Structured titanium surfaces with regular geometry prepared with different technologies and their influence on MG-63 cell morphology

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Introduction

Because of its outstanding material properties like high corrosion and mechanical stability at a relatively low density, titanium is often used as implant material, especially for bone replacement. It is commonly known and applied in practice that a rough implant surface forces the process of its integration in the human body. But the reasons for this topography effect still remain unclear. A lot of publications in the last years are dealing with this problem [1,2]. While stochastic roughened surfaces are predominantly relevant in practice because they can be produced with relatively low costs it seems to be useful to investigate the relations between the material surface and the biosystem with the help of structured surfaces with regular geometry. In the latter case the structure is limited to few structure elements with only few parameters that can vary. This makes it easier to study the influence of the material surface on the cell behavior by stepwise and systematic variation of the structure parameters or to find critical values of structure parameters that can influence cell reaction [3,4].

Material and methods

In our work we tried to prepare large-area titanium samples (10x10 mm^2) with simple pillar structures in the µm range (2-5 µm). In contrast to stochastically structured titanium surfaces it is rather intricate to structure it in a direct and defined manner. That's why at first we went a way round by structuring alternative substrate materials and sputter coating them with titanium. The first substrate material we used was SU-8 (MicroChem), a negative photo resist that can be simply structured by a photolithographic process (fig. 1a). The other substrate material was Silicon that can be structured with common used methods of the semiconductor technology like f.i. reactive ion etching (fig. 1b), [5]. Furthermore in a third step we applied a modificated dry etching process for structuring bulk titanium in the direct way (fig. 1c). Flat silicon samples sputter-coated with 100 nm tianium (not shown) were used as reference sample in cell biological tests.

After preparing the surfaces they were characterized with scanning electron microscopy (FE-SEM Supra 25, Zeiss) and energy dispersive X-ray spectroscopy (EDX) (Quantax, XFlash 3001, Bruker AXS). EDX was predominantly used for the titanium coated material to check if the coating especially at the side walls of the pillars was complete. Furthermore the samples were characterized by electrochemical methods (results not shown). In vitro cell biological tests were made with human osteoblastic cells (MG-63, ATCC). The cells were cultured in DMEM with 10 % FCS at 37 °C and 5 % CO2 and after 24h cell culture they were fixed, dried supercritically and visualized by SEM in the low voltage mode (1-3 kV) without common used gold coating.

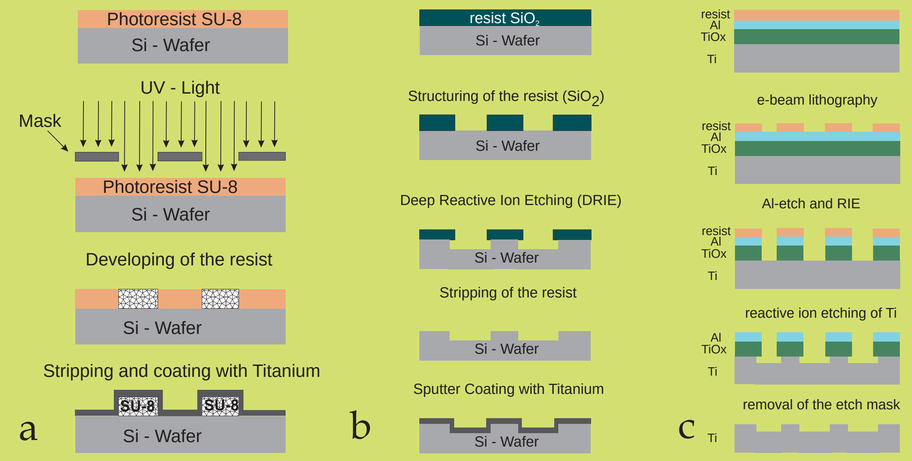


Figure 1: schematic diagram of process step sequence for the production of the samples in three technologies: a) structured photoresist coated with 100 nm Ti, b) structured Si coated with 100 nm Ti, c) structured bulk Ti

Results and discussion

Depending on the preparation technology we got a different quality of the expected pillar structures shown in figure 1. As you can see in figures 2 a-c (above row), all methods delivered a uniform pillar array, but in higher magnification (lower row) there can be observed some differences between the samples caused by the preparation process. The pillars prepared with the first SU-8 based method (figure 2 a) show rounded edges in contrast to the pillars etched in Silicon (figure 2 b). Both samples show a fine structure of the surface especially on the top of the pillars caused by the sputtered titanium film. With EDX (figure 3) we could show that the covering of the side walls with titanium especially at high aspect ratios can be problematic. On the top of the pillars etched in bulk titanium (figure 2 c) you can see a fine structure caused by the polishing pre-treatment. There are also some additional artefacts like sloped edges and side walls and a remaining roughness at the bottom between the pillars.

The results of the cell biological tests are shown in Figure 4. In lower magnification only few differences between the samples could be observed. The MG-63 cells are well spread on the top of the pillars. But the morphology of the cells is adapted to the pillar structure. They grow in the two main directions parallel to the side walls of the pillars. In contrast to the titanium coated samples (figure 4 b,c) it seems that the area of the cells on the structured titanium sample (figure 4d) is greater but the cells are not so widespread as on the reference sample (figure 4a). Some details of cell morphology are shown in the lowest row in high magnification. It can be observed that filopodia of the cells mostly attach to the pillar edges.

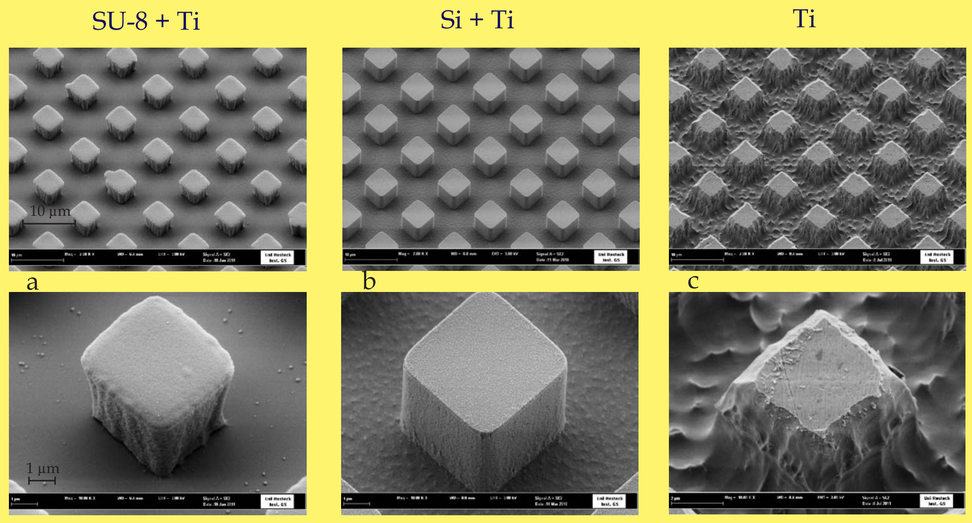
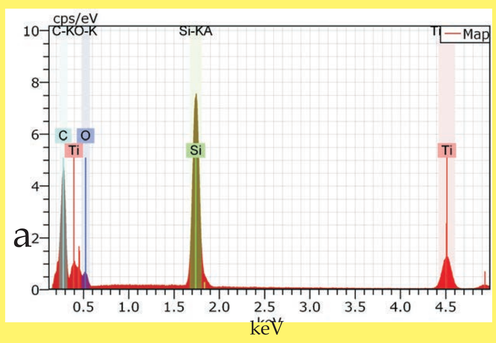
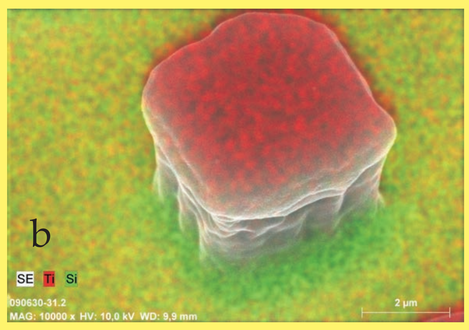


Figure 2: SEM micrographs of the material surface of samples (pillar arrays with requested dimensions: 5x5x5 $µm^3$ cubic pillars in a distance of 5 µm) produced in three technologies: a) structured photoresist coated with 100 nm Ti, b) structured Si coated with 100 nm Ti, c) structured bulk Ti, in two magnifications, respectively





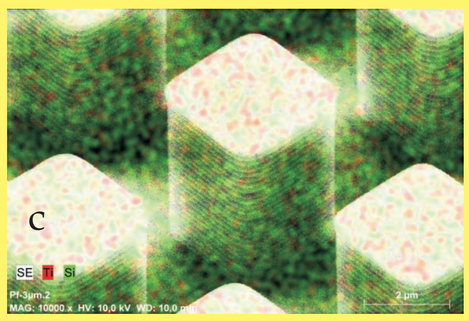


Figure 3: Investigation of the coating of titanium coated samples with EDX: a) measured spectrum of a sample produced with SU-8 technology; b) EDX-mapping of titanium (red) and silicon (green) of the same SU-8 sample (5x5x5 $µm^3$ pillar array); c) dry etched silicon sample sputter-coated with titanium (3x3x5 $µm^3$ pillar array). Note that at the side walls only insufficient titanium could be detected. Especially at the side walls of the high aspect ratio pillars shown in c) (distance between the pillars 3 µm, height of the pillars 5 µm), there could be found only few titanium

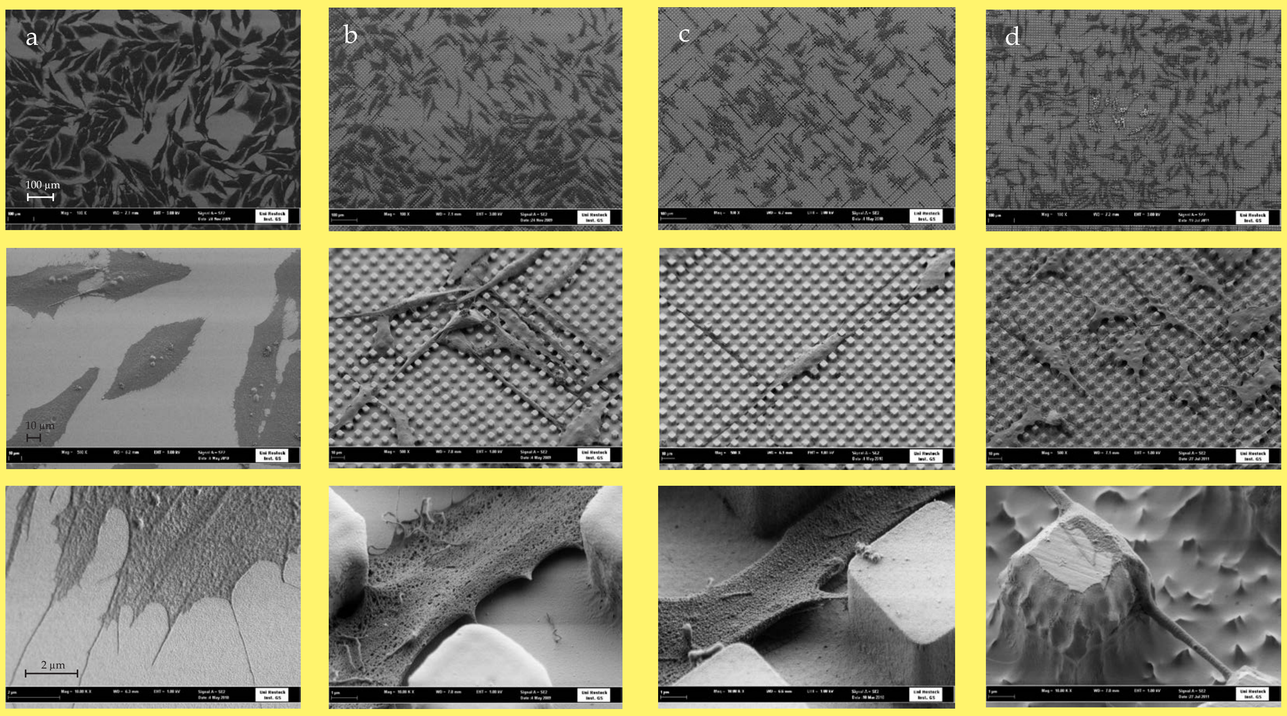


Figure 4: SEM micrographs of MG-63 osteoblast cells grown for 24 h on samples shown in figure 2 in comparison to a) reference sample (100 nm Ti sputtered on a flat silicon substrate); b) structured photoresist coated with 100 nm Ti; c) structured Si coated with 100 nm Ti; d) structured bulk Ti; in three magnifications, respectively

Conclusions and Summary

We could show that the production process influences the quality of the samples. The requested dimensions of the pillar structures not always could be achieved. Especially the edge radius and the fine structure differ from sample to sample. Problems with an insufficient titanium covering detected by EDX can be avoided by etching titanium in a direct way. But this technology has to be further optimized to avoid the described artefacts. At a first glance there are only few differences in cell morphology depending on the preparation technology but this fact has to be investigated in more detail in future. In comparison to flat titanium coated samples it seems there is an influence of the pillar structure on the cell behaviour as can be observed especially at high magnification.

References

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