NANOCAVITATED TITANIUM SURFACES INFLUENCE OSTEOGENIC CELL BEHAVIOR

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BACKGROUND

Cell-substrate interactions and related signalling pathways determine the response of the host tissue and therefore the success of implants. Several studies have shown that when selected nanotopographies are created on implantable metals, the resulting physicochemical surface changes improve osteogenic cell activity. In this context, our group has exploited simple chemical treatments to nanocavitate titanium surfaces and achieve a unique mesoporous surface network that favors cell activity.

OBJECTIVE

The aim of this study with osteogenic cells is to examine how a nanoporous Ti surface affects the formation of adhesion and sensing structures as well as the gene expression of proteins associated with focal adhesions, cellular projections, integrins and Rho Family GTPases. Particular attention is given to filopodia and associated nanoscale lateral protrusions because these could greatly influence the interaction with surfaces by increasing the contact area and interaction force.

MATERIALS AND METHODS

Commercially pure grade II titanium discs

Polished

SiC abrasive paper
Texmet carpet
MicroFloc carpet

H_2SO_4 / H_2O_2
Chemical treatment (1.5h)

Control

Nano

MATERIALS AND METHODS

Control | Nano

Sterilization 70 % ethanol and UV light

Mouse calvaria-derived osteogenic cells MC-3T3

Culture: 6h, 24 h and 72 h
MEM-Alpha + 10% FBS
37°C, 5% CO_2
Cell density: 10.000 cells/well

At 24h RNA extraction with Trizol

Quantitative real-time PCR

Oligonucleotide Primer Sequences for Real-Time PCR

Gene	primer sequence (5′-3′; F: forward; R: reverse) Primers (5′–3′)	bp
GAPDH	F: TGGACAGCACTGACTTCCAG R: CAAAGCATCGACCAGTGCTA	190
Pxn	F: CAGCTCCCCATTTCTGATGT R: ACCTGAGGGTTCACAACCAG	138
Vcl	F: ACCTGCAGACCAAAACCAAC R: CTTACCGACTCCACGGTCAT	134
Tln	F: TCCAGTACCACACTGGGTCA R: CAGATGGTGCTTTCAGCGTA	146
FAK	F: AAGGCTCAGAGGTGCCTGTA R: TGGAGACATCCTGCACAGAG	131
ITGβ1	F: GGGAGGGGCTTTCTTCTATG R: GCATTCACAAACACGACACC	138
ITGα1	F: GCCTTCATGTGTCTGCTTGA R: GGGGAAATCCAAGTTTCCAT	180
ITGα5	F: ACCACCTGCAGAAACGAGAGGC R: TGGCCCAAACTCACAGCGCA	217
ITGαV	F:TCCCACCGCAGGCTGACTTCAT R: TCGGGTTTCCAAGGTCGCACAC	126
ACTN1	F: TTTTGGTAACACCAGGCACA R: CTGCTTGTCATCCGTCTTGA	146
Cdc42	F: CTGCTATGAACGCATCTCCA R: CATGAGTGCATGTGGGTAGG	149
Rac1	F: CCTTGACTCTGCTTGGGAAG R: AGCCAGCTTGTCACTGTCCT	143
RhoA	F: TTTGCATCGTCCTGAGTCTG R: TGAAACCTTCCCACACACAA	143
Rock1	F: CCTGCCCTAGAGTGTCGAAG R: CACACCCTGCACCTTACCTT	102
PTEN	F: AGCATGTTTCTGCGACCTCT R: ACGGTTCCCTCACTGAACAC	114

Cell fixation (Periodate-lysine-paraformaldehyde (PLP))
Permeabilization
Blocking
Immunofluorescence labeling
(Anti-vinculin)

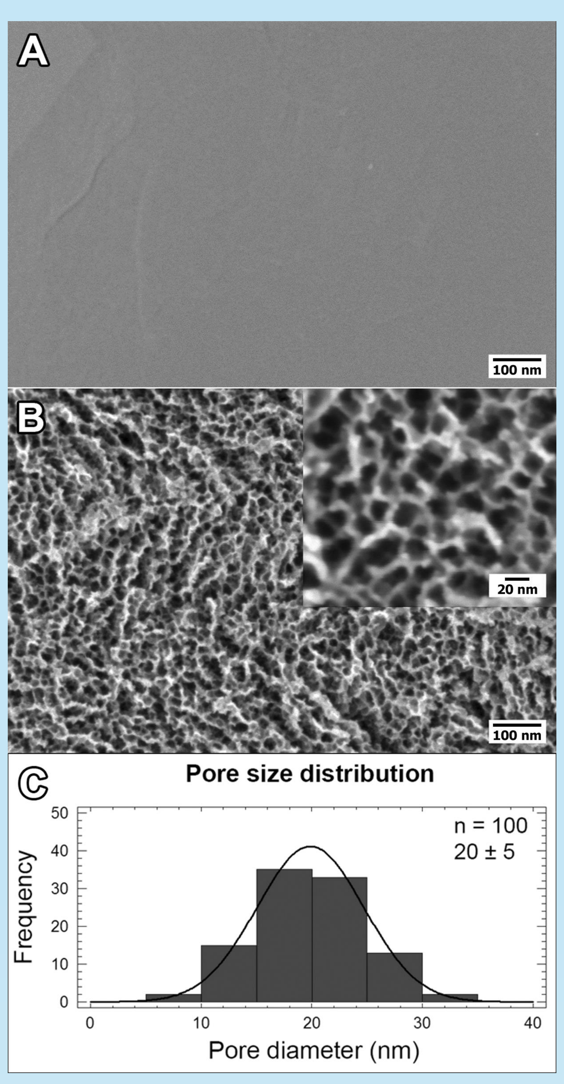
Fluorescence light microscopy (fLM)

Cell fixation (Glutaraldehyde 1h)
Osmification
Dehydration
Critical point drying

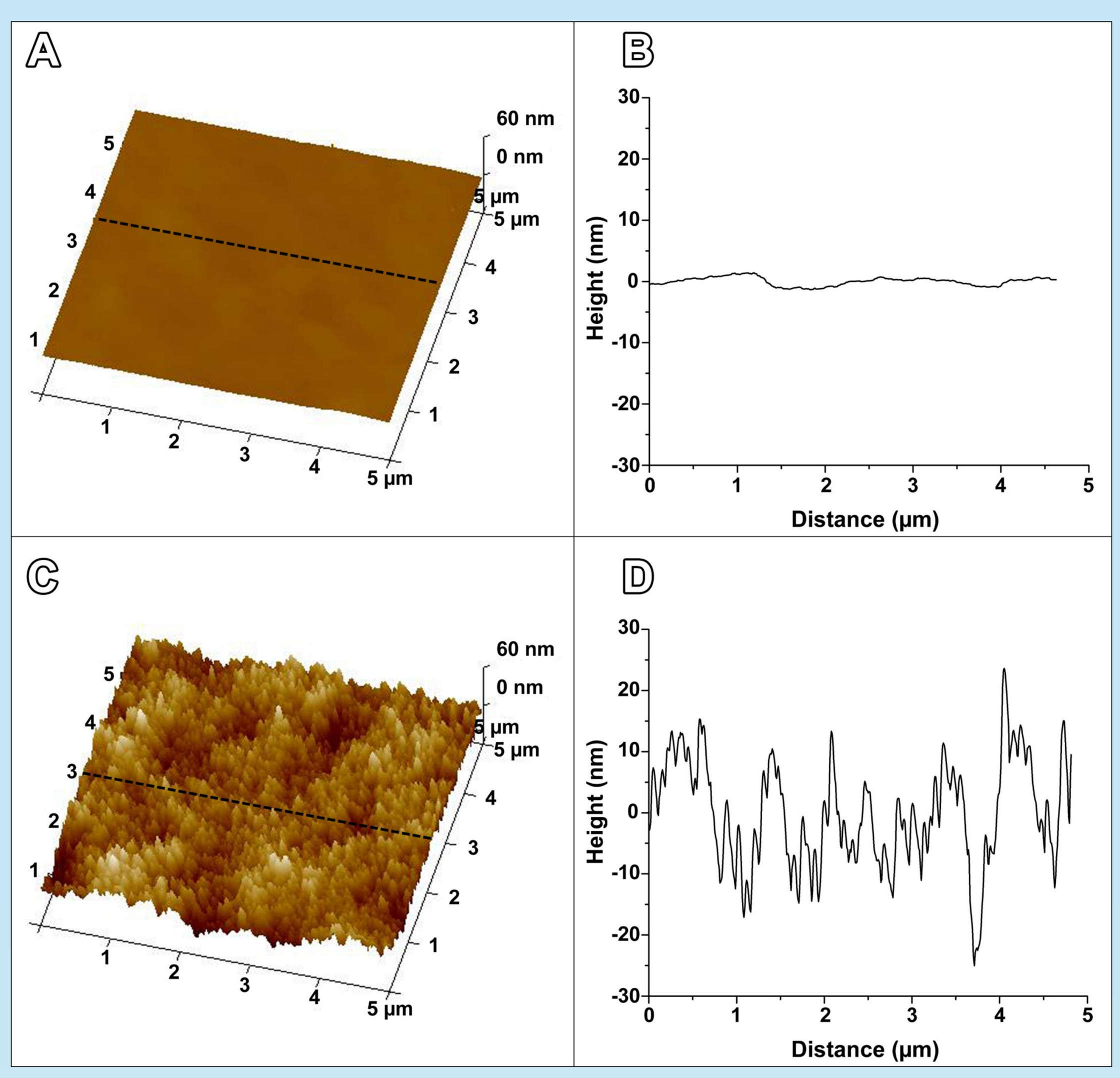
Scanning electron microscopy (SEM)
Helium Ion Microscopy (HIM)
Atomic Force Microscopy (AFM)

RESULTS

Substrate characterization



SEM images of the titanium surface after (A) mechanical polishing and (B,inset) treatment with H_2SO_4 / H_2O_2 for 1.5 h. (C) size distribution of the nanopores.



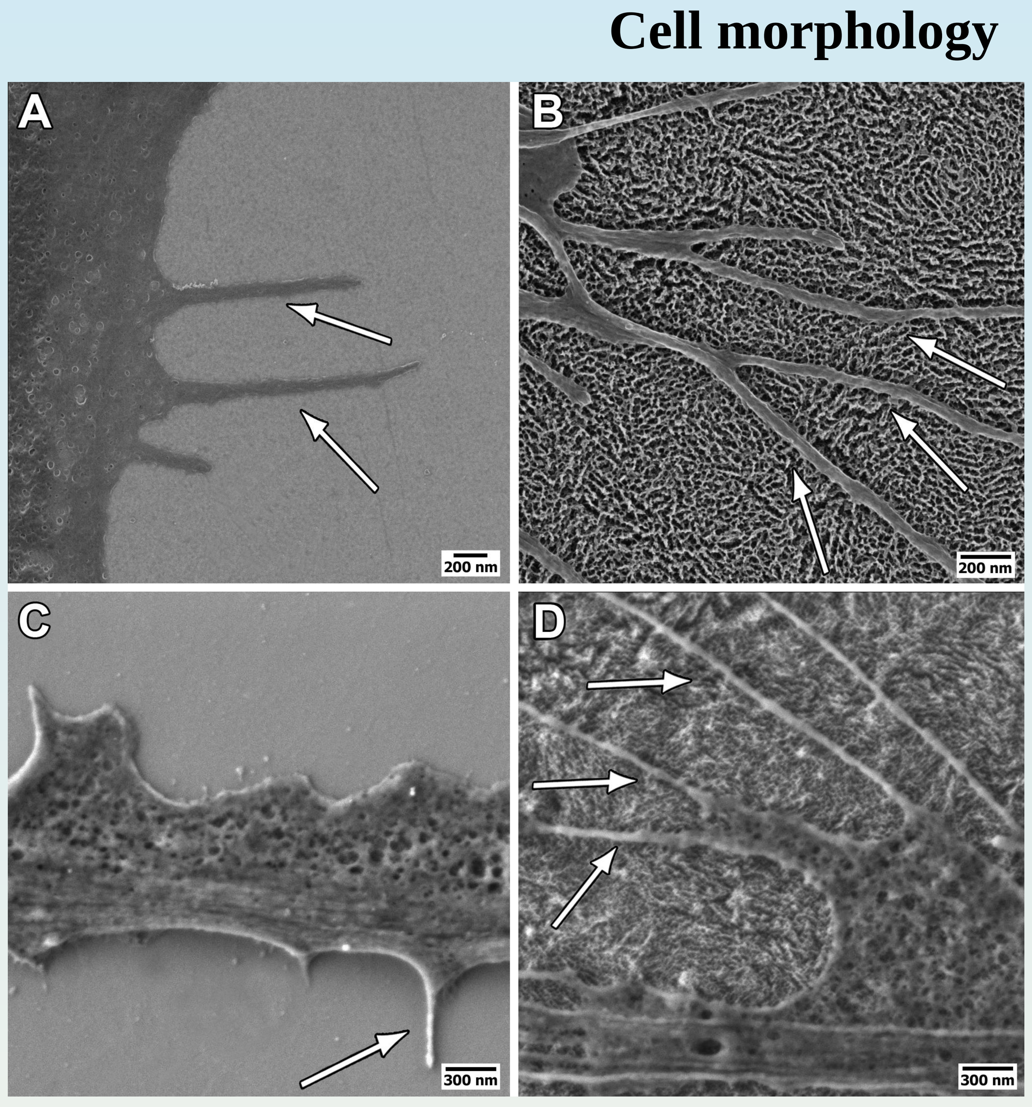
AFM 3D topographies of polished (A) and chemically treated (C) surfaces. Corresponding line sections: (B) and (D).

Profile roughness parameters:

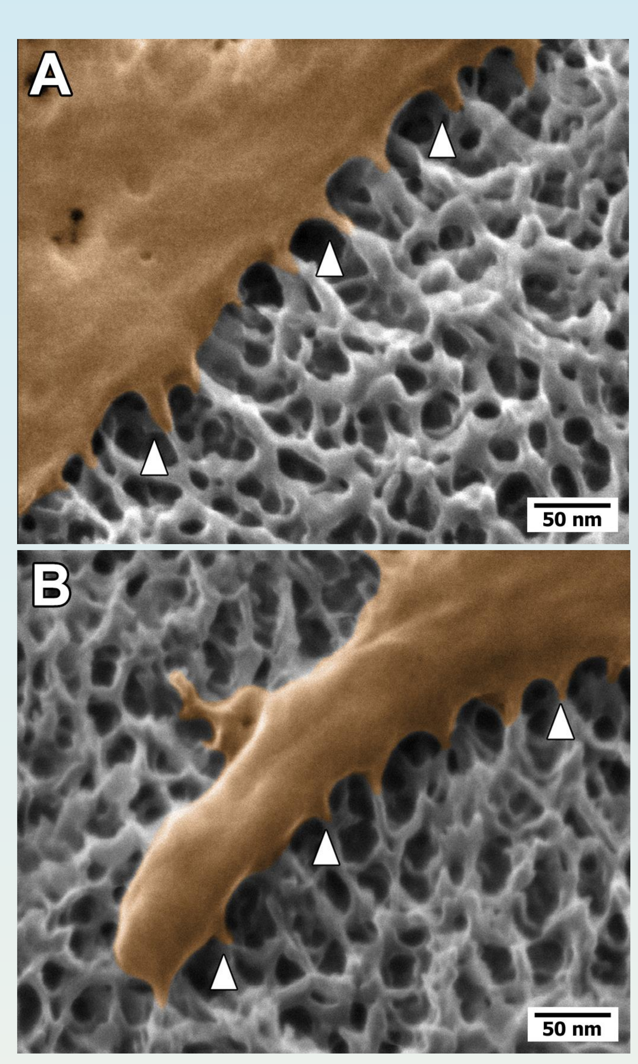
Rq = 11.5 nm (Quadratic mean) Ra = 9.2 nm (Arithmetic mean)

Treatment time of 1.5 h resulted in a planar nanoporous surface. AFM corroborated the overall planar nature of the surface and the pore-size distribution by SEM revealed a mean diameter around 20 nm.

Cell morphology



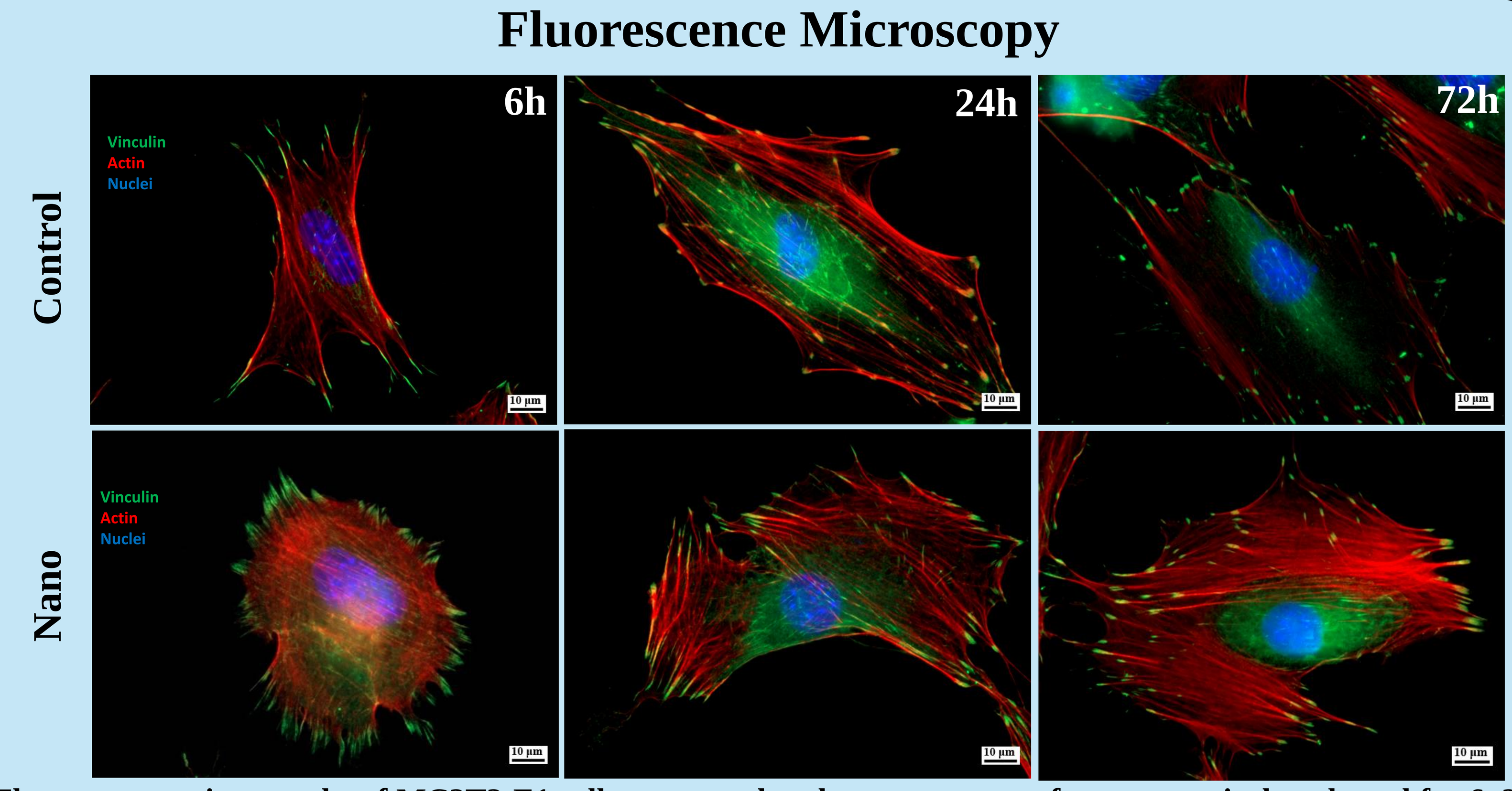
SEM micrographs of MC3T3-E1 cells (A, C) on control and (B, D) nanoporous surfaces grown for (A, B) 24 and (C, D) 72 h. Filopodia are indicated with arrows.



HIM images of MC3T3-E1 grown for 72 h on a nanoporous surface showing the presence of nanoscale protrusions (arrows) emanating from the (A) cell body and (B) form a filopodium.

Cells developed more filopodia on the nanoporous surface as compared with the control surface. Using HIM, it could be seen that the nanoscale lateral protrusions extended along the walls of the nanopores. We believe that this structure contributes an strengthen the adhesive interactions of the filopodia with the surface.

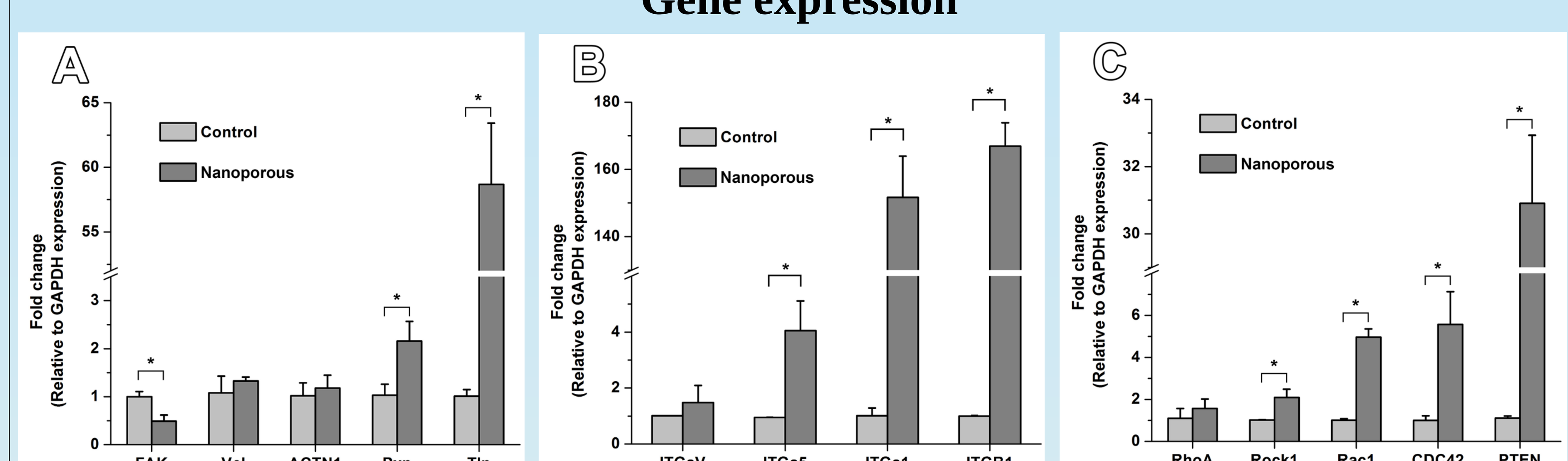
Fluorescence Microscopy



Fluorescence micrographs of MC3T3-E1 cells on control and nanoporous surfaces successively cultured for 6, 24, and 72 h. Stained with DAPI (blue) for nuclei, rhodamine/phalloidin (red) for actin, and anti-vinculin (green) for vinculin.

Analysis revealed increases in focal adhesion per cell area, focal adhesion length, and maturity on the nanoporous surface.

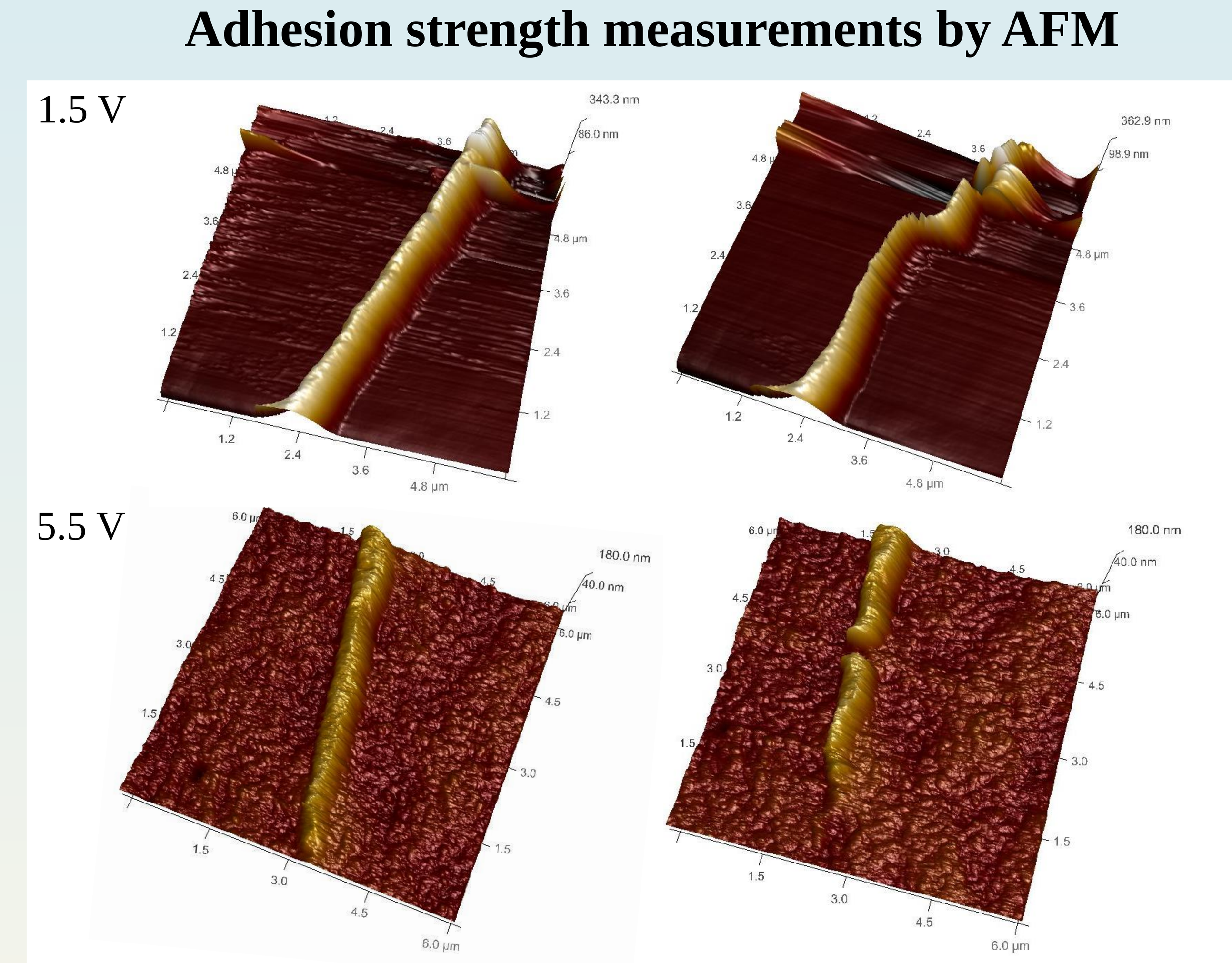
Gene expression



Comparative gene expression profile of (A) focal adhesion markers, (B) integrins, and (C) Rho family GTPases by MC3T3-E1 cells cultured on control and nanoporous surfaces.

There was a significant increase in expression of focal adhesion markers, including paxillin and talin, and of different integrins (e.g. α1, β1, and α5) on the nanoporous surface.

Adhesion strength measurements by AFM



Ongoing analyses suggested that filopodia on the nanocavitated surface require more lateral force to detach.

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CONCLUSIONS

- These results illustrate that by simply controlling the physico-chemical characteristics of surfaces, we can modulate cellular signaling.
- The increase in number of focal adhesions, as well as the abundance of filopodia with nanoprotrusions, that exhibit an apparent ‘stronger’ adhesive strength, altogether likely positively contribute to increasing cell adhesion, and thereby alter the nanoscale biomechanical relationships that regulate cell behavior.

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