Titanium Nanostructured Surfaces Fabricated by Glancing Angle Sputter Deposition: Anti-bacterial Activity (Cicada Wing Effect) and Influence on Human Mesenchymal Stem Cells

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Disclosures:

Introduction: The development of biomaterials with bacteriophobic surfaces which hinder or prevent bacterial colonization and proliferation is still an important challenge in biomaterial research. Very recently an example for an antibacterial effect based solely on a special physical surface structure has been found in locust-like insects [1]. The wings of the Clanger cicada (Psaltoda claripennis) are covered by a periodic topography consisting of hexagonal arrays of nanopillars which are able to destroy gram-negative bacteria by a physico-mechanical effect solely due to the presence of the nanostructures. In this study we aimed to implement the “Cicada wing effect” for the metallic biomaterials by fabrication of titanium nanostructures using glancing angle sputter deposition (GLAD). This effect was proved by analyzing bacteria and tissue cells on Ti GLAD nanostructures, and the results were compared with a dense Ti thin film.

Methods: GLAD is a physical vapor deposition method to fabricate nanostructured thin films. In this method, an obliquely incident particle flux $\beta$ (usually $\beta \approx 80^\circ$, as measured to the substrate normal) facilitates growth of columnar structures as a result of strong influence of the self-shadowing mechanism. The substrate was rotated (25 rev/min) in order to form vertical columnar structures. A dense thin film reference sample was produced by conventional sputter deposition. The wettability of the samples was analyzed by water drop contact angle measurement.

To investigate the antimicrobial activity of Ti nanostructures on gram-positive S. aureus as well as a gram-negative E. coli test specimens were incubated with $10^6$ bacteria per ml by dilution with fresh BHI broth. For analysis of the cell adherence the plates were incubated for one and three hours at 37°C. Subsequently, the suspension was removed and the specimens were stained with BacLight to analyze the bacterial adherence and viability. In order to prove the bacterial adhesion on the nanostructured surfaces the scanning electron microscopy (SEM) was employed.

To investigate the influence of Ti nanostructures on human cells, hMSCs were cultured (1x10^4 per cm^2) on the specimens for 2 days and cultured in RPMI/10% FCS at 37°C. Adherence and morphology of the incubated cells were analyzed using calcein-AM and propidium iodide (PI) fluorescence staining. The adherent cells were analyzed by fluorescence microscopy.

Results: Nanostructured samples exhibited columnar structures with an average height of 478 ± 6 nm and randomly oriented faceted sharp-edged peaks (mean peak distance 158 ± 105 nm, roughness Rq = 26 ± 3 nm). In contrast, thin film control samples exhibited a dense compact surface (63 ± 3 nm thickness, Rq = 3.0 ± 0.2 nm). Contact angle measurements showed hydrophilic surfaces either for the nanostructured sample and the dense film reference sample. In this study, bacterial adherence of S. aureus and E. coli were not significantly different after one hour of incubation for the dense thin film as well as the nanostructured surfaces. In contrast, after a prolonged incubation time of three hours, bacterial adherence and viability of E. coli was significantly decreased on the nanostructured film compared to the dense samples. However, the adherence and viability of S. aureus was not different after 3 hours of cultivation between the nanostructured and dense thin film, with respect to the results after 1 hour of incubation. The rod-shaped E. coli attached to the surface of the nanostructured film exhibited an irregular morphology (Fig. 1B) in contrast to the observed morphologies in the dense film (Fig. 1A). As shown in Fig. 1B, the nanostructured surface making substantial deformation of E. coli (Fig. 1B, white arrow). The morphology of spherical-shaped S. aureus after the attachment on the nanostructured surface (Fig. 1D) was not affected and was similar to the S. aureus cultured on the dense thin film surface (Fig. 1C).

To investigate the impact of nanostructured Ti surfaces also on eukaryotic cells, the cell adherence, spreading and viability of hMSCs (typical fibroblast-like cells) cultured on the nanostructured film and the dense thin film (as a control) were evaluated. After 2 days of cell culture, a layer of adherent and viable hMSCs were observed on the dense thin film and the nanostructured surface.

Discussion: A natural nano-columnar surface architecture was recently identified that exert an antibacterial effect by a physical-mechanical mechanism [1]. Here we have been able to produce an antibacterial effect with a Ti nanostructure made with physical vapor deposition. These fabricated metallic surfaces showed similar antibacterial effects as found in nature. Similar to...
the publications on the natural Cicada wings we have also observed a selective antibacterial effect on E. coli but not on S. aureus. Since the mechanism of killing is obviously related to physical forces which disrupted the cell wall of attached bacteria to the nanopillars a reasonable explanation would be the difference in the mechanical stabilities of both bacterial classes. Additionally, the contact area of the spherical S. aureus is smaller compared to the rod-shaped E. coli. Furthermore, the structural process of cell division is different between both species. In conclusion the size, shape and membrane rigidity of bacteria and the organization and shape of the nano-features play a major role in bacterial attachment and viability. Besides antibacterial activities, we have shown that the adherence and viability of human MSCs on the nanostructured surface and the dense thin film were similar.

**Significance:** The structure-related antibacterial Cicada wing effect is limited to gram-negative bacteria, therefore clinical applications with gram-negative infections should be addressed. Nevertheless, we have shown that the selective antibacterial effect of the Cicada wing could be transferred to a metallic biomaterial by mimicking the natural topographies, thereby; this study demonstrates a successful example of bionic engineering.

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