Multifunctional titanium-based biomaterials – advantages of nanostructures in metallic implants

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INTRODUCTION

Among the biomaterials used for bone or dental implants, the most commonly used are titanium-based[1]. They are biocompatible and relatively resistive to corrosive conditions; however, the fast and firm connection between the implant and surrounding tissue is problematic[2]. The presence of titanium dioxide on the surface allows such osseointegration, which is why the preparation of Ti/TiO_2 material is of great importance. One of the methods used in its synthesis is anodization, which allows the formation of a nanostructured oxide layer with different geometric parameters[3]. Due to the presence of porous structure, such materials may be modified with different antibacterial and antiinflammatory substances (e.g., nanoparticles[4] and drugs[5]), providing multifunctionality of the scaffolds. Moreover, recently 3D-printed scaffolds are being modified with the means of anodization, which gives scientists increased possibilities in designing individual implants[6].

METHODOLOGY, RESULTS & CONCLUSIONS

Ti substrates → anodization process

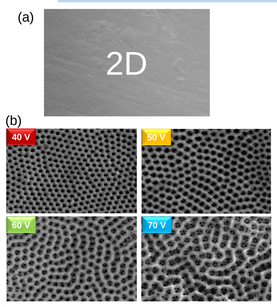

Fig. 1. (a) FE-SEM microphotographs of polished Ti foil; (b) FE-SEM images of TiO_2 nanostructures on Ti foil formed via three-step anodization process in an ethylene glycol-based electrolyte with NH_4F and H_2O under constant voltages of 40, 50, 60 or 70 V.

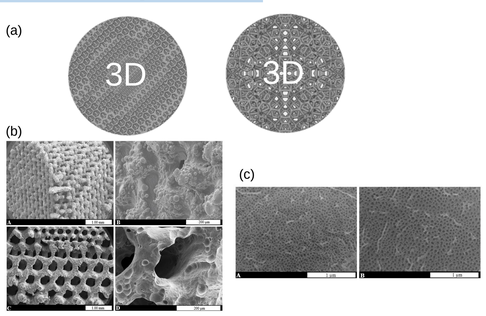

Fig. 2. (a) CAD models of 3D titanium substrate with gradiently and randomly distributed micropores; (b) FE-SEM images of 3D Ti substrate after the post-treatment; (c) FE-SEM images of TiO_2 nanostructures formed via one-step anodization process in an ethylene glycol-based electrolyte with NH_4F and H_2O under constant voltages of 40 V for 5 minutes.

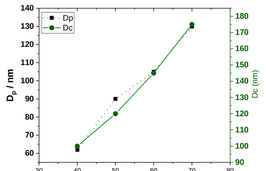

Fig. 3. Graph showing linear dependency between applied voltage during anodization proces and the pore diameter (D_p) and interpore distance (D_c)[3].

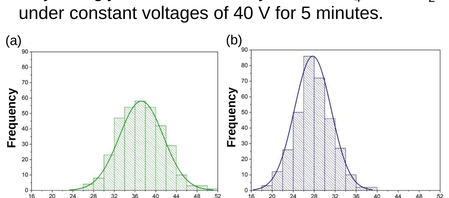

Fig. 4. Distribution of pore diameters for 3D Ti samples with pore distribution: (a) gradient and random (b), anodized under 40 V for 5 minutes.

Physicochemical characterization of tested samples

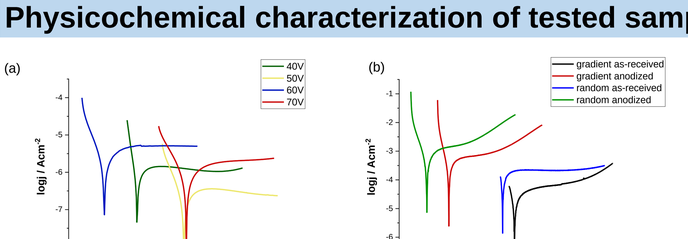

Fig. 5. Tafel plots for the 2D anodized samples (a) and 3D as-received and anodized samples (b) recorded in the phosphate buffer solution at 37 °C.

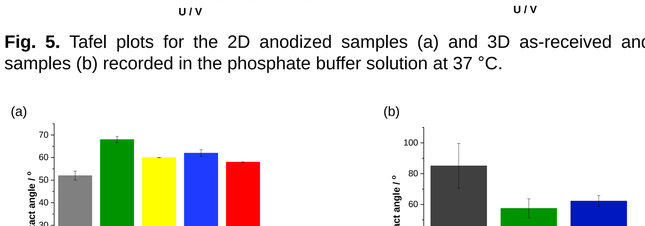

Fig. 6. Water contact angle measurements for (a) 2D as-received and anodized Ti samples; (b) 3D as-received and anodized Ti samples.

Modifications of nanostructured TiO_2 samples – towards multifunctionality

- **with nanoparticles – antimicrobial surfaces**

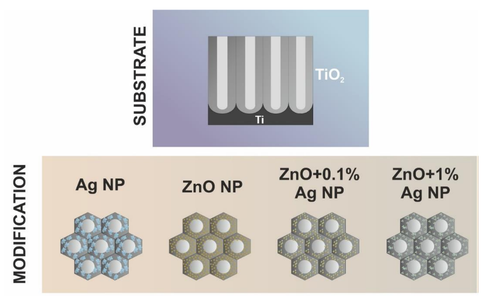

Fig. 7. Schematic representation of nanostructured titanium dioxide modified with different types of nanoparticles with potential antibacterial properties.

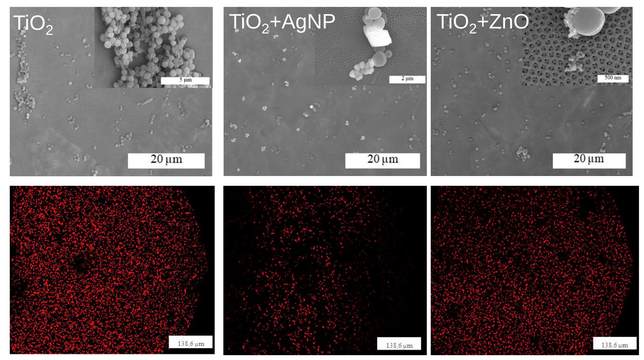

Fig. 8. FE-SEM (top raw) and fluorescent (bottom raw) microphotographs of unmodified and modified TiO_2 layers colonized by *Staphylococcus aureus* for 2 h.

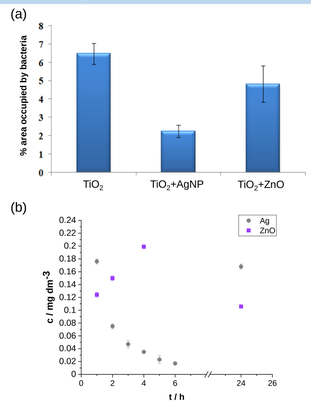

Fig. 9. (a) Area occupied by bacteria S. aureus after 2 h of incubation on unmodified and modified nanostructured TiO_2; (b) concentration of the released Ag and Zn from the modified samples at different time points. The release process was made in the PBS solution with pH = 7.4 and at 37 °C.

- **with drugs – Drug Delivery Systems (DDS)**

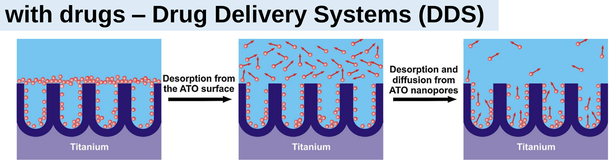

Fig. 10. Schematic representation of the desorption-desorption-diffusion release model from nanostructured TiO_2[5].

Table 1. Summary of the total drug (ibuprofen) loaded inside nanostructured TiO_2 formed on 2D and 3D samples.

	3D		2D
	non anodized	anodized	anodized
Mass of ibuprofen [mg]	106.75 ± 16.82	143.01 ± 3.05	1.23 ± 3.40

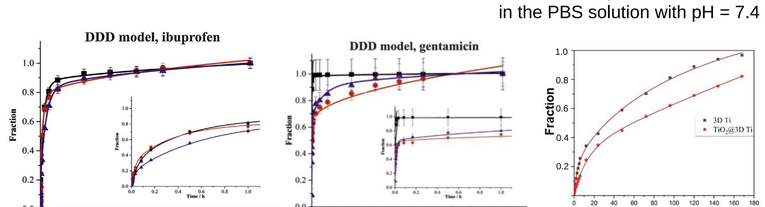

Fig. 11. Release profiles of (a) ibuprofen and (b) gentamicin for 2D nanostructured TiO_2 samples with different crystalline structure.[5] (c) Release profiles from as-received and anodized 3D samples. To all release profiles, the DDD kinetic model was fitted.

Biocompatibility of nanostructured TiO_2 samples

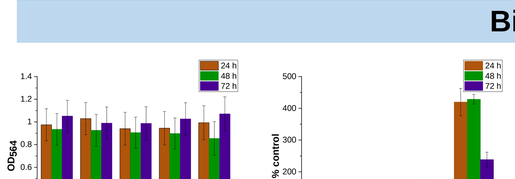

Fig. 12. (a) MTT test results after 24, 48 and 72 h of incubation of MG-63 cells on ATO layers. Plots show the optical density (OD) at the characteristic wavelenght for titanium samples anodized at different potentials. Each measurement was done in triplicates. (b) Adhesion of MG-63 cells on ATO layers after 24, 48 and 72 h of incubation. Results are shown as the % of the control (PS) for the cells stained with crystal violet.

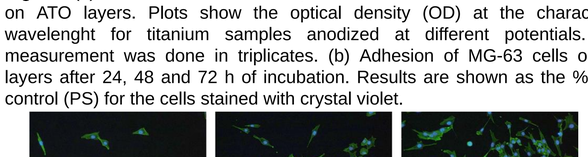

Fig. 13. Fluorescence microscope images of nucleus (blue) and cytoskeleton (green) stained cells after 24. 48 , and 72 h of culturing on ATO layers (40 V).

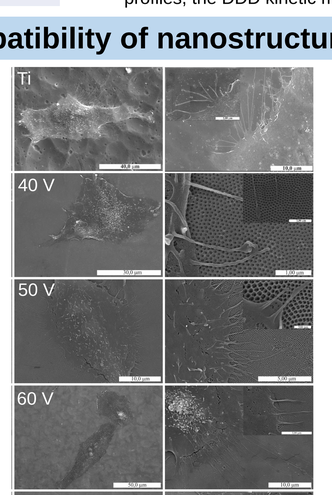

Fig. 14. FE-SEM microphotographs of MG-63 cells amd close ups of filopodia after 24 h incubation on polished Ti and ATO layers fabricated with anodization process under 40, 50, 60, and 70 V.

Conclusions

- anodization is a simple and cost-effective method of fabrication of TiO_2 nanostructures on different titanium-based materials, both two- and three-dimensional;
- corrosion resistance of pure Ti and covered with TiO_2 is similar, though further studies must be provided in order to determine corrosion behavior in details;
- due to the nanostructured morphology, such layers may be enriched with antibacterial (nano)particles that possess antibacterial properties;
- anodic titania nanostructures, both 2D and 3D, may be efficiently used as drug delivery carriers; the release kinetics is described by the desorption-desorption-diffusion model;
- nanostructured anodic TiO_2 is not cytotoxic;
- the adhesion of MG-63 cells is enhanced on the nanostructured surfaces but it depends on the pore diameter of TiO_2; cells are well spread and filopodia are well developed on nanostructured surfaces of TiO_2;

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