Nanopyramids Reduce Inflammatory Reactions

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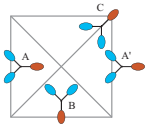
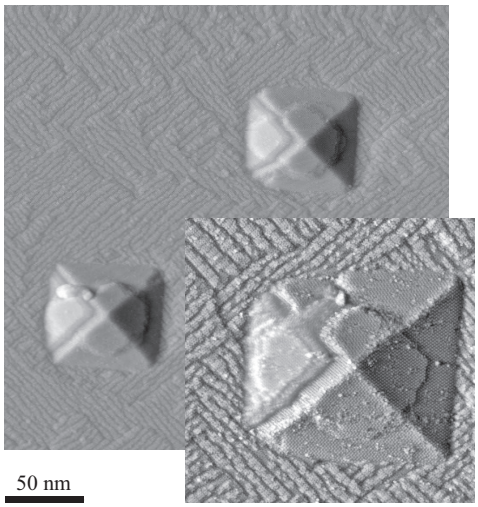
INTRODUCTION

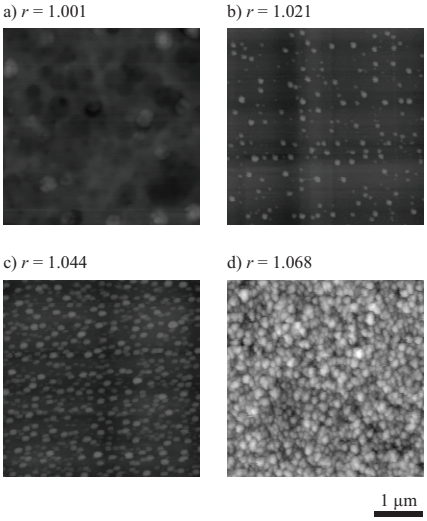


Biocompatibility is understood as surface and structural compatibility of a material integrated in the desired biological environment: The performance of an implant material does not only depend on its physico-chemical nature but also on its surface architecture or mophology. To get an insight into the response of monocytes regarding substrate nanostructures, the adsorption of respresentative proteins: γ-globulin and albumin on Ge/Si and the morphology of the monocytes on Ge/Si, including the composition of the cell culture supernatant, is investigated. In vitro assays with monocytes enable to study specific inflammatory reactions of the body, an important aspect of biocompatibility, mainly regarding rejection. The nanopyramidal surfaces allows quantifying the surface morphology by the Wenzel ratio.

SURFACE ROUGHNESS

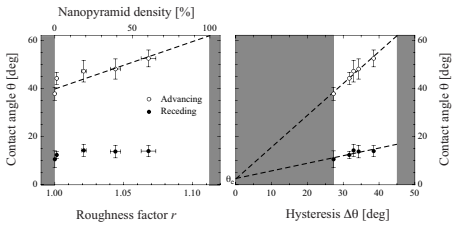


The Ge/Si-nanostructures are generate by epitaxial growth in a natural way without any lithographic technique. Germanium grown on Si(100) by molecular beam epitaxy (MBE) or chemical vapor deposition (CVD) forms well defined islands (nanopyramids) due to the lattice mismatch between both elements. The density of the pyramids is adjusted varying the growth conditions.



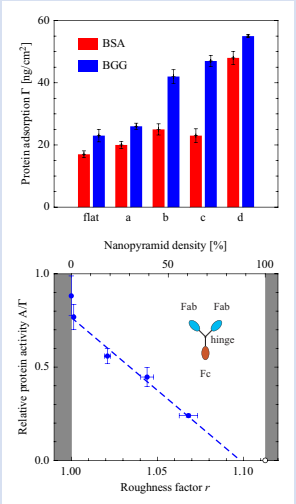
The morphology of the oxidized nanostructures is characterized by ex situ atomic force microscopy (AFM). The AFM images reveal homogeneously distributed nanopyramids of different density, which have been used as substrates for the present study. The Wenzel ratio of the CVD-grown nanostructures is indicated. The gray scale corresponds to 80 nm.

CONTACT ANGLE



Wettability of solid surfaces, as measured by contact angles, plays an important role in processes such as condensation and nucleation. The contact angle of water droplets may give an indication if cell shape and function can be altered by nanoscale roughness. Six independent measurements each on the bare Si wafer (not shown), on a 3 monolayers thick, flat germanium film on Si(100), and on the 4 nanostructured surfaces consisting of 60 angles at advancing and receding water droplets have been performed. The data, given show an increase in the hysteresis between advancing and receding contact angle, which is characteristic for the surface roughness of chemically almost identical substrates.

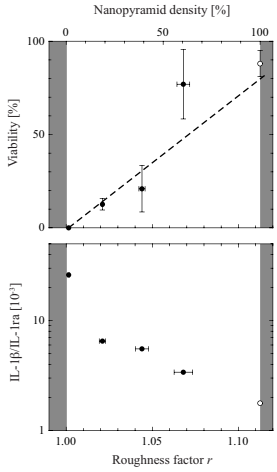
PROTEIN ADSORPTION



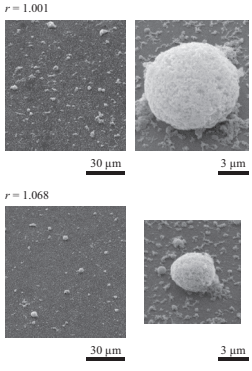
Another experiment performed for substrate characterization is protein adsorption. Combined with biological activity the protein adsorption gives rise to the biocompatible properties of the substrate. The diagram shows the adsorption of two representative proteins: FITC-marked albumin (BSA) and FITC-marked γ-globulin (BGG) on the substrates with different roughnesses. The related biological activity of BGG detected by anti-γ-globulin (anti-BGG) is given below.

MONOCYTE ACTIVATION

The biocompatible properties of the Ge/Si substrates with their different roughness on the nanometer scale are characterized by the attachment and activation of monocyte-like cells from the cell line U937. The number of viable cells is a result of both cell proliferation and activation. During activation cytokines are expressed including the highly cell-toxic protein TNF-α. Therefore, low viability means high activation. The density of cells after 5 days was about 1.5 x 10^6 per mL for all samples. The initial density of cells was determined to (4.2 ± 0.3) 10^5 per mL.



In addition, the amount of IL-1β, one of the most crucial cytokines in inflammatory reactions, decreases with pyramid density. Even more important for the inflammatory behavior is the ratio of the cytokines IL-1β and its receptor antagonist IL-1ra. This ratio exhibits a more than exponential decay with the roughness factor. This behavior can be qualitatively supported by electron microscopy images of the cells and their fragments. The figure below represents images of the substrate with the lowest and highest density on different scale showing an increased amount of cell fragments and more expanded cells.



CONCLUSION AND ACKNOWLEDGEMENT

Epitaxial growth of germanium on Si(001) yields a natural way to tailor nanopyramids of identical shape with different density and without the use of any lithographic technique. Counting the nanopyramids, the effective surface and the roughness factor (Wenzel ratio) are determined with high precision. Since the study is focused on dome clusters with facets, which form an angle of about 26° with the substrate, the roughness factor can be varied between 1.0000 and 1.1126. The nanopyramids give rise to a strong interaction between BSA and BGG on one hand and the substrate on the other hand, changing the protein conformation. BGG adsorbed on the nanopyramids becomes inactive. Related experiments with monocyte-like cells reveal a strong correlation between nanopyramid density and cell viability as well as protein release responsible for inflammatory behavior. Along these lines, features on the nanometer scale such as nanopyramids reduce the inflammatory reactions of various implants applying structural compatibility.