

TIME-DEPENDENT ADHESION AND MORPHOLOGY OF OSTEOBLASTIC CELLS ON TITANIUM MODEL SURFACES FEATURING SCALE-DEPENDENT TOPOGRAPHY

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INTRODUCTION: The biological performance of implantable titanium devices in medicine and dentistry depends critically on their surface topography in the micro and nanometer range [1,2]. In order to obtain precise information about the effects of the surface topography on cell behavior, one should be able to study independently the micro topography (structures larger than 1 micron), the nano topography (structures smaller than 1 micron) and the superposition of both, without changing the chemical characteristics of the surfaces. A preferred approach is the use of model surfaces. To study the interaction of cells with titanium implants, different model surfaces presenting scale-resolved surface topography were fabricated on bulk titanium and tested using human bone-derived cells.

METHODS: First, to produce well-defined titanium microstructures comparable to cell size, through-mask electrochemical micromachining (EMM) was used. Mechanically polished titanium disks were coated with a negative photoresist. This latter was exposed using a standard UV mask aligner and developed to define the initial patterns. Anodic dissolution of titanium through the patterned photoresist was performed in a methanol-ethanol based 3 M sulfuric acid electropolishing electrolyte cooled at -10°C [3]. To minimize Joule heating, a sample holder cooled from the inside was used and the electrochemical parameters were optimized using a custom potential function [4]. Secondly, submicrometer structures were produced by two different methods: porous anodization and chemical etching [4]. The electrolyte used for the porous anodization consisted of 1 M H_2SO_4 in osmotically purified water at 25°C . The anodization procedure consisted of sweeping the potential at 20V/s from 0 V to 125 V. Chemical etching of titanium was performed according to a proprietary process of Institut Straumann AG (Waldenburg, Switzerland). It involves immersing the titanium workpiece (mechanically polished or microstructured) several minutes in a mixture of concentrated HCl and H_2SO_4 heated above 100°C .

The early phase of the cell-implant interactions was studied using a kinetic morphological analysis of adhesion, spreading and proliferation of MG63 cells. These parameters were evaluated after 4 hours, 24 hours, 3 days and 7 days of cell culture. Scanning electron microscopy (SEM) allowed the appreciation of the morphology and the proliferation of the cells. Immunolabeling of F-actin allowed the visualization of the cytoskeleton of the cells and then the appreciation of the morphology and the organization of cells in function of the underlying surface, as well as the quantification of cell proliferation. Immunolabeling of vinculin allowed the visualization of the focal contacts distribution of the cells in function of the underlying surface and then the appreciation of their adhesion capacity on the tested surfaces.

RESULTS & DISCUSSION: Optimized through-mask EMM of mechanically polished titanium surfaces enabled to produce well-defined microstructures comparable to cell size. Hexagonal arrays of closely spaced smooth hemispherical cavities of 10-100 μm diameters were made reproducibly using computer-controlled dissolution and precise charge control. Porous anodization and chemical etching, respectively, permitted to generate nano topography on flat and on previously microstructured surfaces [Figure 1].

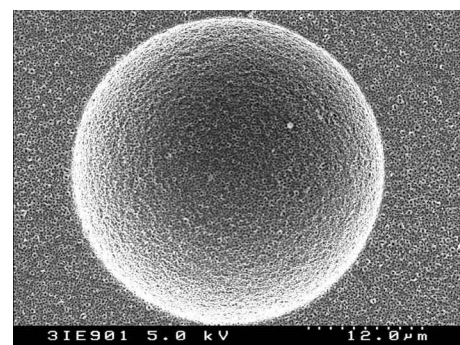


Fig. 1: SEM picture of an anodized titanium surface showing a 30 μm cavity produced by EMM and nanopores resulting from anodic oxidation. (Image courtesy of RG Richards, AO Research Institute, Davos)

Thanks to SEM and double immunofluorescent labeling of vinculin and actin, the cells were found to respond to rough nanostructured titanium surfaces by a higher cell thickness and a delayed apparition of the focal contacts. A singular behavior was also observed on the nanoporous oxide, where the cells were more spread and displayed longer and more numerous filopods than on the other nanostructures. On microstructured surfaces, MG63 cells went inside, adhered and proliferated in cavities of 30 μm and 100 μm in diameter; whereas they did not seem to react to the 10 μm cavities [Figure 2].

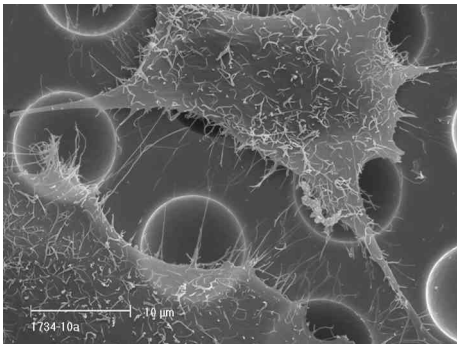


Fig. 2: High magnification SEM picture of MG63 cells cultured 3 days on smooth cavities 10 mm in diameter. Flattened cells covered the cavities, as they did not recognize them as a significant relief.

The threshold in response between 10 and 30 μm diameter cavities could be related to the size of the cells. Condensation aspects of actin cytoskeleton on cavity edges associated with vinculin-positive focal contacts were observed on all microstructured surfaces. Furthermore, cells adopted a 3D shape when attaching inside the 30 μm diameter cavities only, probably due to the curvature of the surface. Nano topography, chemical etching or porous anodic oxidation, superposed on 30 μm micro topography had little effects on cell morphology compared to flat nanostructured surfaces [Figure 3].

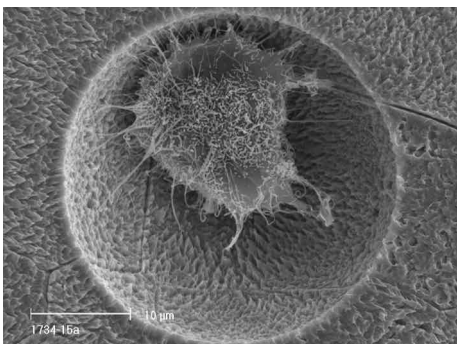


Fig. 3: SEM picture of an MG63 cell, after 3 days of culture, found inside an etched cavity 30 mm in

diameter. The cells adopted a 3D shape when attaching inside the 30 μm diameter cavities only, these latter being smooth, etched or anodized

However, a synergistic effect of nano and micro topography was observed in cell proliferation when combining nanoporous anodization and 30 μm cavities [Figure 4].

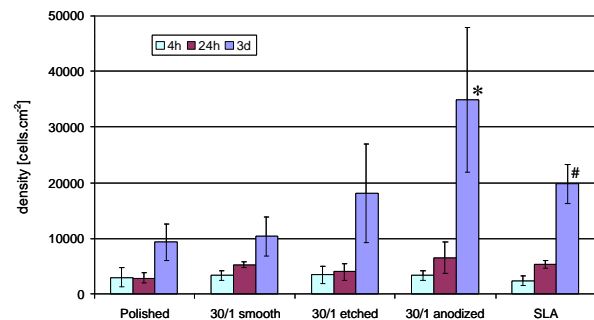


Fig. 4: Cell density on Polished, 30/1 smooth, 30/1 etched, 30/1 anodized and SLA surfaces, after 4 hours, 24 hours, 3 days or 7 days. After 3 days, the cell density on the 30/1 anodized samples is statistically significant compared to all other surfaces tested (marked by *).

CONCLUSIONS: By producing scale-resolved surface topographies on bulk titanium and using selected biological experiments, osteoblast-like cells were shown to respond to surface topography with altered morphology, proliferation and adhesion. More specifically, the behavior of the cells can be influenced differently by nano topography, micro topography or their combination.

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