Design of surfaces with mechanical nanoheterogeneities for a better control of cell-material interactions

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Introduction: Growing cells are sensitive to the chemical composition, the topography and the mechanical properties of their substrate surface. Chemical composition and topography of surfaces used for interaction with biological matter become more and more controlled, either at the supra- or the subcellular level. However, control of the mechanical properties of a surface at the subcellular scale remains challenging. The aim of this study is to prepare surfaces presenting mechanical cues at the nanometer scale, and to evaluate cell behavior in contact with these surfaces. The adopted strategy consists in coating a substrate showing rigid topographical nanostructures with a thin layer of an elastomer (Figure 1). This layer ensures the homogeneous chemistry and topography of the surface, while the rigid structures underneath provide mechanical contrast.

Materials and Methods: *Preparation of substrates with mechanical nanoheterogeneities*: The combination of colloidal lithography and layer-by-layer assembly allows creating a defined topography over wide areas. Glass surfaces were first covered by positively charged polyallylamine, and then put in contact with a solution of 500 nm silica colloids. Particle distribution was examined using scanning electron microscopy (SEM). To obtain a homogenous chemistry and topography, thin films of poly(dimethylsiloxane) (PDMS) were spin-coated on top of the silica colloids. The stiffness of PDMS was adjusted using different cross-linking agent concentrations. Atomic force microscopy (AFM) was used to investigate the topography and the mechanical properties of the obtained substrates, by acquiring topographical images and mapping force-distance curves, respectively. Surface chemical composition and wettability were checked using X-ray photoelectron spectroscopy (XPS) and water contact angle measurements.
Cell culture experiments: Adhesion and proliferation of MC3T3 preosteoblasts, gingival fibroblasts and C2C12 skeletal myoblasts were examined after 4h and 72h in culture on the elaborated substrates, and the observed behavior was compared to the one found on thin PDMS layers, devoid of silica colloids. Cell motility was also investigated using time-lapse imaging.

Results: Particle deposition is regulated by electrostatic interactions, and a regular distribution of the silica colloids was obtained (see SEM image on Figure 1, left). Spin-coating parameters could then be adjusted to produce surfaces with a very limited topography and a homogeneous surface chemistry. The thickness of the PDMS layer was of ~50 nm on top of the 500 nm colloids (see SEM image on Figure 1, right). AFM force maps clearly showed the mechanical contrast produced at the interface by the embedded rigid particles.
Cell response is shown to be affected by the mechanical nanoheterogeneities, in a cell type-dependent manner. Global trends may however be extracted. Cell adhesion tends to increase on the heterogeneous substrates. Moreover, cell proliferation is found to be always maximized on heterogeneous substrates, although the used cell types proliferate better on homogeneous substrates of different stiffness. This suggests that on mechanically nanoheterogeneous substrates, cells can make use of areas of the most favorable stiffness, which will then control their response. Finally, cell motility was enhanced in presence of rigid mechanical nanocues. This can be attributed to enhanced formation of focal adhesions owing to the presence of more rigid domains, but the limited size of these domains prevents extensive maturation of these focal adhesions

Conclusion: Taken together, these results demonstrate that cells are sensitive to mechanical heterogeneities at the subcellular level, which opens the way to novel strategies for a better control of cell-material interactions.

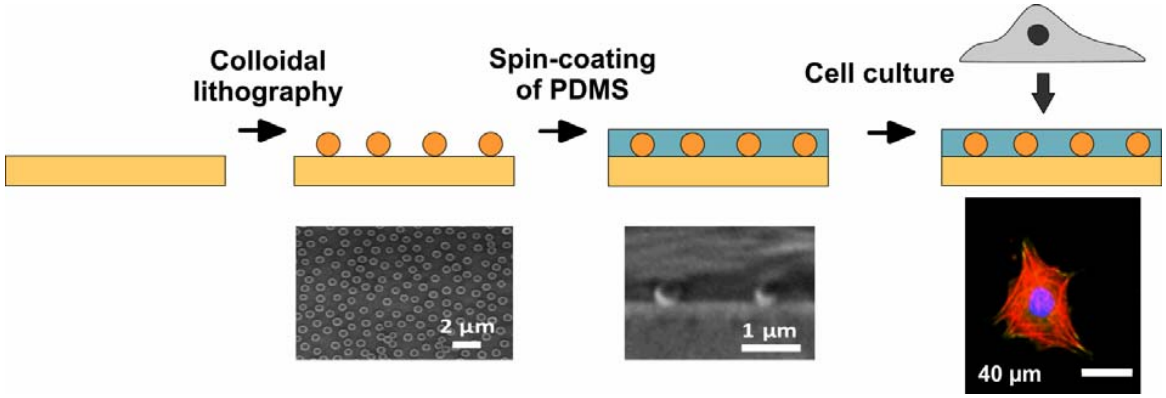

Figure 1. Strategy adopted to study the effect of nanomechanical heterogeneities on cell response.