TOPOGRAPHIC FEATURES AT THE MICROMETER SCALE INDUCE CHANGES IN CELL CYTOSKELETON AND GOLGI COMPLEX MORPHOLOGIES

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It is now well recognized that cellular behaviour on topographically modified materials is of great importance for fields as tissue engineering and regenerative medicine. Surface features at the micro and nanometer scale have been shown to influence and even determine cell behaviour and cytoskeleton organization [1]. However, a poorly studied area is the response, function and distribution of sub-cellular organelles to topographically modified surfaces.

Nanostructures and microstructures are present in the natural environment of the cells: cell membranes contain nanosize molecules and the ECM is formed by biomolecules configured in different geometrical arrangements (nanopores, nanofibers, nanocrystals…). Therefore, it is of particular interest to study the effect of nano and microscale topographic structures on the cell behaviour and its internal structure. Indeed, several techniques derived from the microelectronic industry have been applied to create topographically modified substrates that are used in cell culture systems. The effects of micro and nanostructures in cell orientation and adhesion and the cytoskeleton organization have been widely studied [2,3]. However, very little has been reported about the influence of different topographies in the disposition of subcellular elements. The fact that on one hand, the cell adhesion and morphology depend on the substrate topography and on the other hand there is a tight relationship reported between the morphology and function of the Golgi complex with the actin cytoskeleton organization and its dynamics[20] led us to postulate a correlation between topographically modified substrates and the subcellular positioning and organization of this organelle.

In this study, we have used nanoimprint lithography to generate a variety of physical features on poly (methyl methacrylate) PMMA at the microsize range with geometries of post and holes, specifically chosen for their complementary geometries and the same specific area. Normal rat kidney fibroblasts (NRK) were cultured onto the patterned substrates during 24 hours and their morphology was studied by means of scanning electron microscopy (Figure 1). Single or double immunostaning assays were performed to visualize the Golgi complex, the microtubules, the intermediate filaments and the actin cytoskeleton organization and focal contacts.

The experiments showed a direct correlation between the proportion of compact Golgi and the alteration in the cytoskeleton organization in cells cultured onto microstructured samples (Figure 2). Based on the obtained results, which suggested that Golgi complex contain assembly complexes to link to actin microfilaments [4], our hypothesis is that microstructures may induce physicochemical alterations in the F-actin that disrupts the Golgi complex

mechanical stability, which ends with its collapse. To our knowledge, up to now, naturally derived substances obtained from fungi and sponges that contain toxins that either depolymerise (latrunculus, cytochalasin) or stabilize (jasplaknolides) were the only experimental tools used to study dynamics of actin cytoskeleton and its involvement in many cellular events such as changes in cell shape, motility or polarity by dramatically disturbing its structure and organization [5]. Here we show that physical agents such as topographically modified surfaces at the micrometer range can also be used to induce alterations in the actin cytoskeleton, then changing dramatically Golgi complex morphology without adding chemical substances. Results showed that physical modifications on polymer surfaces alter internal organization of the cell thus they are a tool that can be applied to study cellular events without adding chemical agents.

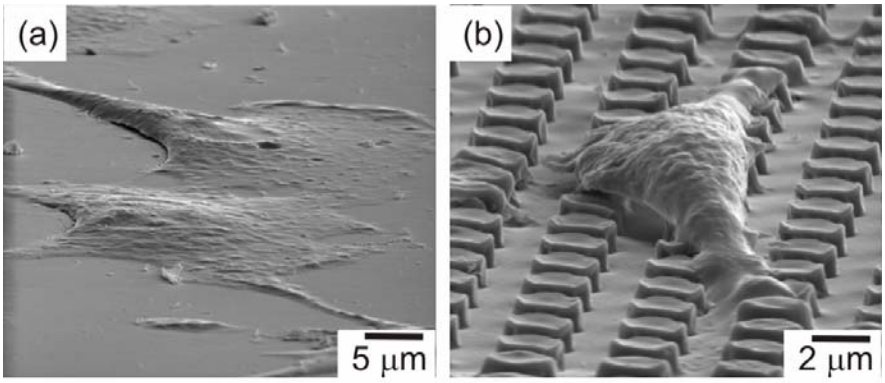

Figure 1. SEM image of NRK cells cultured on a) flat PMMA, b) posts 2 µm

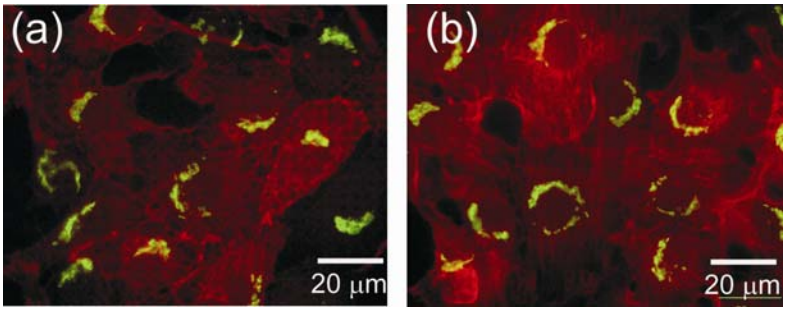

Figure 2. Double staining of actin filaments and Golgi complex of (a) sample structured with post 2 micrometer in size, (b) flat sample

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