Controlled Adhesion and Proliferation of a Human Osteoblastic Cell Line by Tuning the Nanoporosity of Titania and Silica Coatings

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Electronic Supplementary Information (ESI)

ESI-1: Experimental procedures.

ESI-2: Fluorescent microscope images of DAPI stained cells adhered to Si films at various times.

ESI-3: Fluorescent microscope images of DAPI stained cells adhered to Ti films at various times.

ESI-4: Statistical analysis of cell adhesion and proliferation.

ESI-5. Structural information of mesoporous samples.

ESI-1: Experimental procedures.

Film Synthesis: TEOS was prehydrolyzed by refluxing for 1 h in a water/ethanol solution; H_2O]/[Si])1; [EtOH]/[TEOS])5. To this prehydrolyzed solution was added surfactant (SF), alcohol, and acidic water in order to prepare the precursor solutions, with final composition TEOS:EtOH:H_2O (0.1 M HCl):SF equal to 1:40:5:0.0075 mol ratios. These solutions were aged 72 h at room temperature. Titania solutions were used without aging, and the films were made at 30 ºC solution temperature. The final composition of the precursors solution were $TiCl_4$:EtOH:H_2O:SF equal to 1:40:10:0.0075. The composition used was $TiCl_4$/EtOH/H_2O 1:40:10.

Film characterization: Film thickness and reflective index values were obtained from the ellipsometric parameters ψ and Δ at each P/Ps (Ps being the saturation water pressure), which was varied from 0 to 1 using a SOPRA GES5A apparatus, equipped with microspot optics. Film porosity was evaluated by the WinElli 2 software (SopraInc), which transforms the variation of n with P/Ps into filled pore volume by using a three-medium BEMA treatment. Pore and neck size distributions are derived according to a Kelvin model. Contact angles were determined by averaging measurements on five distilled water droplets using a Ramé-Hart 190 contact-angle apparatus. Micrographs were obtained using a Philips EM 301 transmission electron microscope (TEM) operating at 60 kV. All samples were analyzed in triplicate. Data are represented as means $\pm$ SE.

Cell culture: The human osteoblastic cell lines Saos-2 (ATCC, Rockville, MD) were cultured as monolayers in a humidified 5% CO_2 atmosphere at 37°C in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY) supplemented with 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS) (Gibco), 100 U ml^{-1} penicillin, and 100 µg ml-1 streptomycin. Before confluence, cells were removed from culture flasks by treatment with 0.1% trypsin and 0.02% EDTA. Cells were counted after Trypan Blue staining in a Neubauer chamber.

Cell adhesion and proliferation:

Cell adhesion: To test cell adhesion on the different materials, we added 1 x 10^3 Saos-2 cells to the surface of each type of coated glass. After 6hs the non adhered cells were

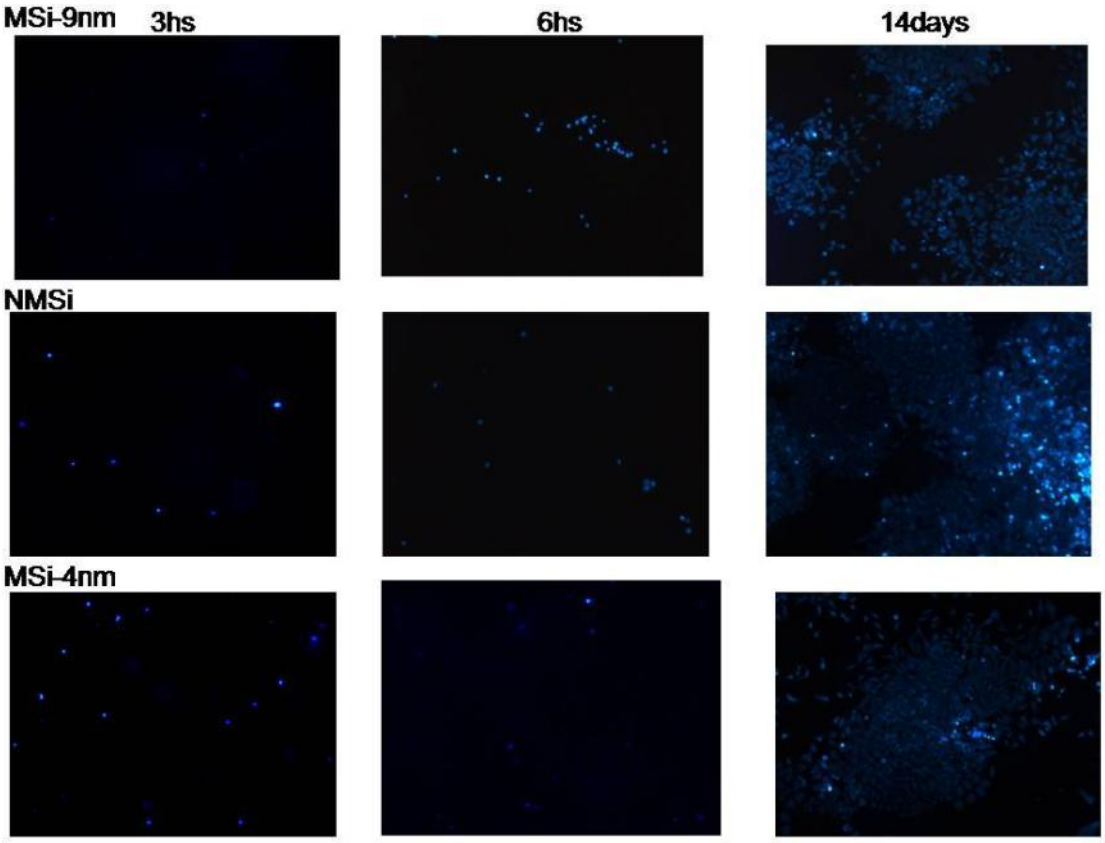
removed and fresh complete medium was added. For cell counting, at different times after cell seeding, the glasses were rinsed three times with PBS and fixed with 4% paraformaldehyde for 1 h. Samples were again washed three times with PBS. Cells were permeabilized by Triton X-100 (0.05% in PBS-1% bovine serum albumin) for 10 min and the nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI) (5 μg mL^{-1} in PBS) for 2 min.

Cell proliferation: MTT test. This colorimetric assay is based on the ability of the mitochondrial dehydrogenase enzymes of living cells to convert 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) into an insoluble formazan. The medium was removed and a 5 mg/mL solution of MTT in PBS was added to the glasses and incubated at 37ºC in a humidified 5% CO_2 air atmosphere for 4 h. Afterward, MTT solution was removed, the glasses were washed three times with PBS and DMSO was added for 30 min. The optical density was recorded at 570 nm.

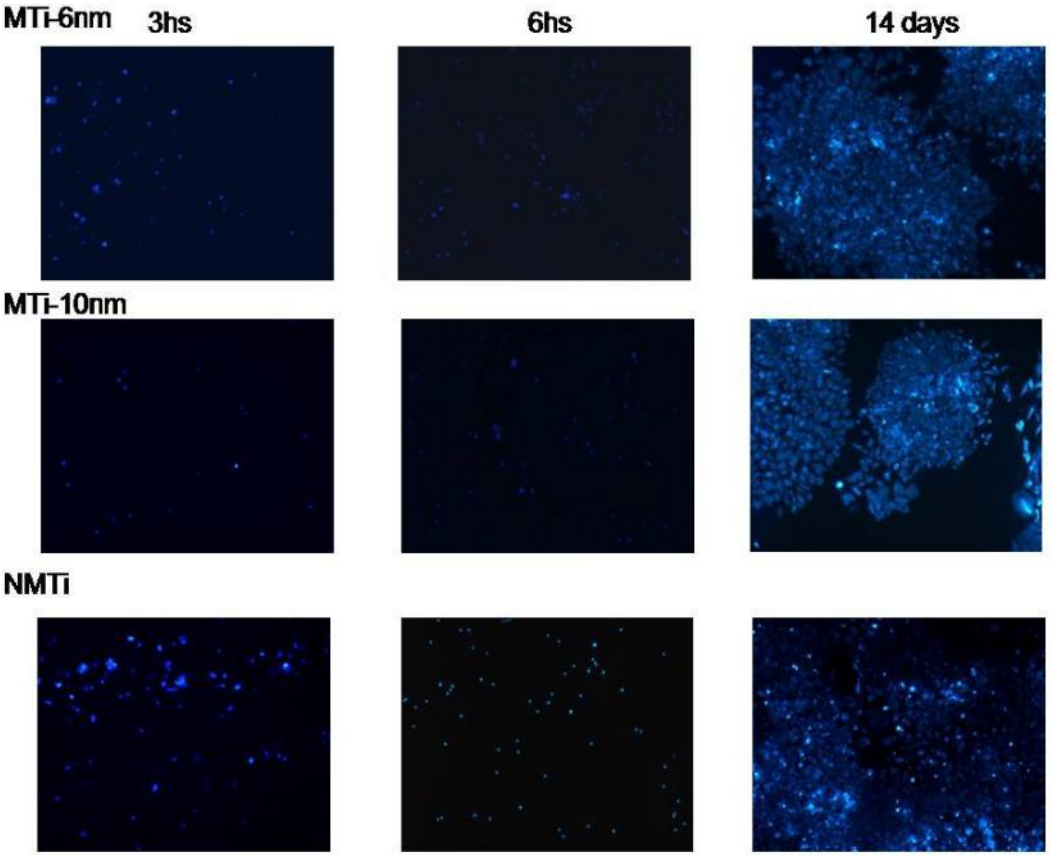
Statistical analysis:

All experiments were performed in triplicate and statistically analyzed by one-way ANOVA. Data are represented as means $\pm$ SE. The differences were analyzed using one way ANOVA, followed by Bonferroni's Multiple Comparison Test, when $p < 0.05$ difference was considered significant.

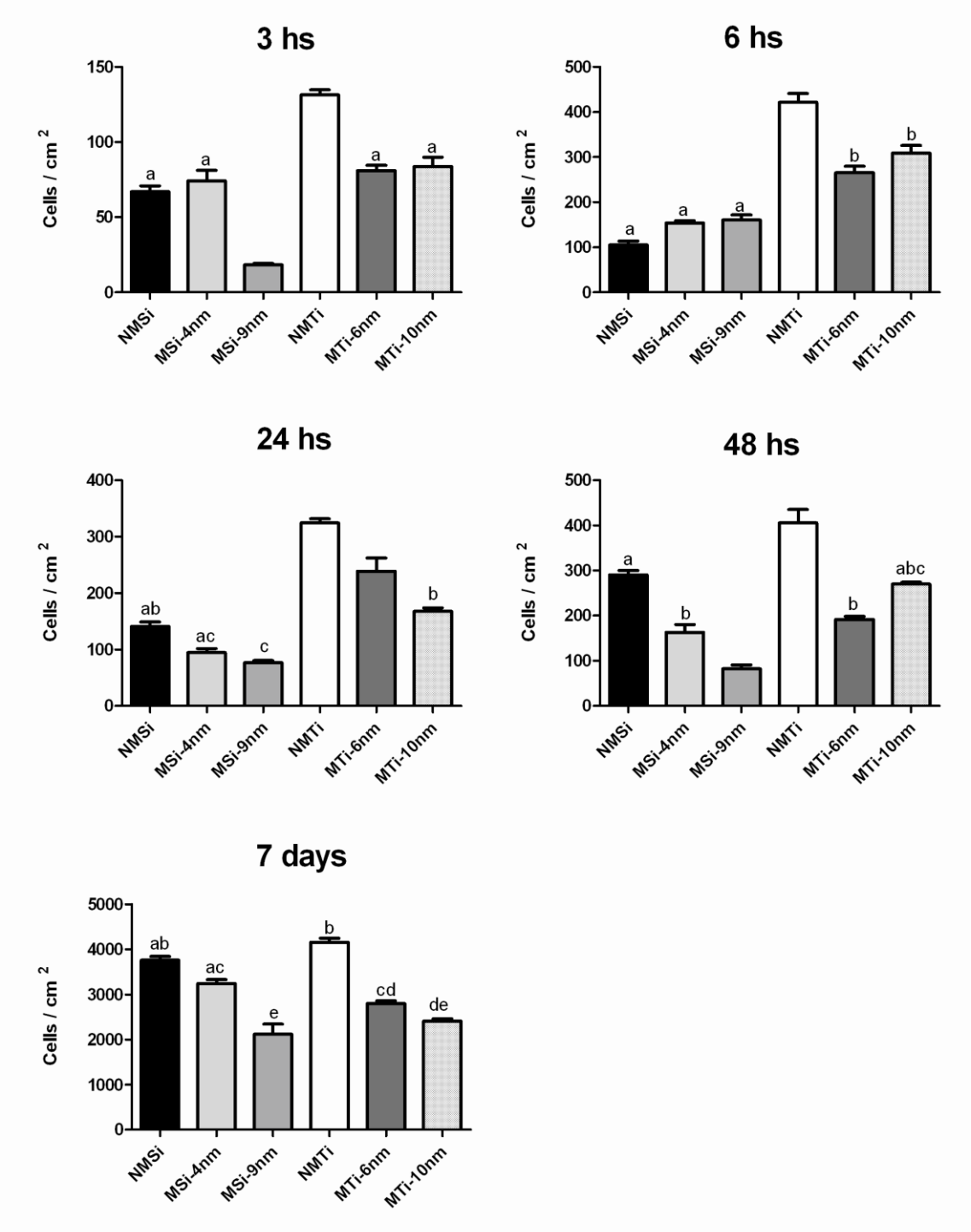
ESI-2: Fluorescent microscope images of DAPI stained cells adhered to Si films at various times.



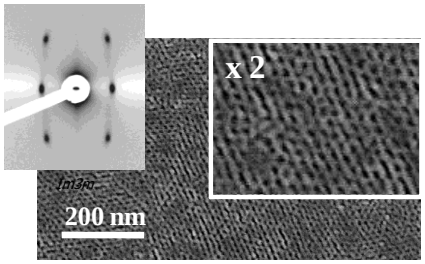
ESI-3: Fluorescent microscope images of DAPI stained cells adhered to Ti films at various times.



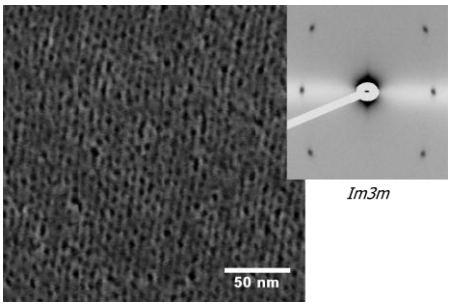
ESI-4: Statistical analysis of cell adhesion and proliferation. Samples marked with the same letter, at each time point, denote that there are no significant differences between them. There are statistical differences between the samples without the same symbol ($p<0.05$)



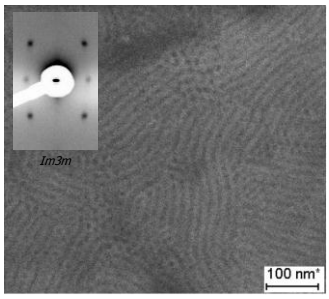
ESI-5. Structural information of mesoporous samples.



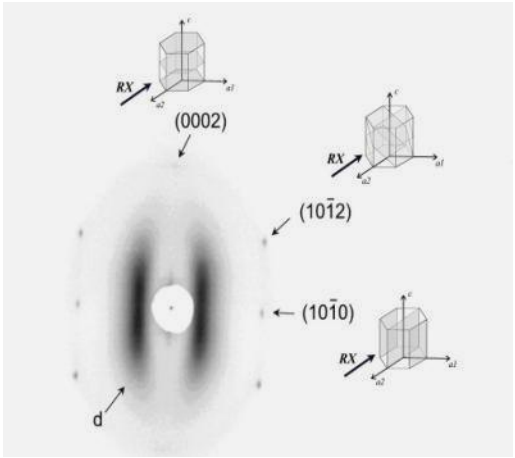
SAXS pattern and FE-SEM micrograph of MTi-10nm sample showing the Im3m cubic mesostructure



SAXS pattern and FE-SEM micrograph of MTi-6nm sample showing the Im3m cubic mesostructure



SAXS pattern and FE-SEM micrograph of MSi-9nm sample showing the Im3m cubic mesostructure



SAXS pattern of MSi-4nm sample showing the 3D hexagonal mesostructure.