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Cellular Adhesion and Response to Thermally Modified, Sol-Gel Derived TiO_2 Thin Films

Megan Coe[1], Diana DeRosa[1], Jeanette Blaine[2], David Musgraves[2], Mark Leick[1], Felipe Lee Montiel[1], B.G. Potter, Jr.[2], and Mark Riley[1,2]

[1]Agricultural and Biosystems Engineering

[2]Materials Science and Engineering

The University of Arizona, Tucson, AZ

Motivation for Research

Develop modifiable surfaces that encourage cell attachment and spreading, but that decrease basal level of cell activity

Goal is to modify the distribution of cell surface receptors so as to increase the susceptibility to viral infection

TiO_2 (titania)

- TiO_2 commonly used as a surface for bone implants (hip, knee, etc.)
- Osteoblast and osteoblast-like cells grown on titanium conform to the irregular substrate surfaces maximizing the contact between the cell membrane and the substrate
  - Surface chemistry can alter cell adhesion and function
  - Surface structure (shape, feature size, roughness) can modulate cell adhesion and function
    - Nanotexturing of TiO_2 surfaces upregulates expression of bone sialoprotein and osteopontin by cultured osteogenic cells [de Oliveira and Nanci, 2004]

Approach

Create surfaces with varying topology and chemistry

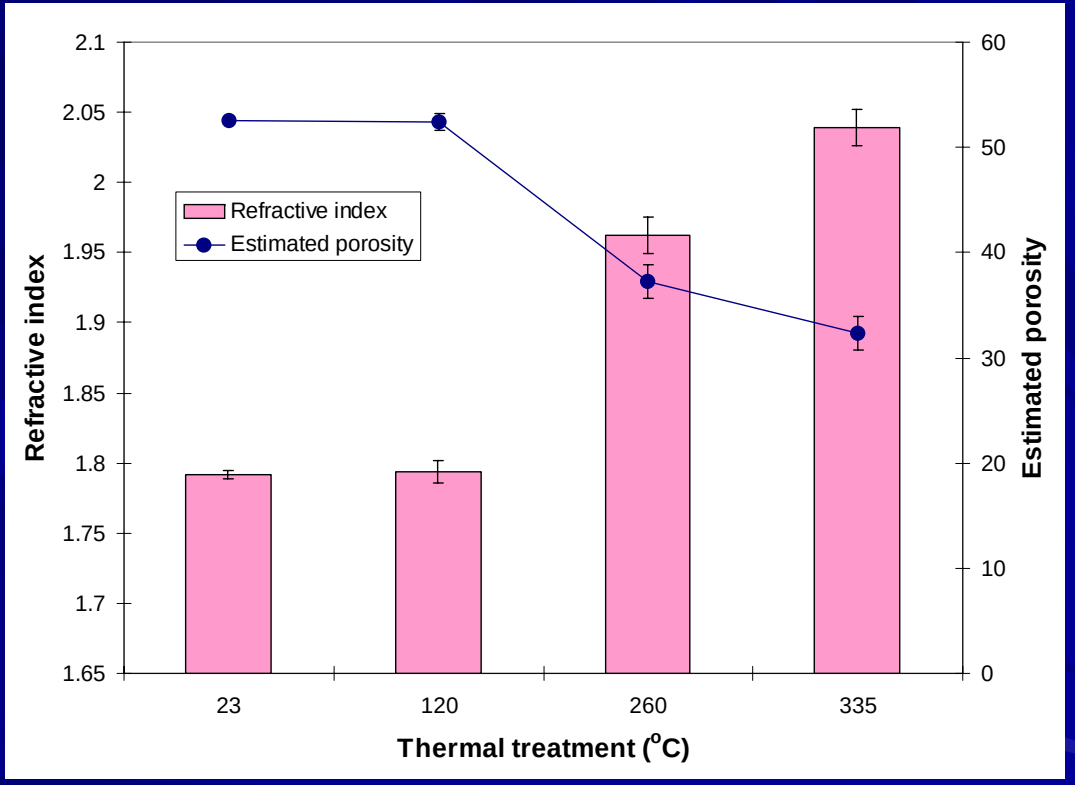
- Substrate is fused silica
- 2 methods
  - sputter coat followed by thermally-induced condensation
  - laser deposition (1 step)
  - Films made in this way typically have a thickness of 35 nm and varying surface structure

Characterize cell response

- Cell attachment and spreading
  - Fluorescence and confocal microscopy
    - Stains for actin and vinculin
- Cell activity
  - Metabolic rates
    - w/ and w/o activation by bacterial lipopolysaccharide (LPS)

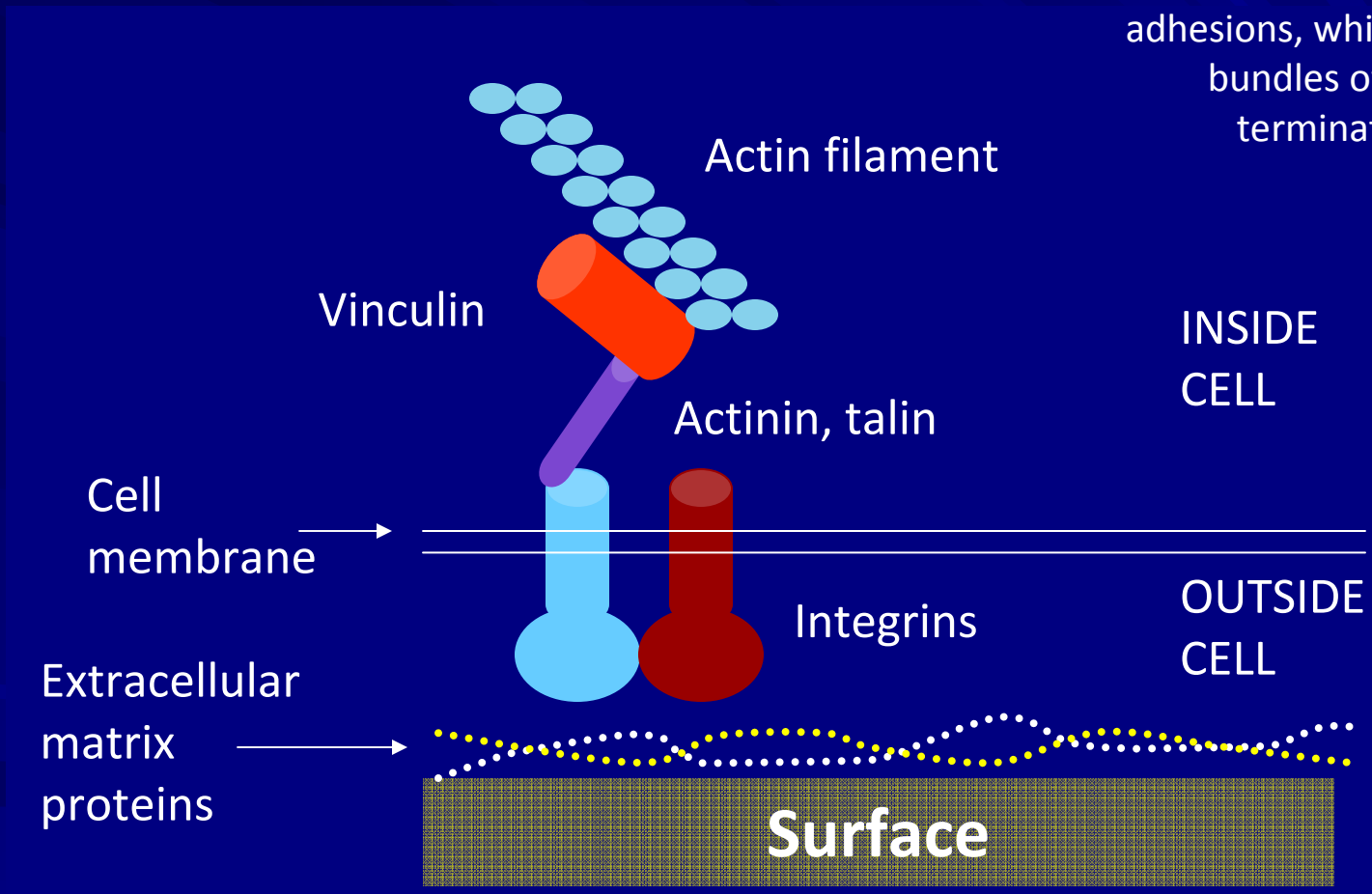
Ellipsometry shows that the porosity decreases with thermal treatment

- Porosity estimated using the Lorentz-Lorentz equation, decreases with thermal treatment.
- This data is consistent with increased film density with increasing thermal treatment
- Refractive index of fully dense material was assumed to be n=2.38 for calculations



Adherence to surfaces – focal adhesions

Vinculin is located only at focal adhesions, which is also where bundles of actin filaments terminate at the plasma membrane.

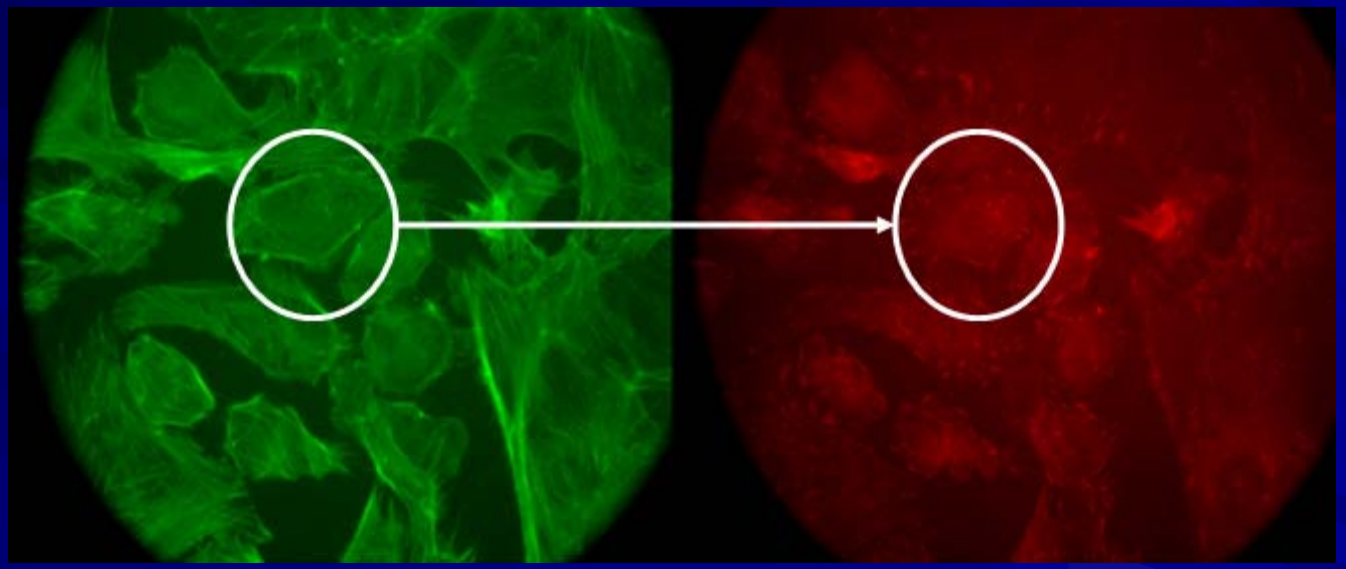


Adapted from Alberts, et al., Molecular Biology of the Cell, 4th Ed.

Epithelial cells on a control surface (tissue culture treated plastic)

Actin Vinculin

Mag: 40x.



Vinculin found at actin termini indicates strong binding of cells to surfaces

The photo on the left shows actin, a filamentous cytoskeletal protein stained with a green fluorescent dye. These cells are on an tissue culture treated plastic surface.

The photo on the right shows the same location of the fluorescent-tagged vinculin.

Epithelial on untreated glass (a poor surface for adhesion)

Actin

Mag: 40x.

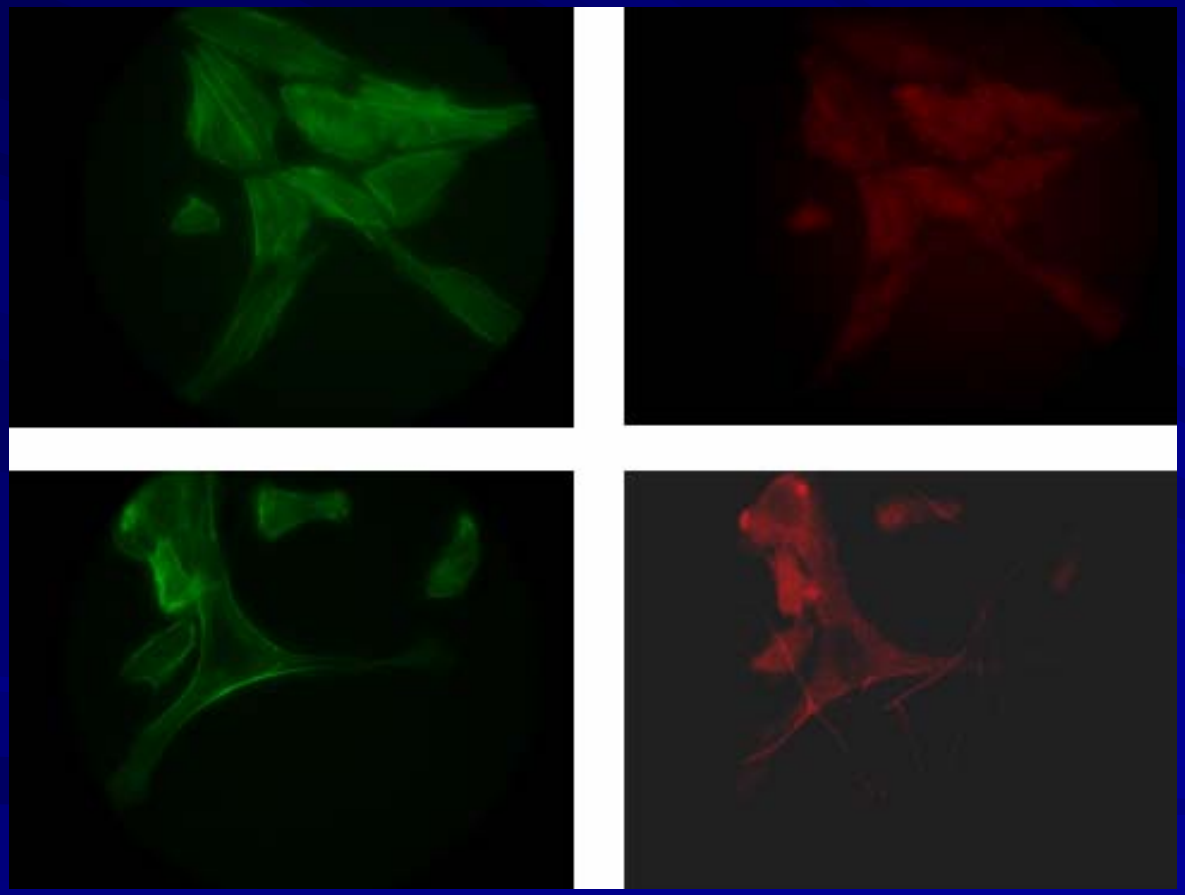
Vinculin

The photo on the left shows actin, a filamentous cytoskeletal protein stained with a green fluorescent dye. These cells are on an untreated glass a surface.

The photo on the right shows the same location of the fluorescent-tagged vinculin on the last slide.

A594 Lung epithelial cell spreading

Actin Mag: 40x. Vinculin

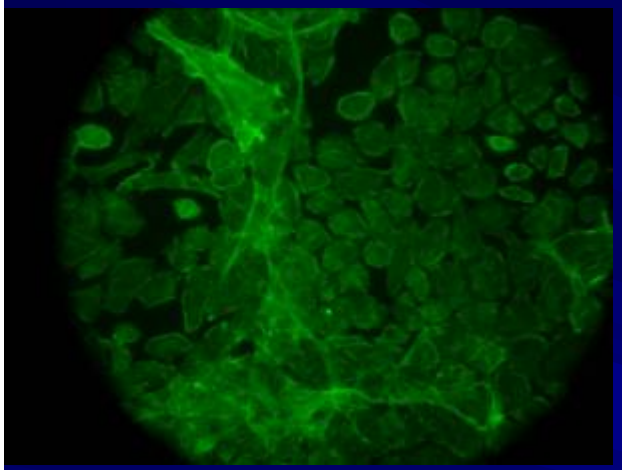


TCP
plastic

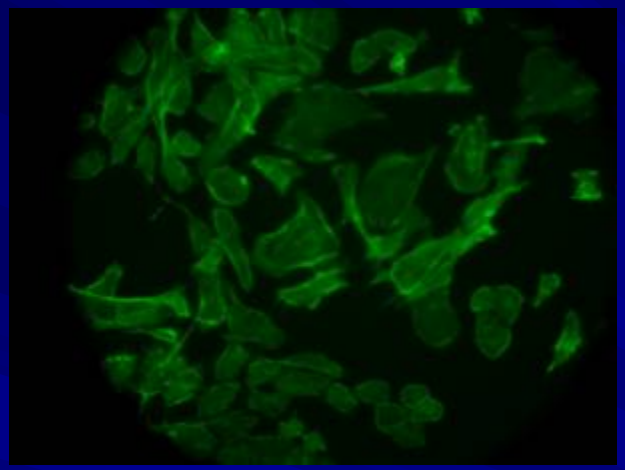
TiO_2 that has been heat treated at 260 ^oC

Effect of TiO_2 treatment on A549 Type II Lung Epithelia

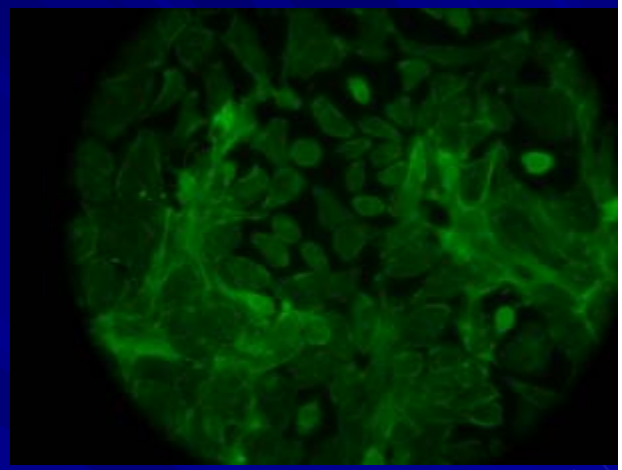
Tissue Culture Polystyrene



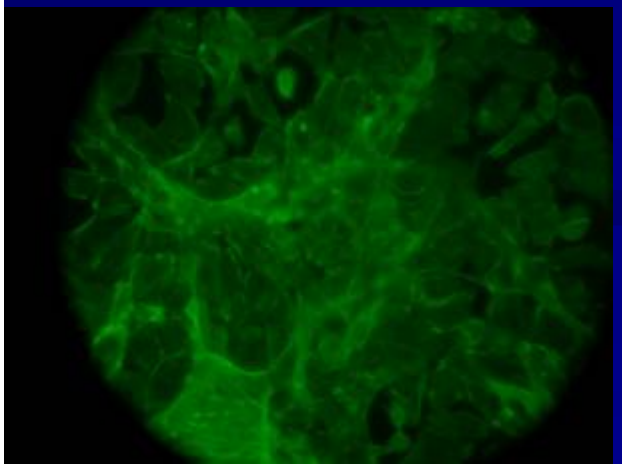
Glass



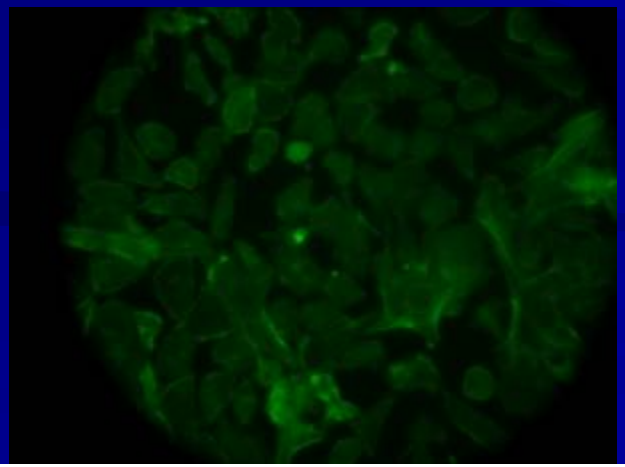
A, no heat treatment



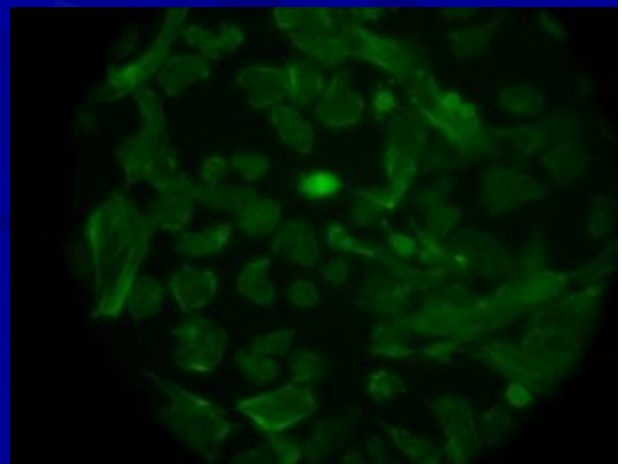
B, 120°C heat treatment



C, 260°C heat treatment

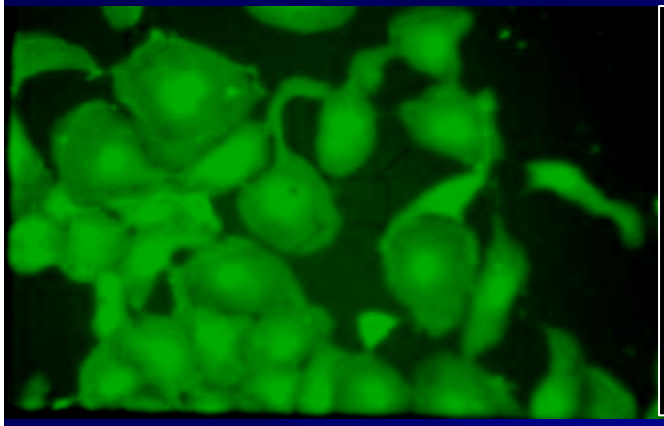


D, 325°C heat treatment

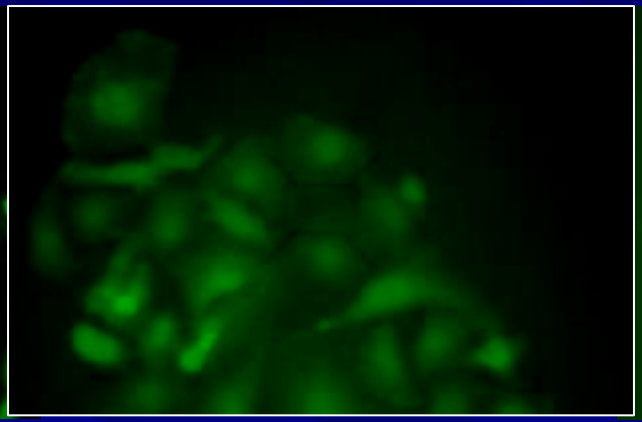


BGMk (kidney) cells on TiO_2

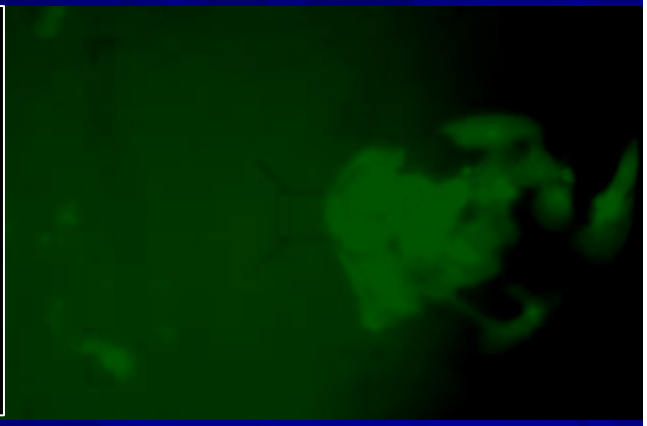
Tissue Culture Polystyrene



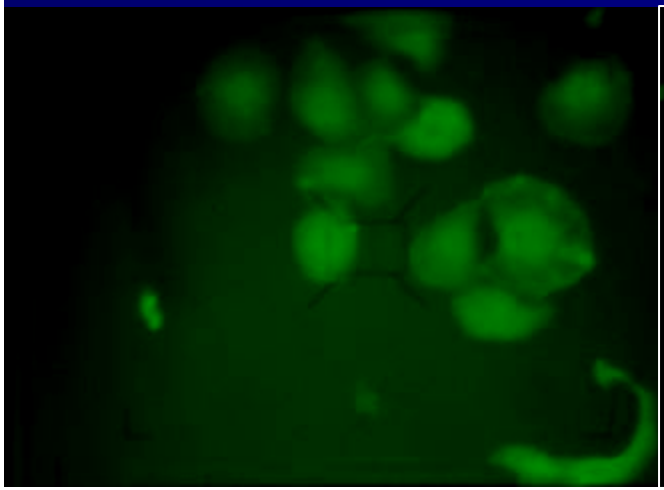
Glass



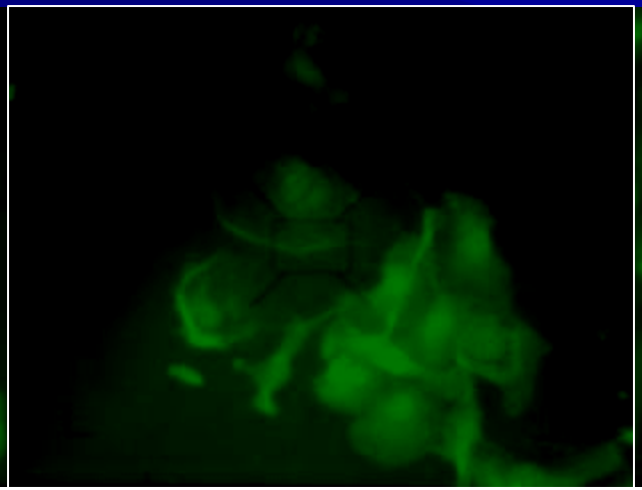
23 °C



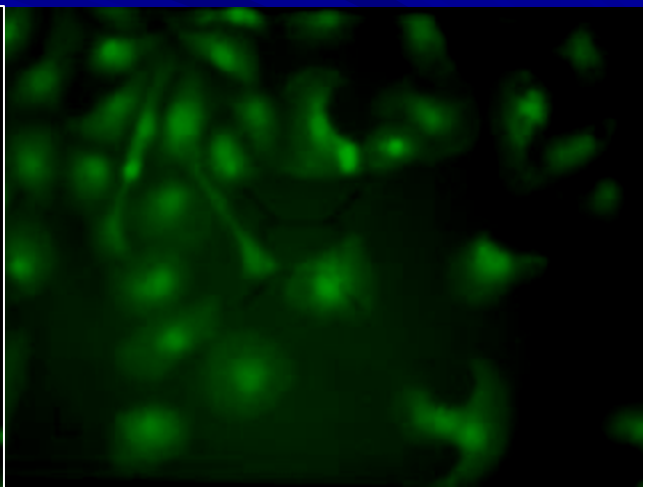
60 °C for 10 min



120 °C 10 min.,
200 °C 10 min



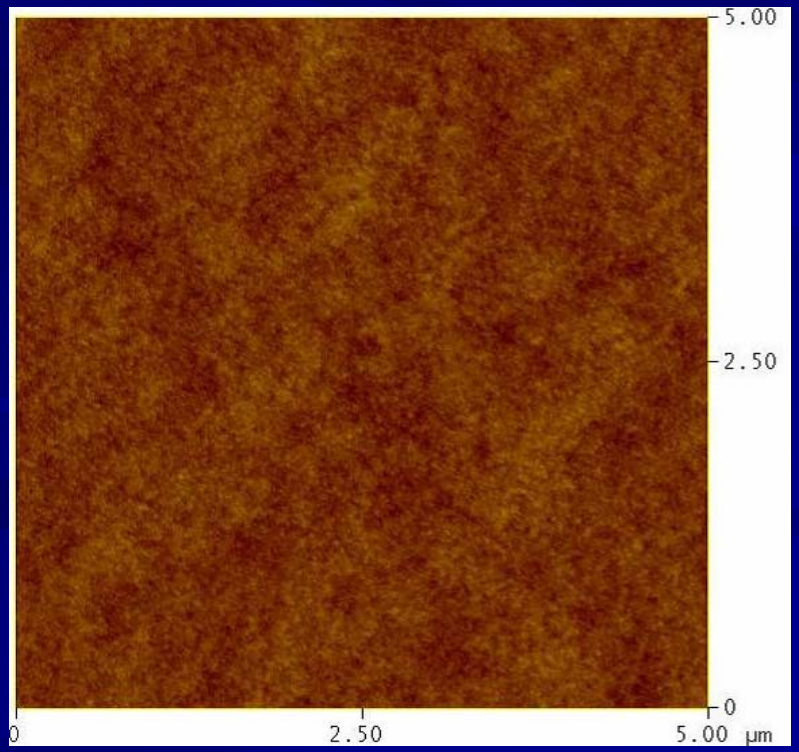
120 °C 10 min.,
335 °C 10 min.



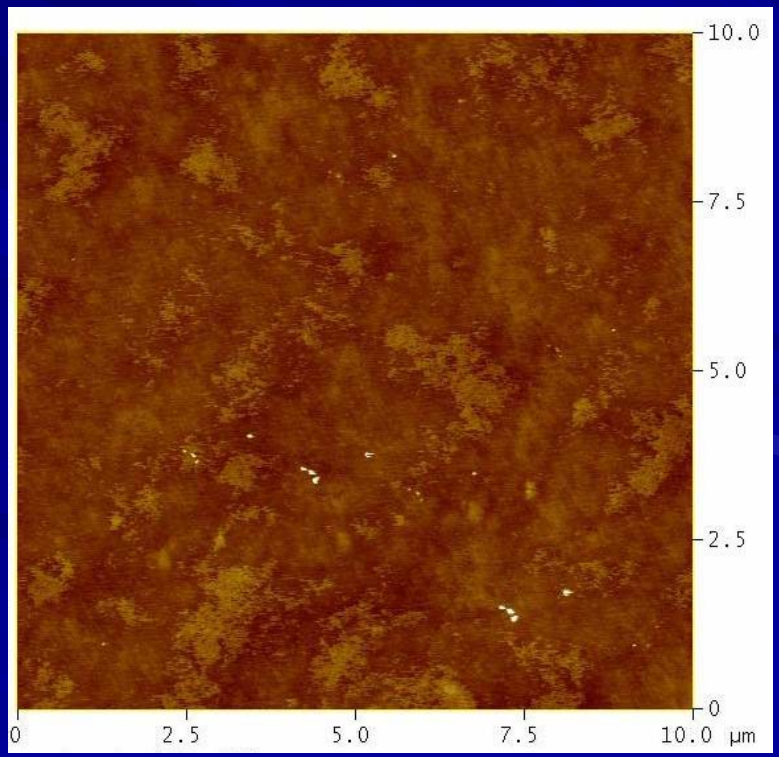
AFM analysis of surface roughness

Surface	Heat treatment	RMS Surface roughness
A	23 °C	0.401 nm
B	120 °C	0.345 nm
C	120 °C / 260 °C	0.442 nm
D	120 °C / 335 °C	0.414 nm

23 °C

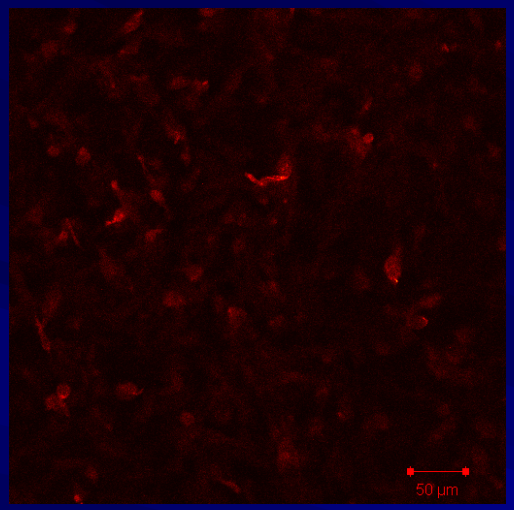


120 °C / 335 °C

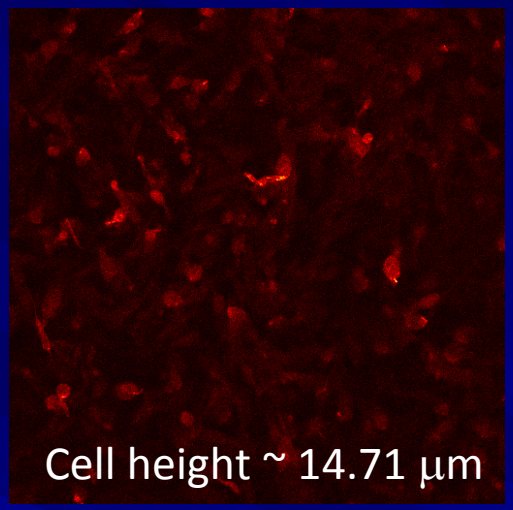


Confocal images of BGMK cells with labelled actin - TAP1 @534 nm

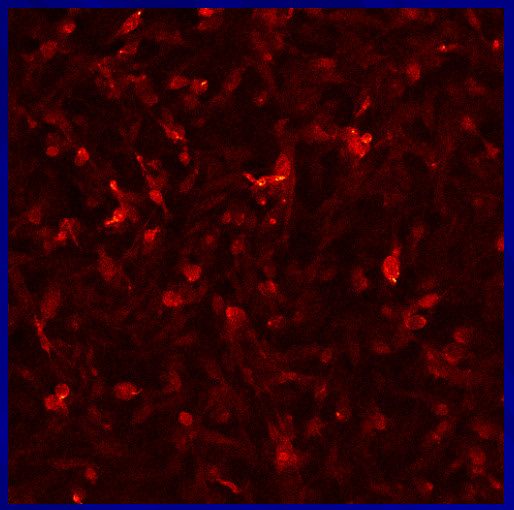
14.71 um



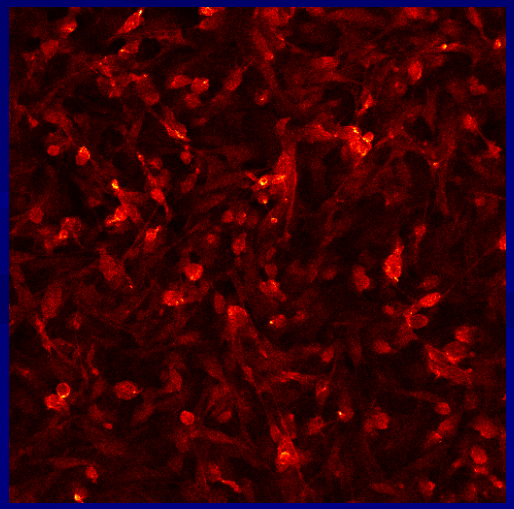
11.77 um



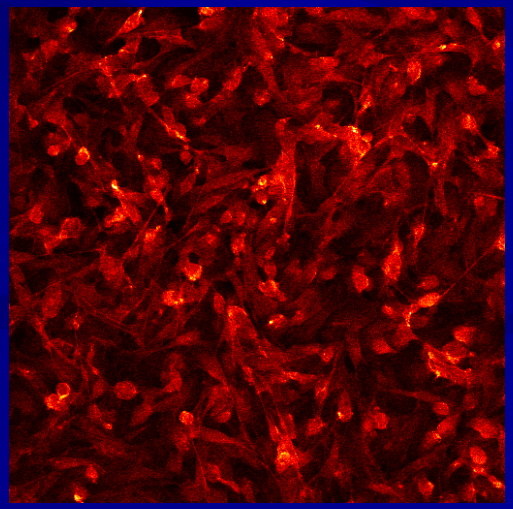
8.83 um



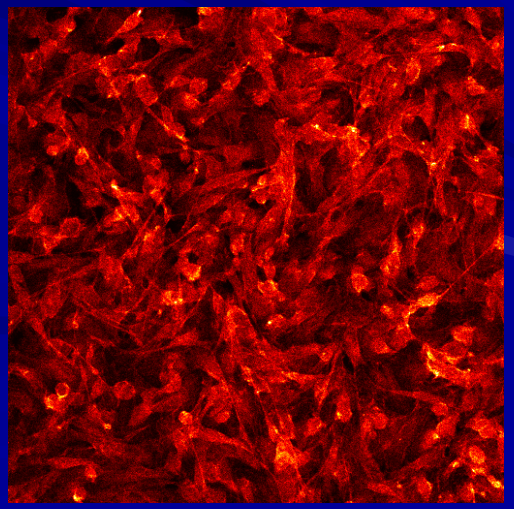
5.89 um



2.94 um

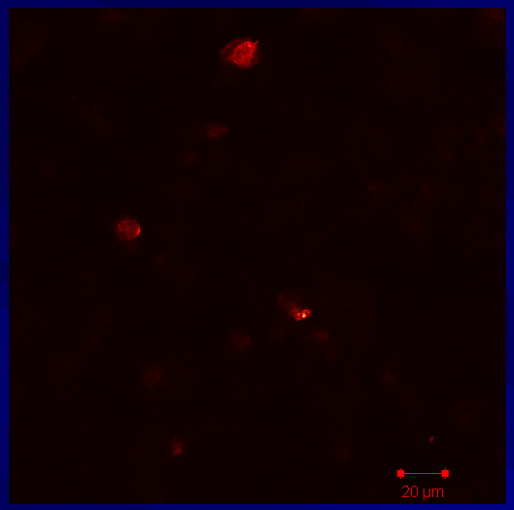


0 um



Confocal images of BGMK cells with labelled actin - TAP3 @534 nm

11.77 um



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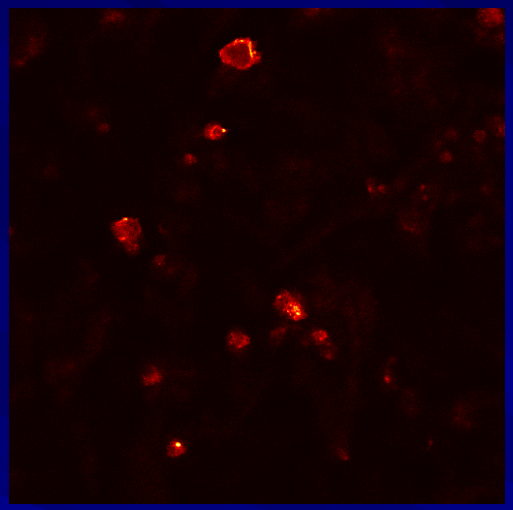

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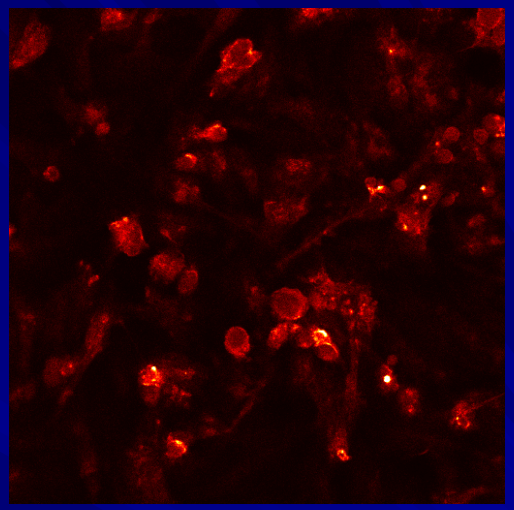

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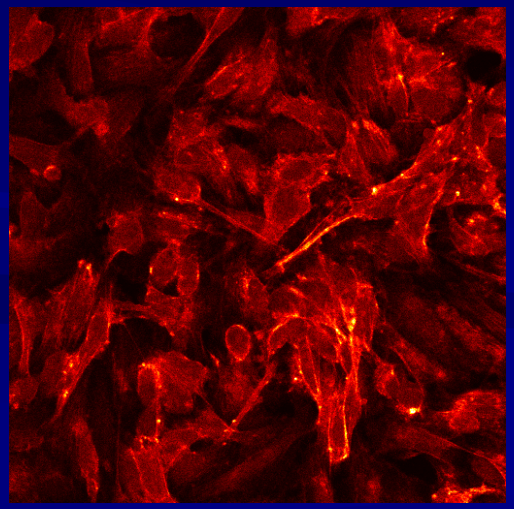
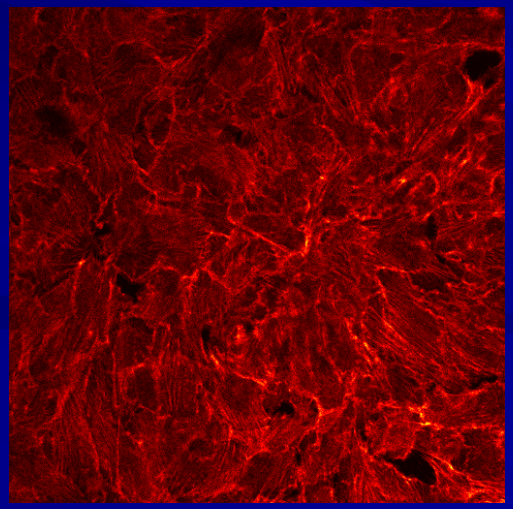


0 um

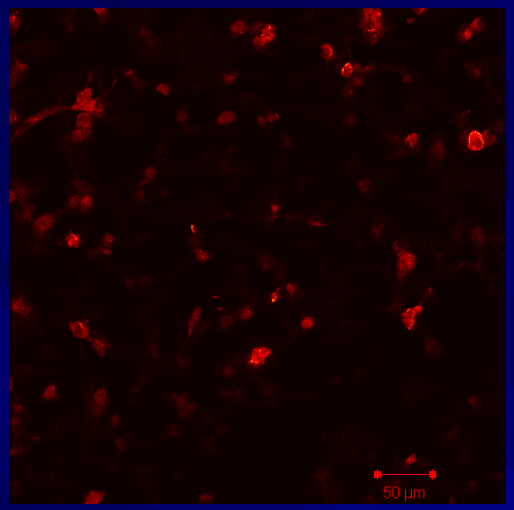


Note, scale is ½ that of previous slide images

Cell height ~ 7.85 µm

Confocal images of BGMK cells with labelled actin - TAP5 @534 nm

11.77 um



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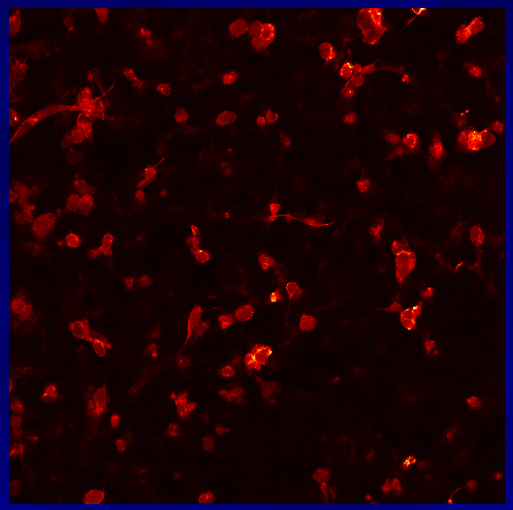

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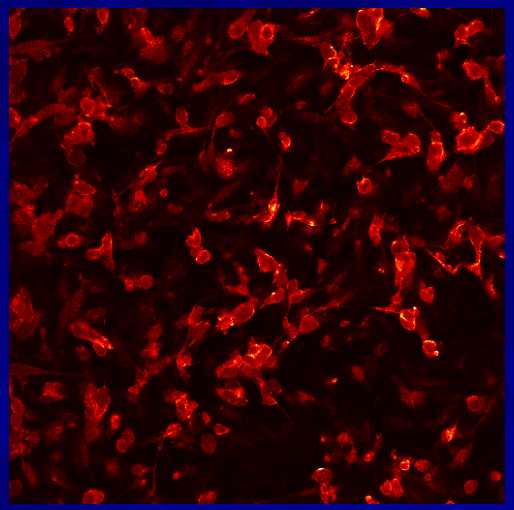
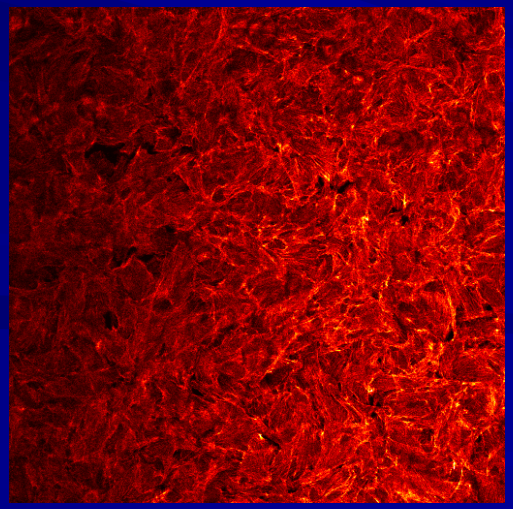
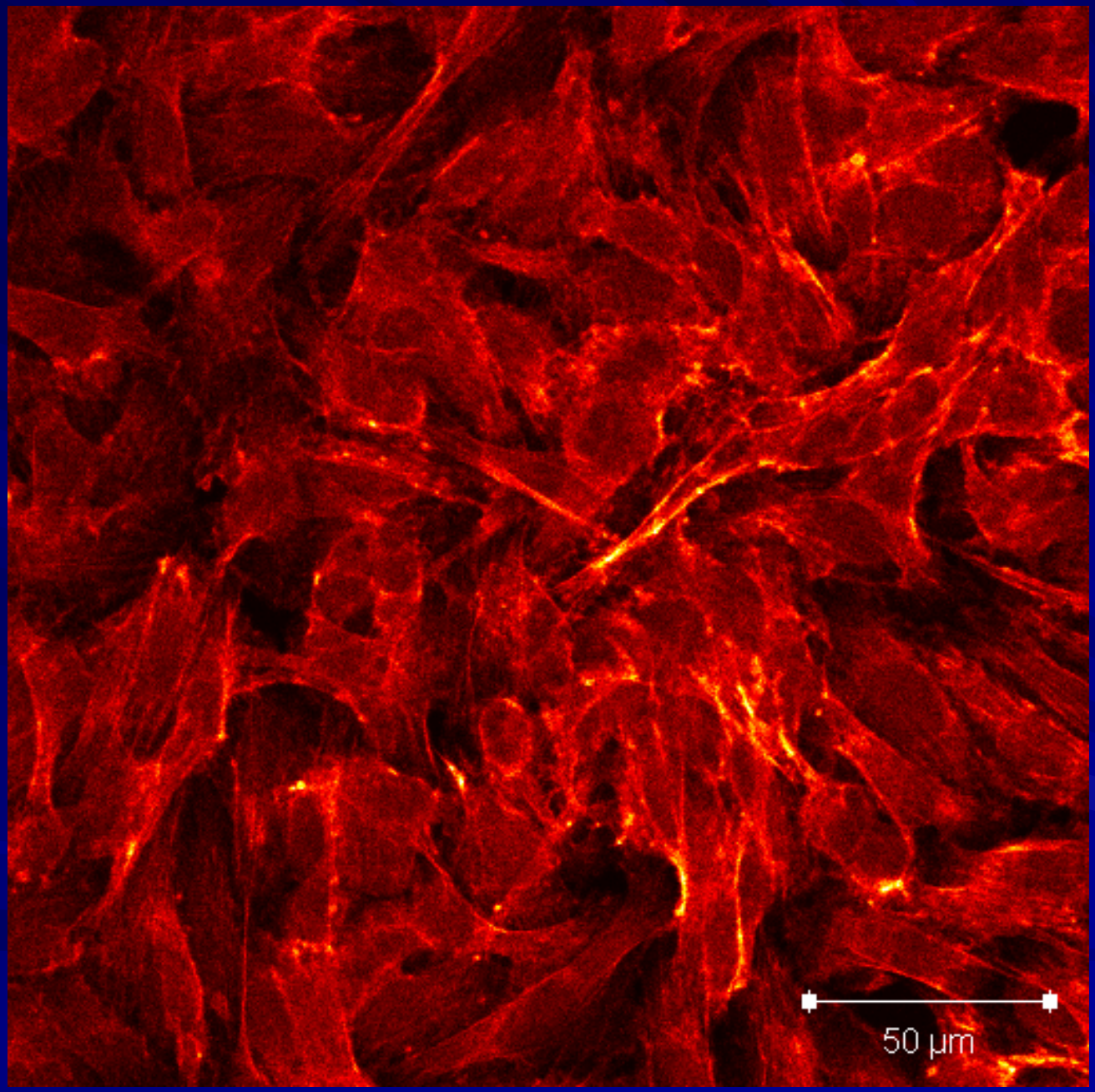


2.94 um

0 um

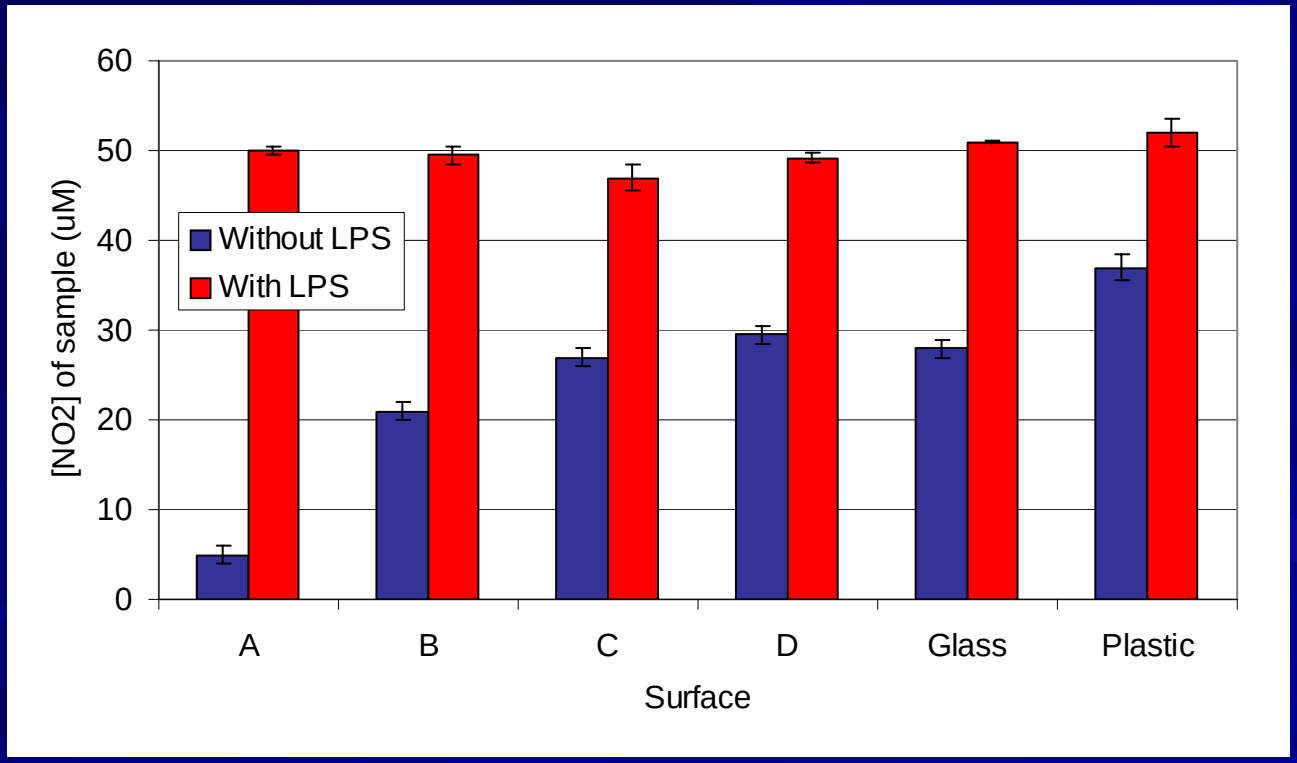


Cell height ~ 11.77 µm



TAP 3

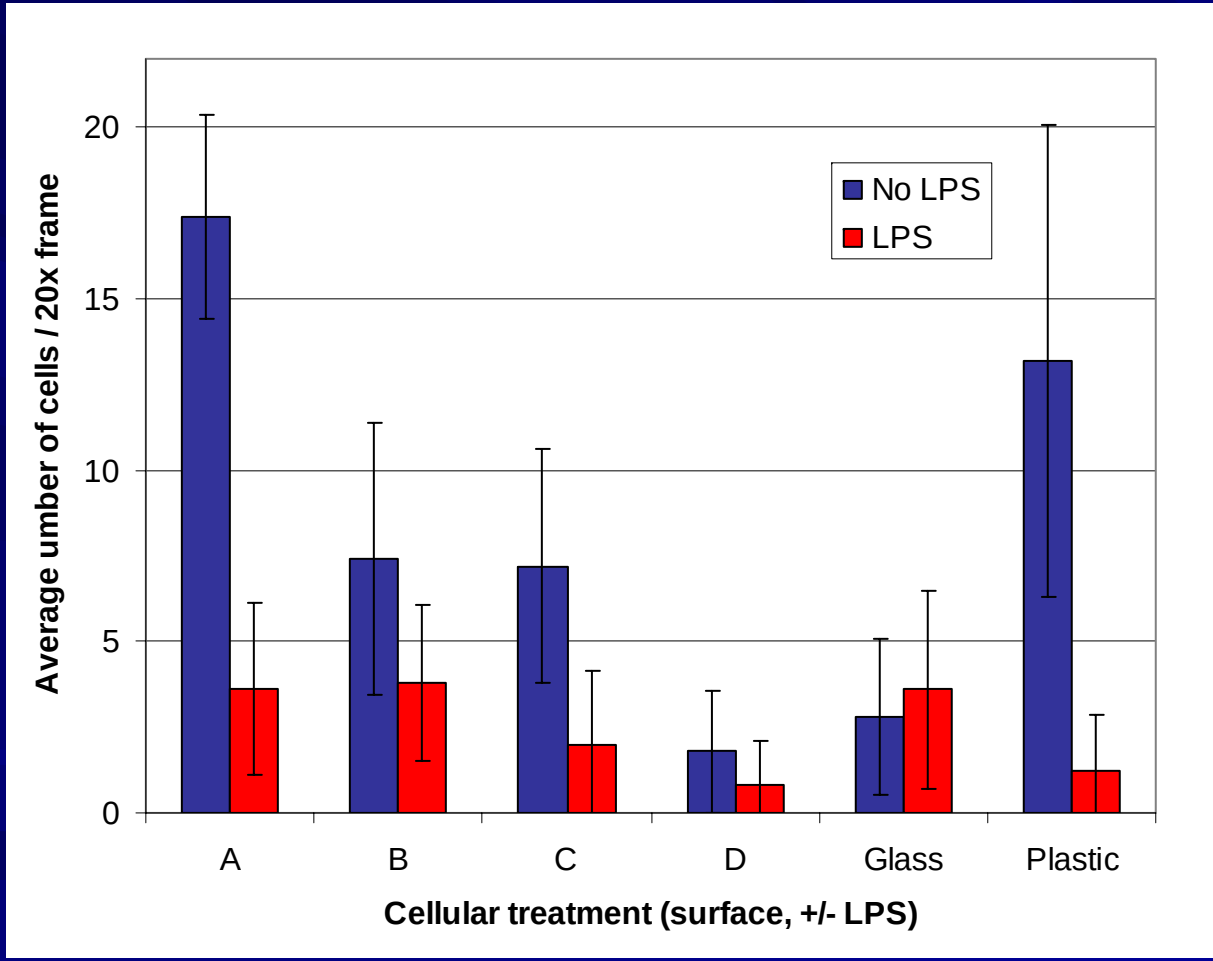
NRL8383 Macrophage activation (nitrite release) in response to surface roughness and LPS challenge



Surface	Heat treatment
A	25 °C
B	120 °C
C	260 °C
D	325 °C

10.67 ug/mL LPS applied for 24 hrs

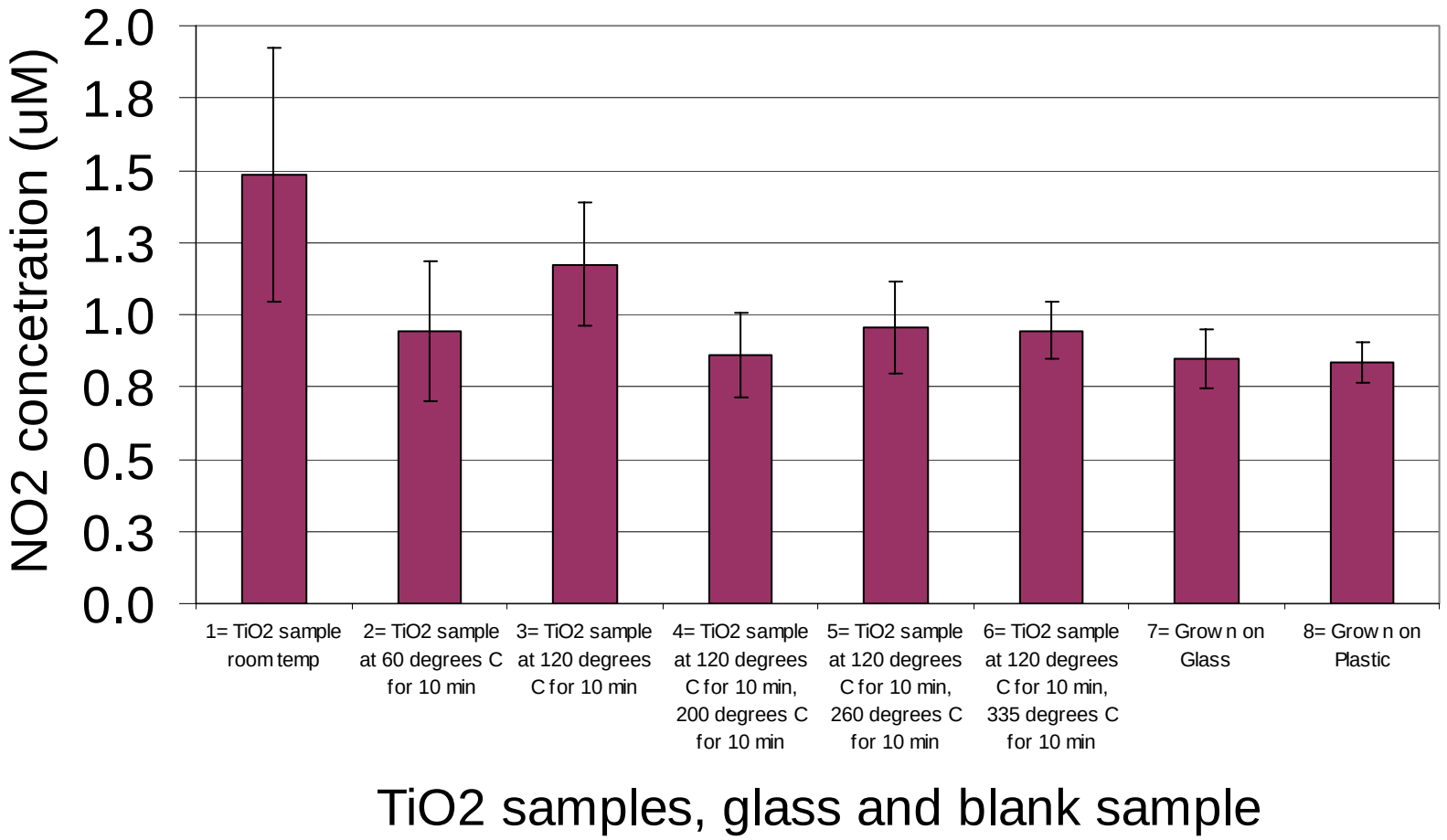
Cellular attachment w/ and w/o LPS



Surface	Heat treatment
A	25 °C
B	120 °C
C	260 °C
D	325 °C

10.67 ug/mL LPS applied for 24 hrs

Basal activation of RAW macrophages (anchorage-dependent)



No LPS applied

Summary

- Photodeposited TiO_2 films support the growth of a variety of mammalian cell types
- Adhesion can be modified through condensation of films.
  - In general, better cell adhesion at intermediate levels
  - Substantial differences across cell types
- Cell function can also be modified
  - Basal activation low on most rough surfaces
  - Bacterial challenge leads to similar excitation
  - Substantial differences across cell types.

Acknowledgements

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