Microbial and cell growth on nanoporous anodic titanium oxide (ATO) layers

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1. Anodic titanium oxide preparation

The most commonly used materials for bone-implants are titanium and its alloys. It is due to their good biocompatibility, high strength to weight ratio and excellent corrosion resistance. Such materials are therefore widely used in orthopedic, dental and other implants, as well as in medical devices. Unfortunately, there are some drawbacks connected with titanium implants, e.g. their inertness and long-term osseointegration via natural oxide (TiO_2) existing on the surface. Nanoporous anodic titanium oxide (ATO) layers on Ti formed by electrochemical anodization have been proposed as a potential nanostructured material for bone implants. The main advantage of such materials is direct growth of TiO_2 on Ti surface, using a simple and cost-effective method such as anodic oxidation. Anodization allows to precisely control nanopore size and structure porosity. The presence of nanoporous layer guarantees an excellent inertness of its surface, allowing it to readily heel into the bone tissue.

1a

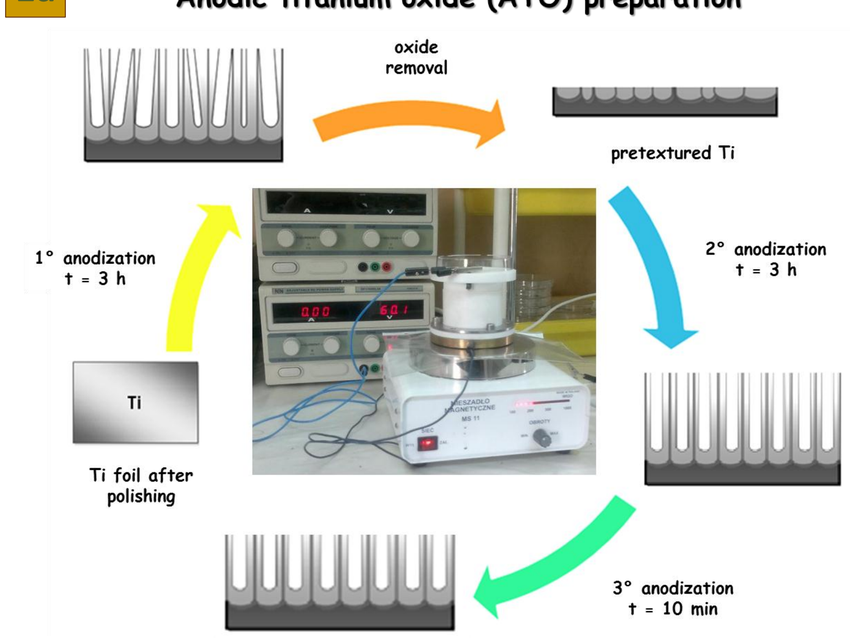
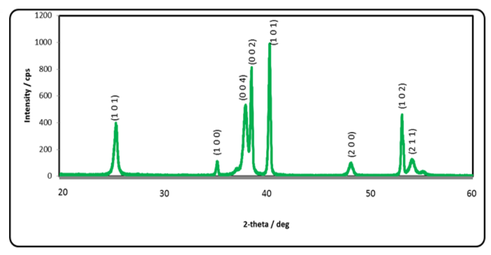


Fig. 1a. Schematic procedure of TiO_2 preparation. Anodic titanium oxide templates were prepared via three-step anodization under a constant voltage of 40 V in an ethylene glycol solution containing NH_4F (0.38 wt. %) and H_2O (1.79 wt. %) at 20 °C.

1b

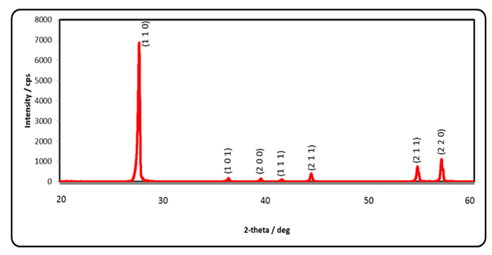
ANNEALING

400 °C anatase



2-theta(deg)	d (A)	FWHM(deg)	Phase name
25.517(4)	3.4879(5)	0.350(4)	Anatase, syn, (1,0,1)
35.281(5)	2.5418(3)	0.192(4)	alpha-Ti, (1,0,0)
37.25(3)	2.4116(16)	0.94(3)	Anatase, syn, (1,0,3)
37.994(4)	2.3663(2)	0.433(4)	Anatase, syn, (0,0,4)
38.593(2)	2.33093(12)	0.1935(17)	Anatase, syn, (1,1,2), alpha-Ti, (0,0,2)
40.360(2)	2.23291(10)	0.1904(17)	alpha-Ti, (1,0,1)
48.258(8)	1.8843(3)	0.422(10)	Anatase, syn, (2,0,0)
53.1618(16)	1.72145(5)	0.1644(16)	alpha-Ti, (1,0,2)
54.141(7)	1.6926(2)	0.551(7)	Anatase, syn, (1,0,5)
55.266(17)	1.6608(5)	0.52(3)	Anatase, syn, (2,1,1)

1000 °C rutile



2-theta(deg)	d (A)	FWHM(deg)	Phase name
27.6673(12)	3.22154(14)	0.236(3)	Rutile, syn, (1,1,0)
36.282(3)	2.4740(2)	0.198(3)	Rutile, syn, (1,0,1)
39.405(4)	2.2848(2)	0.198(3)	Rutile, syn, (2,0,0)
41.444(4)	2.1770(2)	0.202(5)	Rutile, syn, (1,1,1)
44.270(3)	2.04430(11)	0.180(2)	Rutile, syn, (2,1,0)
54.5187(19)	1.68177(5)	0.1708(19)	Rutile, syn, (2,1,1)
56.8329(14)	1.61866(4)	0.1566(13)	

Fig. 1b. XRD data after annealing nanoporous TiO_2 in two different temperatures: 400 °C (anatase) and 1000 °C (rutile). XRD measurements confirmed presence of both anatase and rutile phases.

2. Cell growth on as received ATO layers

2a

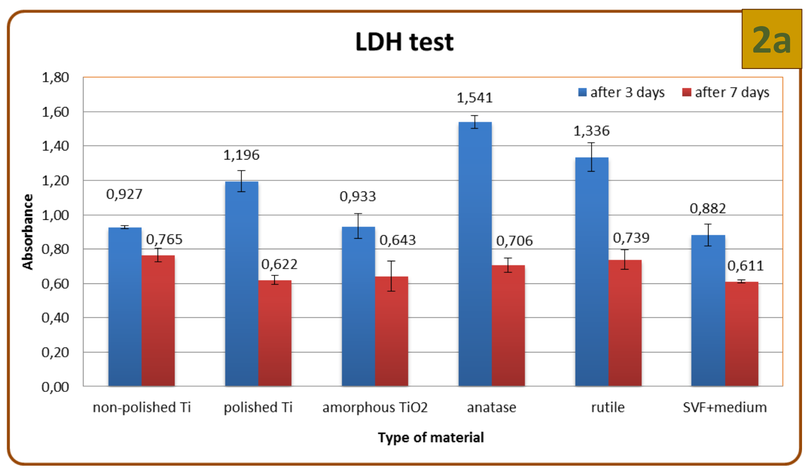
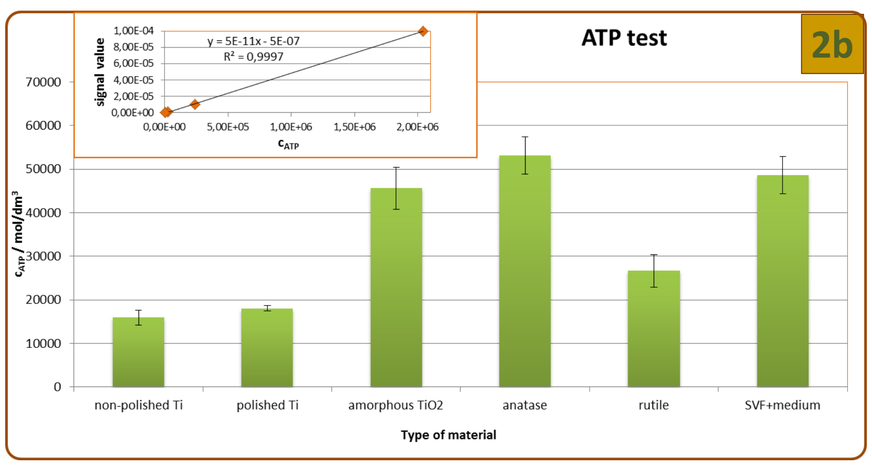


Fig. 2a. Diagram of LDH activity of cells on different surfaces. Activity was measured in 3rd and 7th day of the experiment.

Fig. 2b. Diagram of ATP production. Cells were seeded onto different surfaces. Inplot shows a calibration plot of standard ATP solution.

2b



There are two main aspects connected with bone-implants, osseointegration and risk of post-operative infections. The osseointegration, especially its speed, is a major factor for implant success. The surface topography and surface chemistry are crucial for the short- and long-term success of the osseointegration process. In terms of biomaterial development and implant technology, the cellular response can be affected by topographical structure of the surface. It has been proven that cells sense and react to nanotopography in vitro by exhibiting changes in cell morphology, orientation, cytoskeletal organization, proliferation, signaling and gene expression. Adipocyte derived stem cells obtained from abdominal liposuction were seeded onto Ti foils and nanoporous TiO_2 surfaces. Alkaline phosphatase (ATP) production was assessed using the ATPLite™ kit. For the cytotoxicity determination, the CytoTox96® Radioactive Cytotoxicity Assay was used.

3. Microbial growth on as – received ATO layers

3a

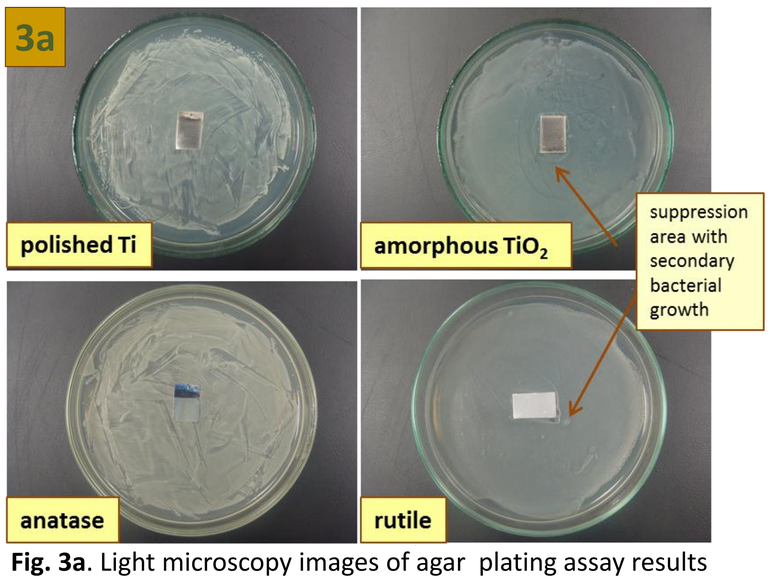


Fig. 3a. Light microscopy images of agar plating assay results after 24 h of incubation for Ti foil and TiO_2 nanostructures. Images show samples that were examined against *Staphzlococcus aureus* bacteria. All samples were put parallel to the surface.

One of the most serious side effects connected with implant surgeries is a very high risk of post-operative infection. It is well-known that formation of biofilms by human pathogenic bacteria on medical titanium-based implants can be dramatic, leading to failure of the devices and resulting in the necessity of implant removal. Strains of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* are reported to be significant contributors to infections associated with orthopedic implants. Hence, finding materials that are less adherent to bacteria, yet good for cell growth are of great importance.

Staphylococcus aureus bacteria were used for examining an antimicrobial character of Ti foil and nanoporous TiO_2 surfaces on Ti. The evaluation of the inhibition of microorganisms growth and possibility of a biofilm formation on various nanoporous TiO_2 surfaces were examined. The cultivation of microorganisms on broths was carried out in incubators Mermmet at 37 °C for 24 h. For biofilm formation tests, Ti foil and nanoporous TiO_2 surfaces were placed in liquid broths with suspension of microorganism. The cultivation at 37 °C were continued for 5 days. After that time the occurrence of biofilm on plates was investigated with a microscopic and UV radiation methods.

3b

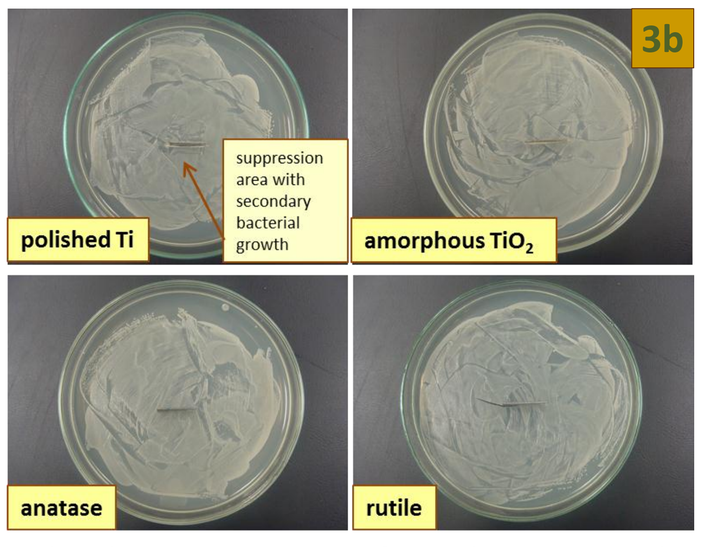


Fig. 3b. Light microscopy images of agar plating assay results after 24 h of incubation for Ti foil and TiO_2 nanostructures. Images show samples that were examined against *Staphzlococcus aureus* bacteria. All samples were put vertically in microbiological base.

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