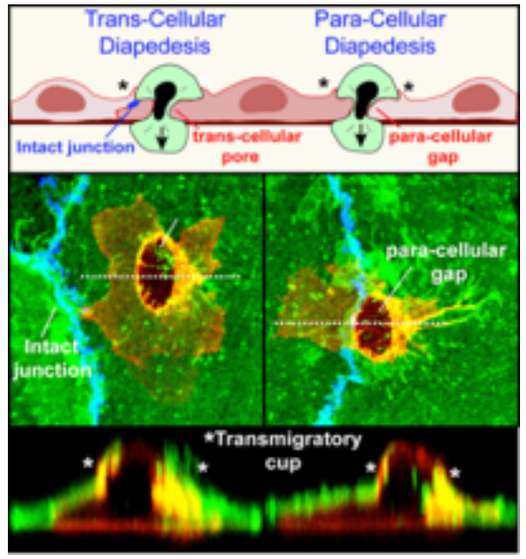
What is a cellular barrier?

- An interface between two cellular or tissue environments - skin, lung, gut, vasculature, eye…
- Critical interface for disease entry, gas exchange, nutrient uptake, drug delivery…

In vitro Model Systems

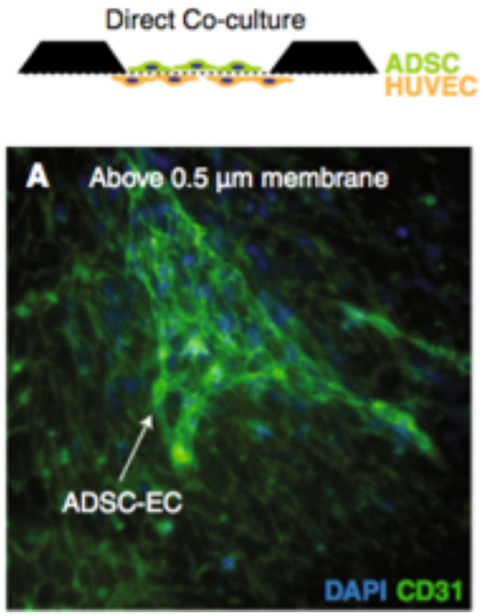
- Transmigration
- Tissue barrier models
- Cellular co-culture

Transmigration Across Vascular Endothelium



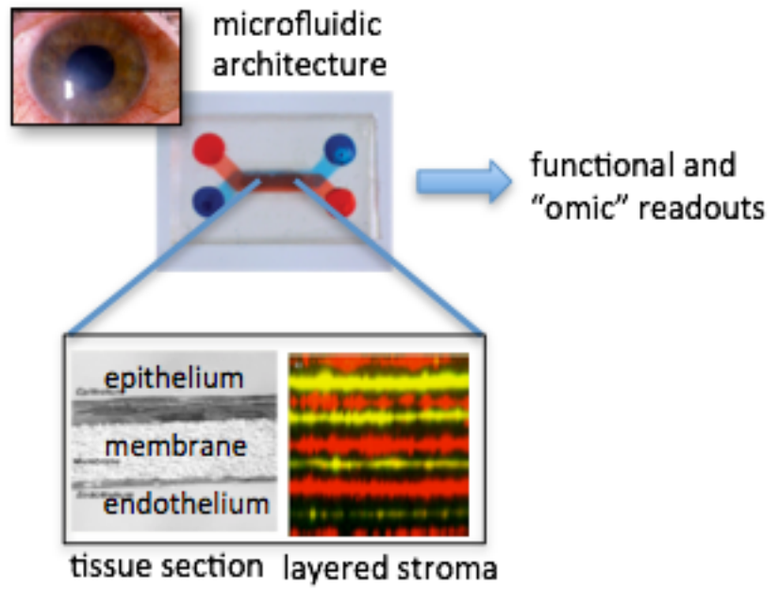
(Carman, Harvard)

Vascular Differentiation of Mesenchymal Stem Cells



(Gaborski, RIT)

Human Cornea Drug and Cosmetic Testing Platform



(Abhyankar, UT Arlington)

Artificial Barrier

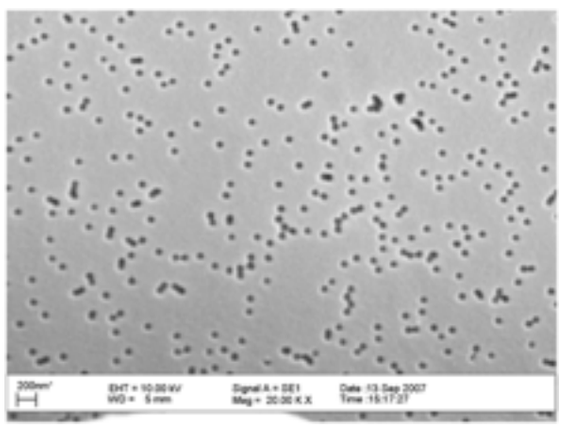
How do we create an artificial barrier *in vitro*?

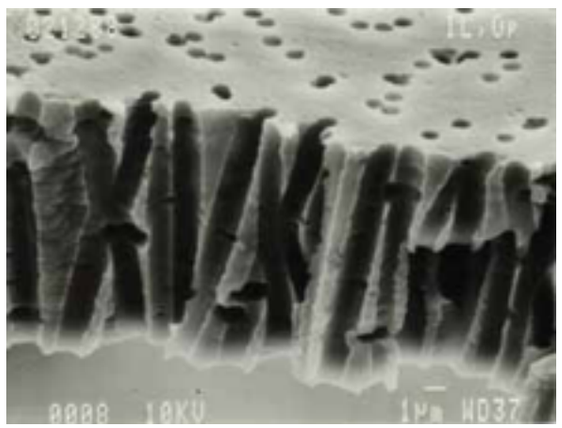
- Semi-permeable (pores - what size?)
- Separate two or more “compartments”
- Support natural cellular growth and communication

Why are better models needed?

Track-Etched Membranes

- Thick (~10 microns)
- Low pore density
- Doublet pores
- Random pore distributions

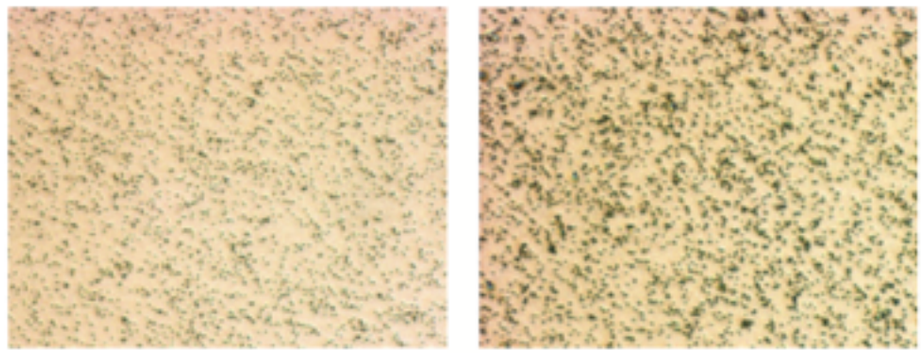




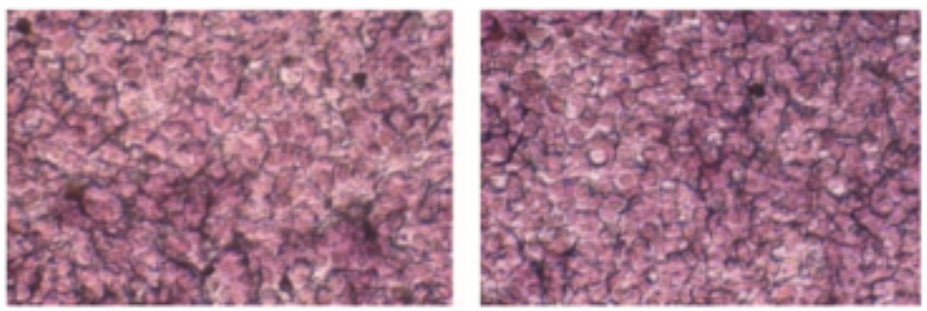
CORNING

Membrane Clarity

Optical clarity and visualization of monolayer is maintained with new membrane.



Empty current PET membrane (l) and new membrane (r), 40x magnification.

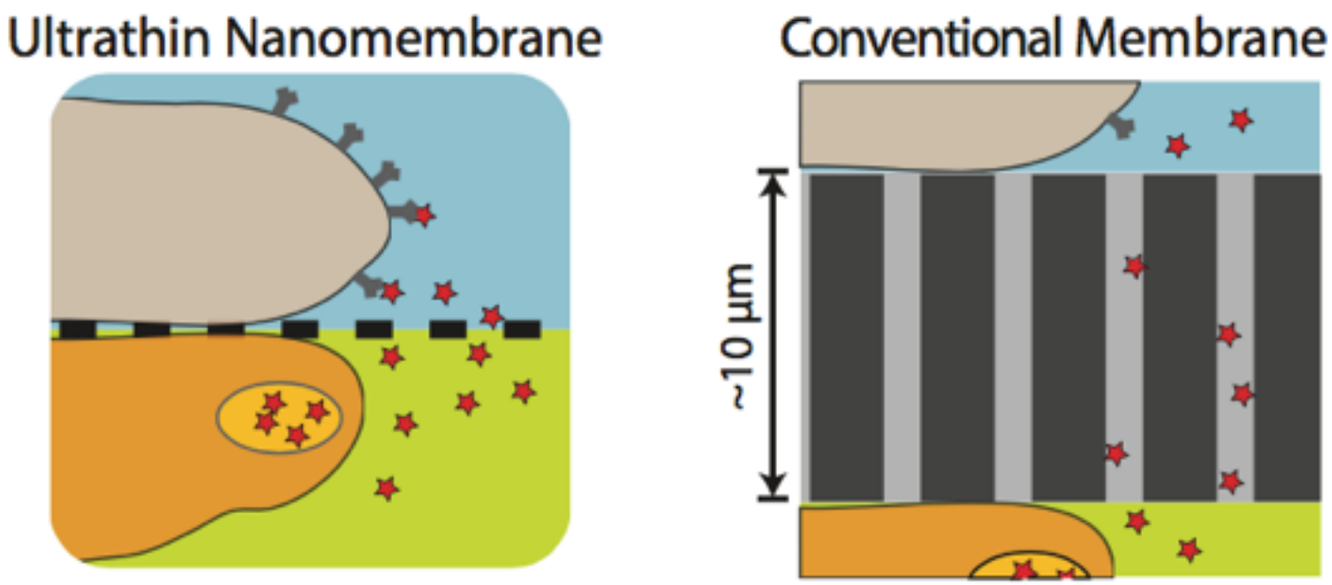


Fixed and stained MDCK monolayer on current (l) and new (r) PET membrane (400 x magnification).

Why are better models needed?

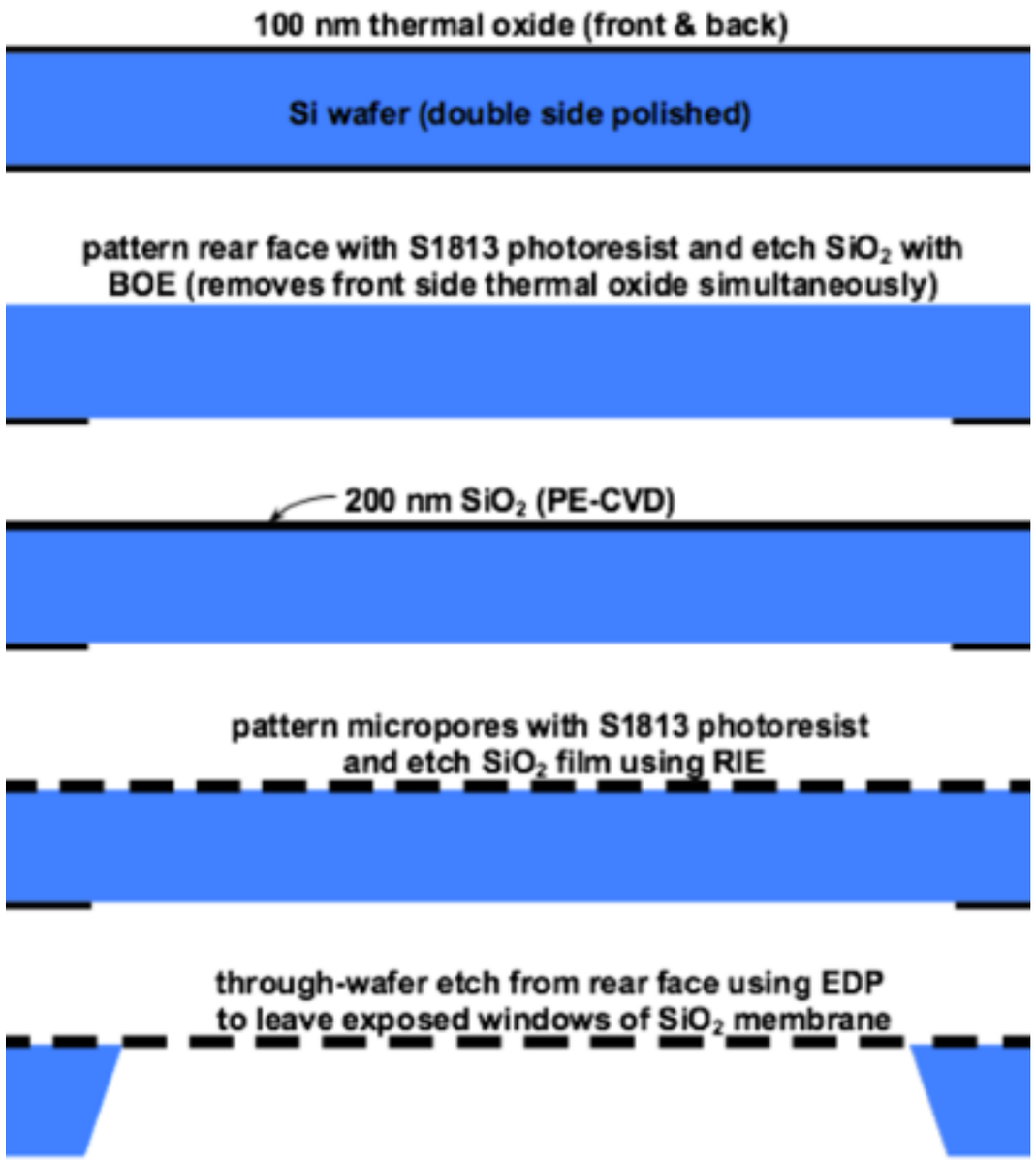
An Ultrathin Membrane can

- Improve cell-cell communication (biochemical and physical)
- Improve real-time and post-fixation readouts



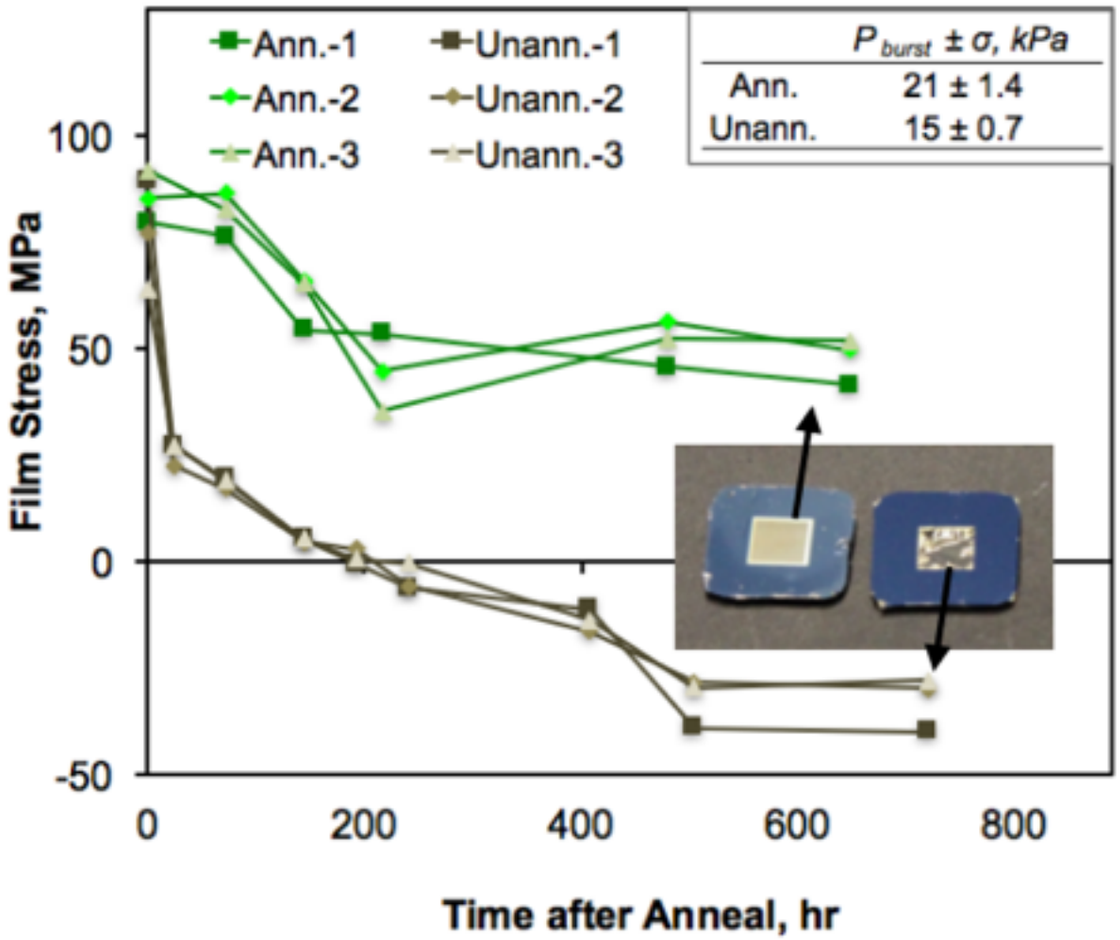
Transparent SiO_2 Membranes

- Wafer Level Process (4-inch, 6-inch, etc.)
- Most steps can be batch processed (ex. 25 wafers at a time) and banked for future development and completion



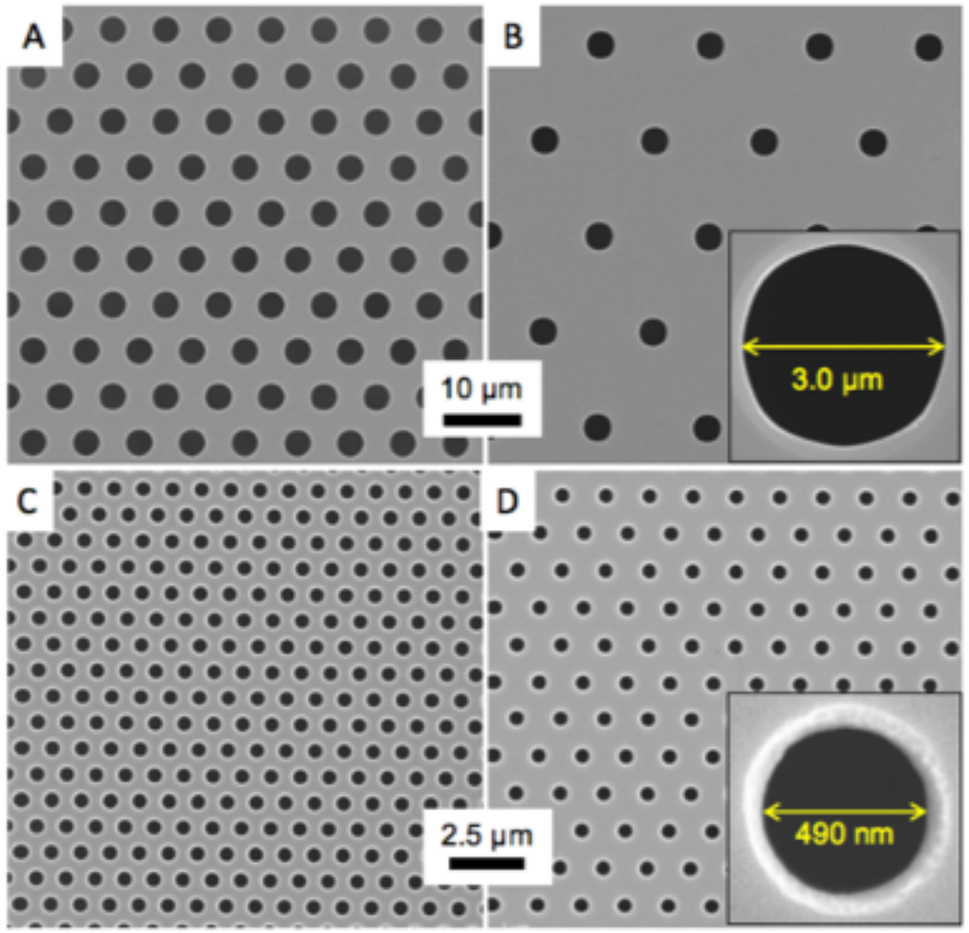
Flat and Tensile SiO_2 Membranes

- Thin SiO_2 membranes are typically compressive films
- Compressive films are wrinkled and weak when “released” from the silicon wafer substrate
- Annealing at 600°C for 1-8 hours results in stable tensile films with increased robustness



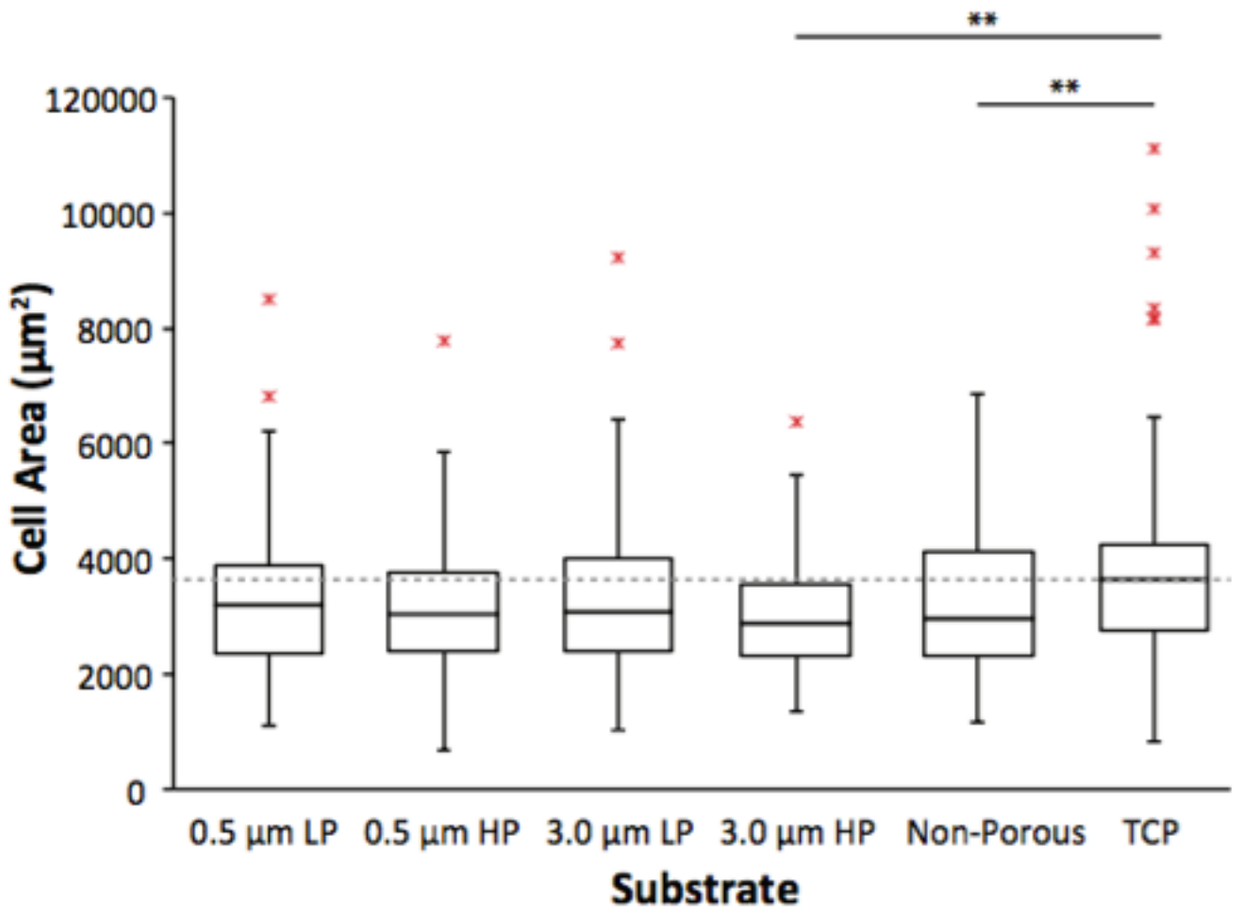
Patterning Pores

- Photolithography allows for precise patterning of pores
- Lithographic resolution limited to >0.1 μm with affordable methods
- E-beam lithography very expensive & time consuming, but truly nanoscale possible



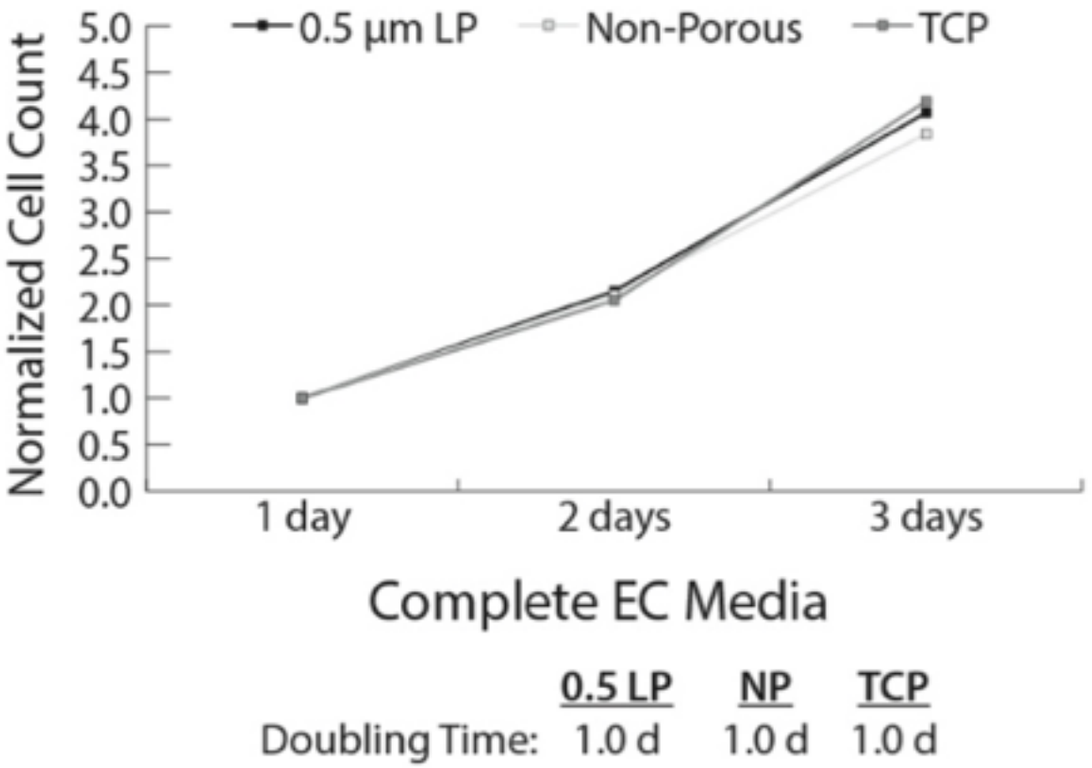
Cell Spreading

- Important that cells attach and spread naturally on the surface
- Large pore sizes and high porosity limits cells' ability to attach and spread
- "Roughness" due to pores may aid in attachment



Cell Proliferation

- Cells proliferate on SiO_2 membranes similarly to control substrates
- Upon reaching confluence, common cell-cell junctions are visible



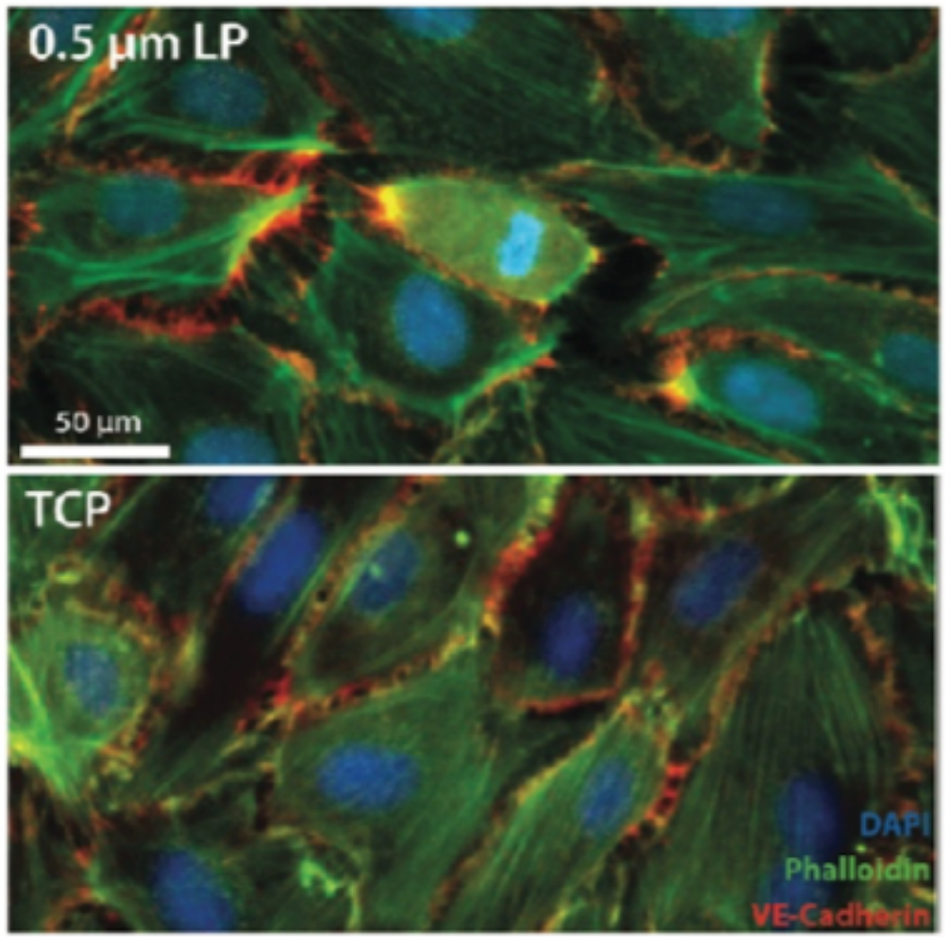
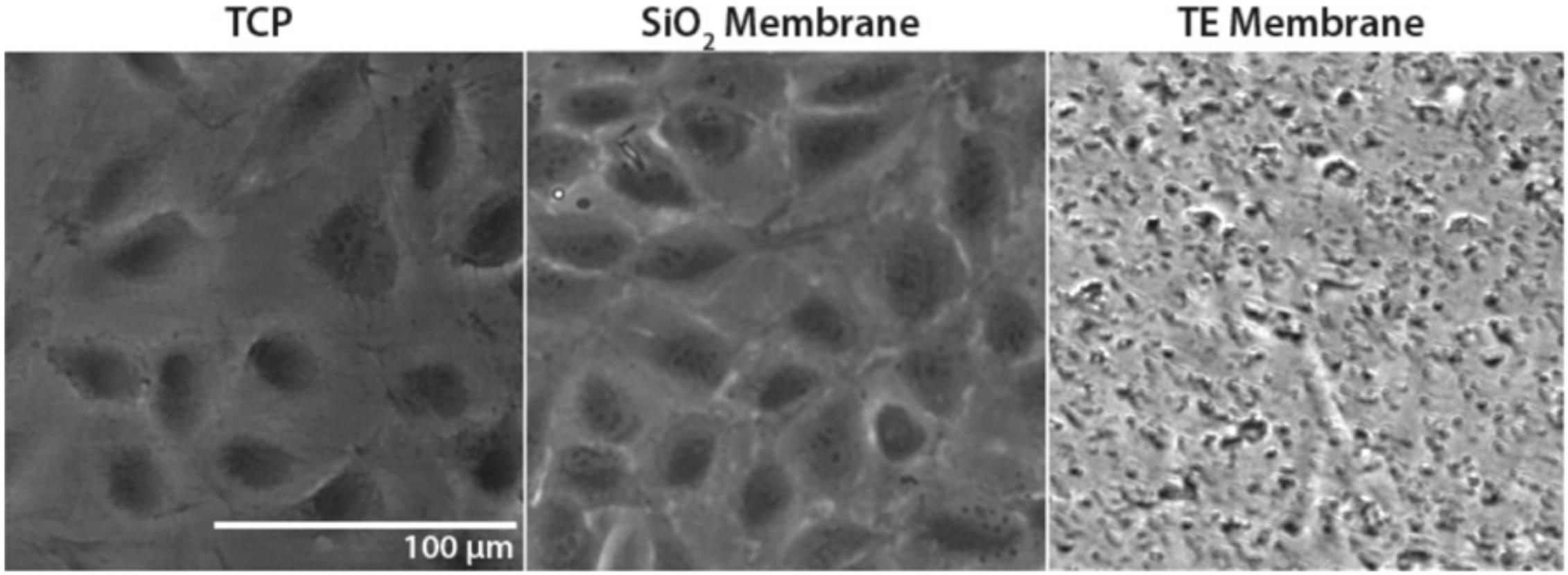
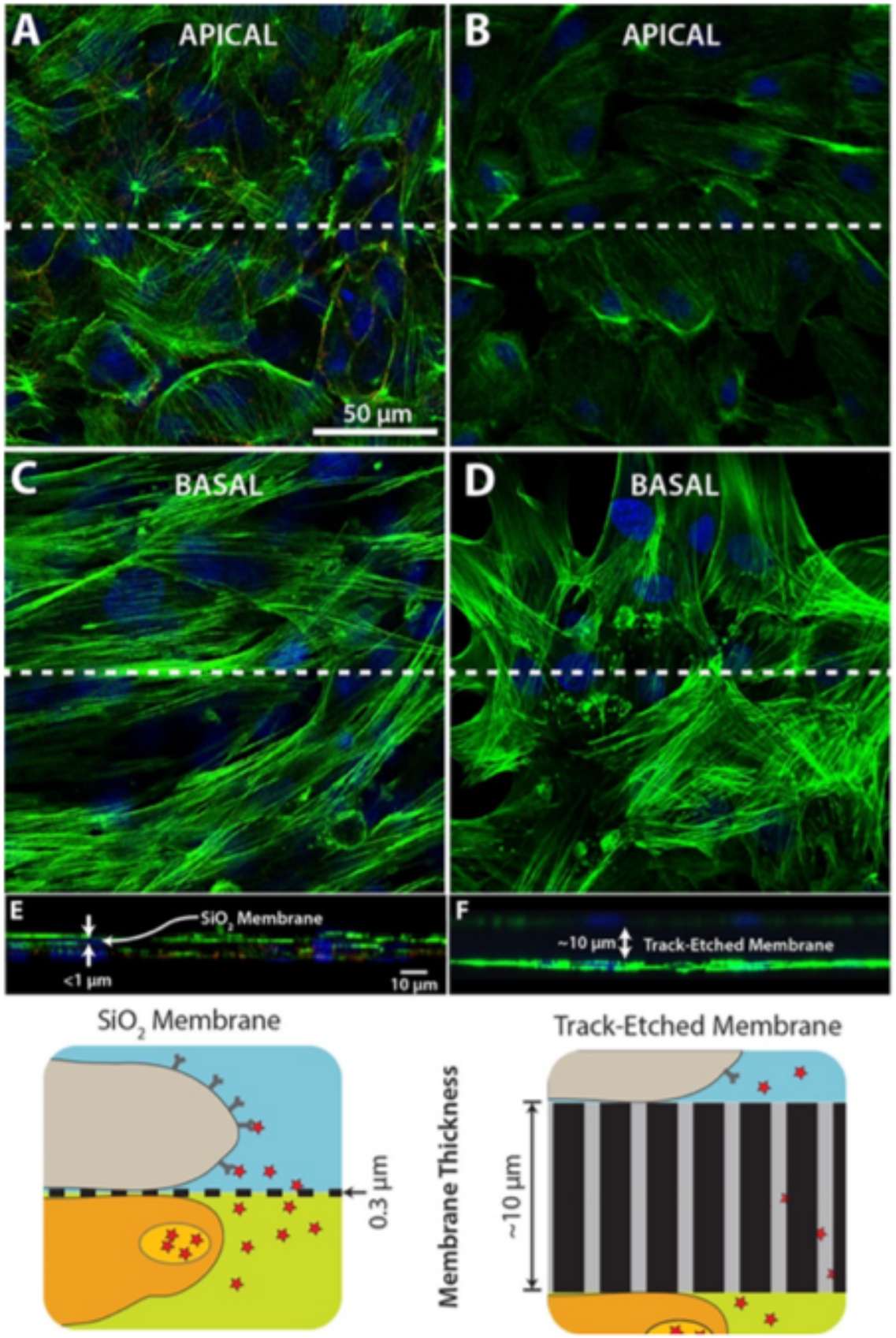
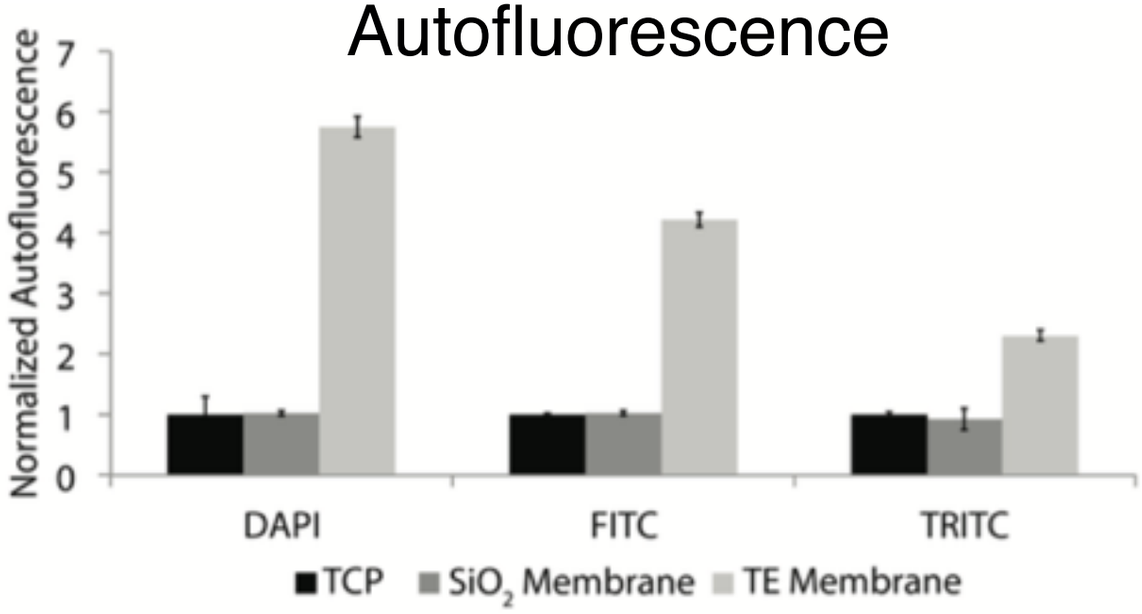


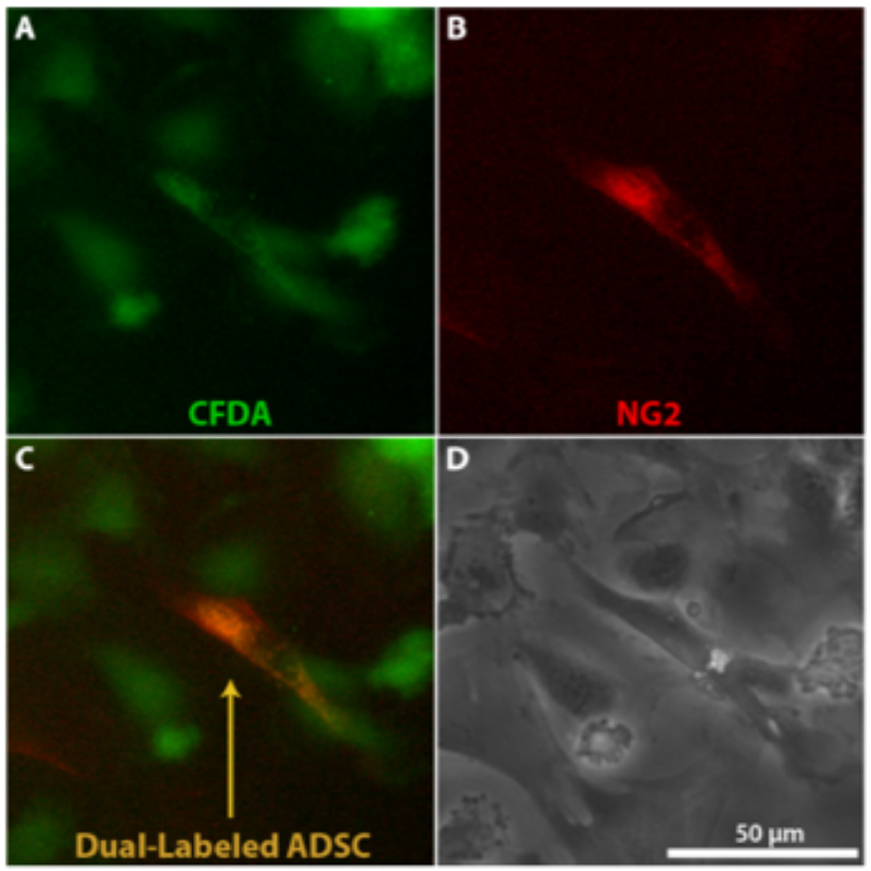
Image Quality

Representative phase contrast image of confluent endothelial cells imaged above the substrate using an standard inverted microscope



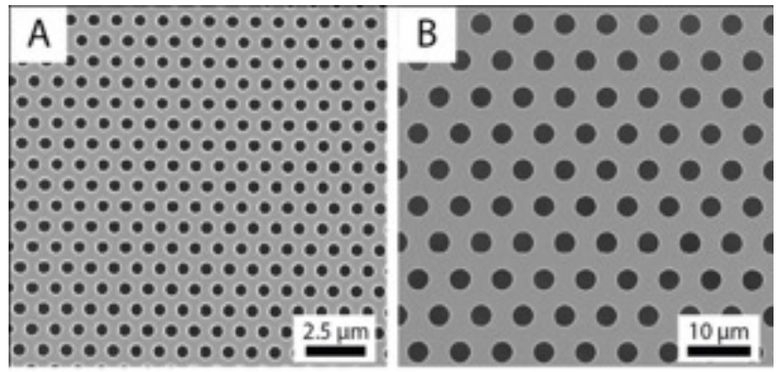

A
APICAL
B
APICAL
50 µm
C
BASAL
D
BASAL
E
SiO2 Membrane
<1 µm
10 µm
F
~10 µm
Track-Etched Membrane
SiO2 Membrane
0.3 µm
Membrane Thickness
~10 µm
Track-Etched Membrane

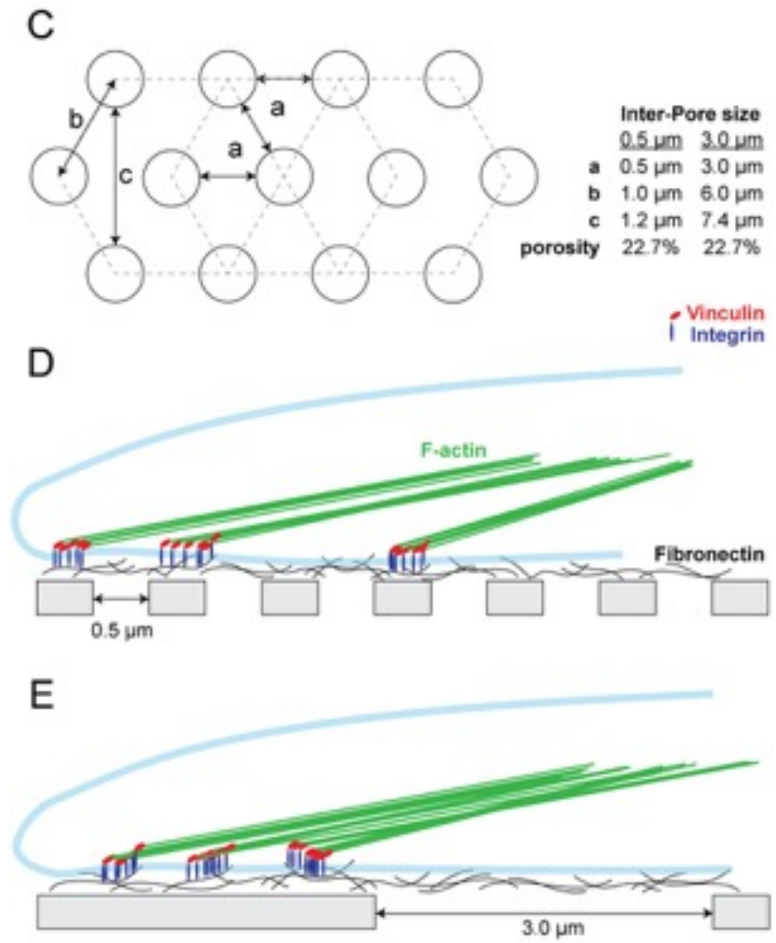

Autofluorescence
Normalized Autofluorescence
0
1
2
3
4
5
6
7
DAPI
FITC
TRITC
TCP
SiO2 Membrane
TE Membrane


Gap-Junction Formation
A
CFDA
B
NG2
C
Dual-Labeled ADSC
D
50 µm

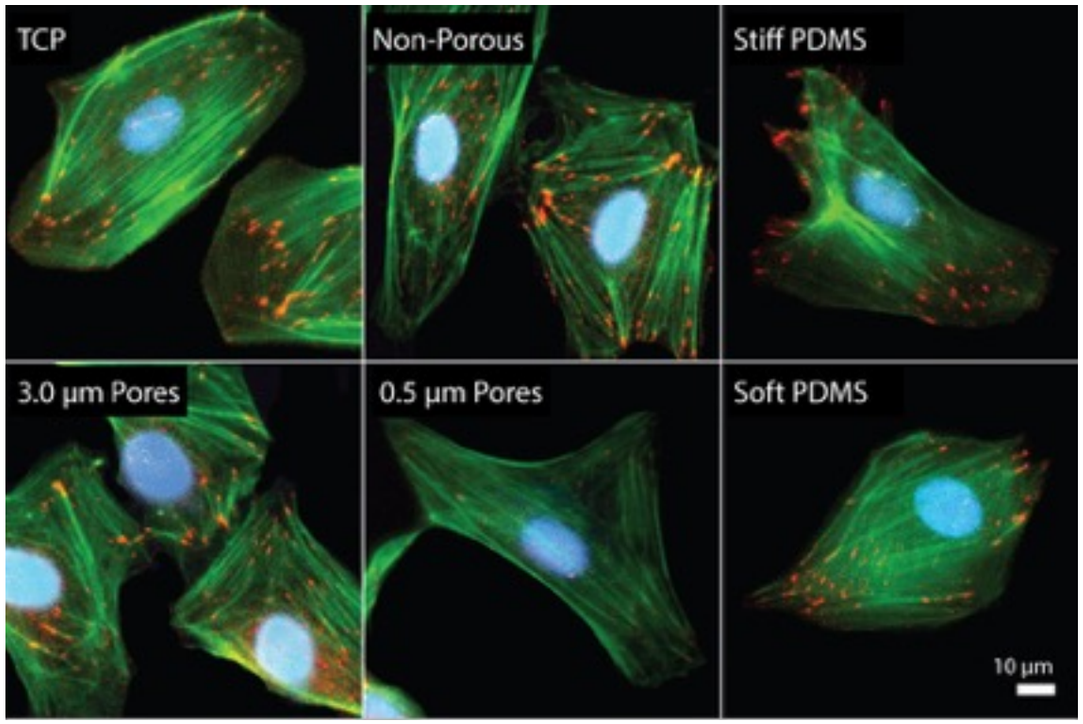
Porosity Affects Behavior

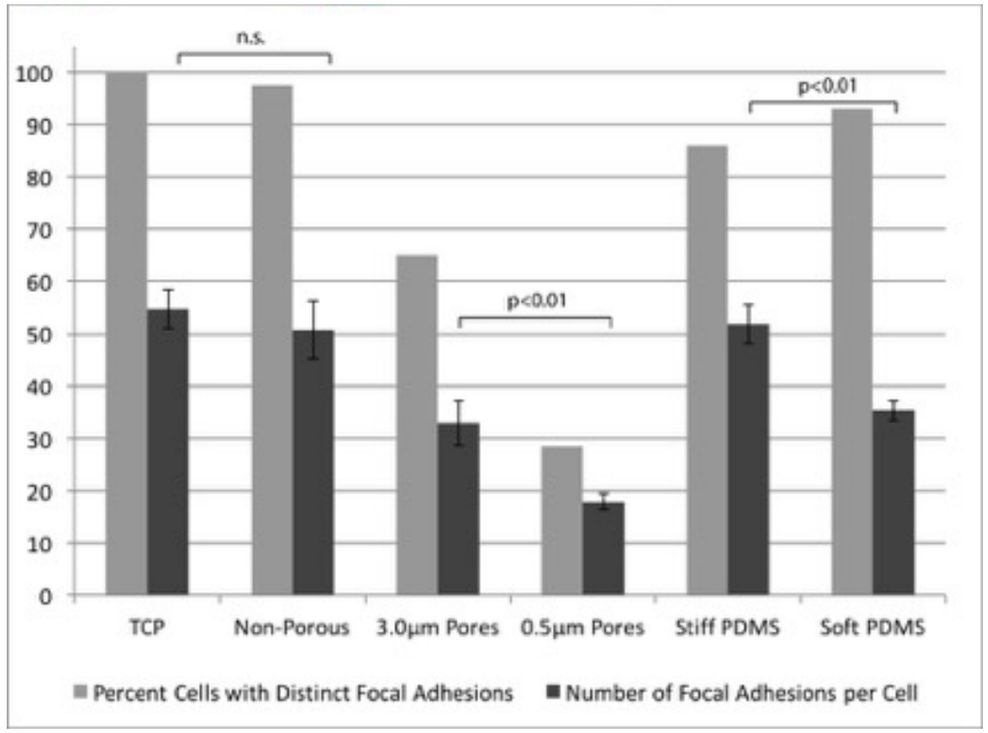
Contact Area & Porosity
can modulate **Cell-Substrate**
as well as **Cell-Cell** interactions





Focal Adhesion Formation

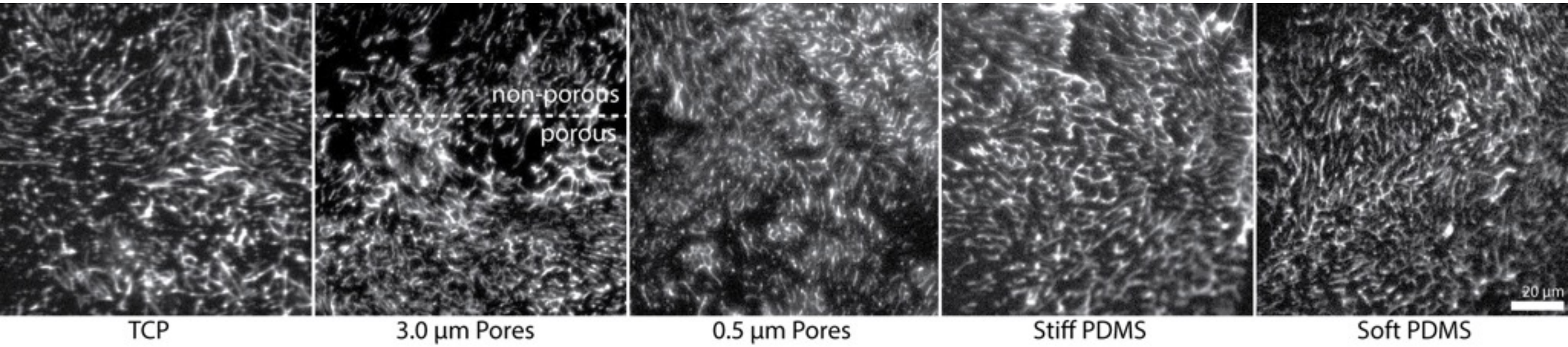




Substrate characteristics influence formation of focal adhesions

Soft Substrates - Fewer Focal Adhesions
Porous Substrates - Fewer Focal Adhesions

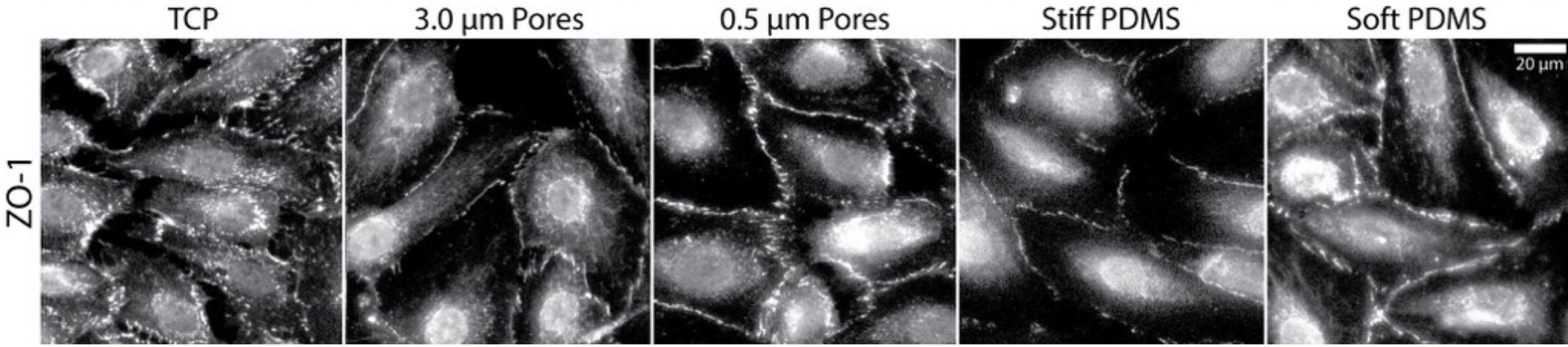
Fibronectin Fibrillogenesis



Substrate characteristics influence ECM formation

Soft Substrates - Less Fibronectin Fibrillogenesis
Porous Substrates - Less Fibronectin Fibrillogenesis

Cell-Cell Junctions



Weak cell-substrate interactions promote cell-cell interactions

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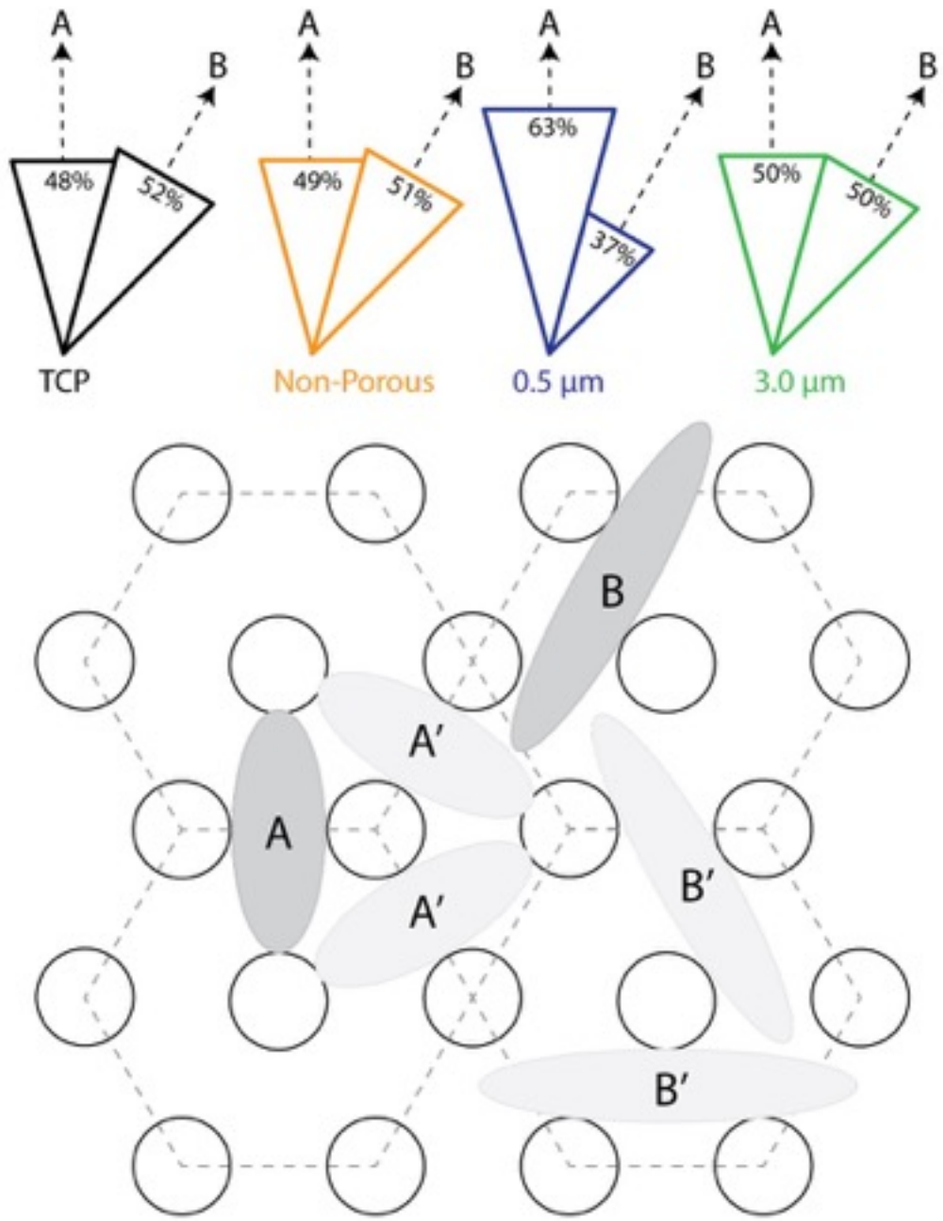
Extra Slides

F-actin Orientation



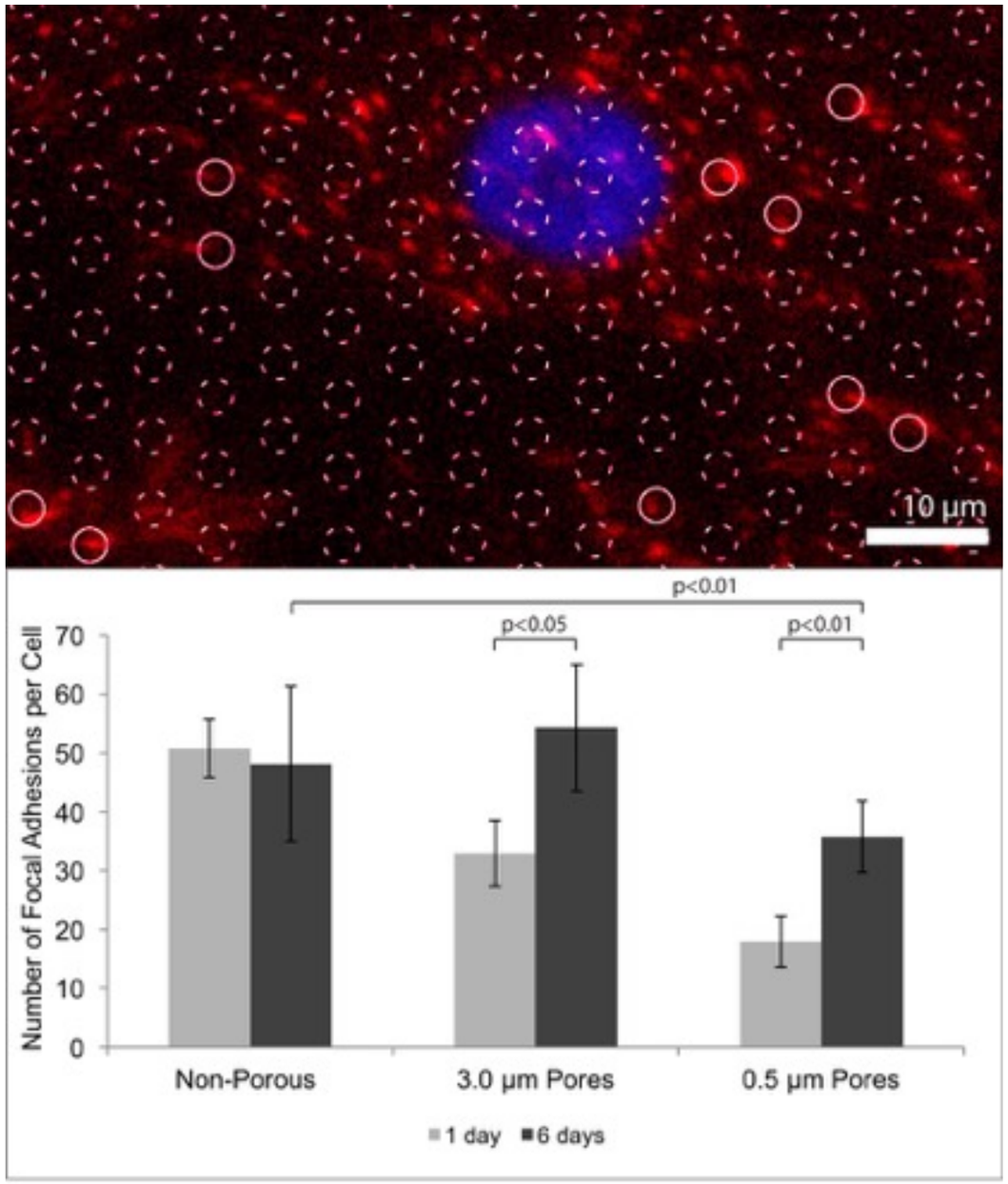
Contact Areas

	0.5 µm	3.0 µm
A	1.0 x 1.2 µm	6.0 x 7.4 µm
B	<0.4 x inf µm	2.2 x inf µm

Focal Adhesion Formation

- After 6 days, cells develop additional FAs on porous membranes

- FAs form over the pores likely due to pore-spanning ECM proteins



Adherens Junctions