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Corrosion, antibacterial activity and haemocompatibility of TiO_2 nanotubes as a function of their annealing temperature

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ABSTRACT

The present work reports on the corrosion behavior and antibacterial activity of self-organized TiO_2 nanotubes in dependence of annealing temperature. TiO_2 nanotubes are grown by anodic oxidation of Ti and are further annealed (350–750 °C). Besides the morphological and compositional changes due to annealing, the corrosion resistance and antibacterial activity are significantly improved. Namely, antibacterial activity of 650 °C and 750 °C annealed nanotubes shows the best results for *Staphylococcus aureus* and *Pseudomonas aeruginosa,* with 40% and 80% increase in the growth inhibition index, respectively. Furthermore, such nanostructures are non-haemolytic and present enhanced haemocompatibility compared to amorphous or lower-temperature annealed nanotubes.

1. Introduction

Over the past years, Ti and Ti alloys have been extensively used as implant materials. This is due to the native protective oxide layer (TiO_2) formed on its surface [1,2] that leads to remarkable corrosion resistance and biocompatibility. Furthermore, the formation of nanostructures on the surface of Ti and its alloys can enhance their biocompatibility and osseointegration, nano-architectures such as nanotubes [3–5] or nanowires [6].

The high specific surface area of nano-architectures combined with a good control over their nanotopography expanded their potential use in the bio applications field [3,7]. The advantage of nanostructures obtained via electrochemical anodization, besides the good control over morphology and ease of application, is the reduction of the delamination tendency (as structures are directly coated onto the metal) [3,8]. Additionally, TiO_2 nanostructured materials have shown a positive effect on the cellular response, i.e. enhancing adhesion, proliferation and differentiation for a large variety of cells (including osteoblast, osteoclast, stem cells, etc.), thus promoting osseointegration [5,9–11]. Moreover, nanostructures can be easily obtained on Ti alloys (TiTa alloys, TiMo alloys, Ti6Al7Nb, TiZr alloys, etc.) [3]; for example, nanotubular structures on Ti50Zr alloys maintained a good biocompatibility while significantly enhancing the antibacterial efficiency [12]. The photoactivated antimicrobial activity of TiO_2 and their applications and limitations have been investigated as well [13].

Generally, as-formed nanotubular structures are amorphous and can be converted to a crystalline structure (either anatase or anatase–rutile mixtures) by annealing; it was also shown that the crystallinity of TiO_2 nanotubes has a positive effect on their bioperformance [14,15]. Still, a wide range of annealing treatments was not investigated, especially as the resulting crystallinity and the morphology of annealed nanotubes are directly dependent upon both annealing temperature and nanotube morphology. The crystallization of amorphous nanotubes to anatase occurs at ≈300 °C, while rutile is observed starting at ≈450 °C [14,15]. By further increasing the annealing temperature, the rutile amount increases as well; moreover, the growth of the rutile layer starts from the metal substrate and through the bottom of the nanotubes, leading to a sintering of the nanotubular structure [16–18]. Annealing of TiO_2 nanotubes leads to a better performance of nanotubes and

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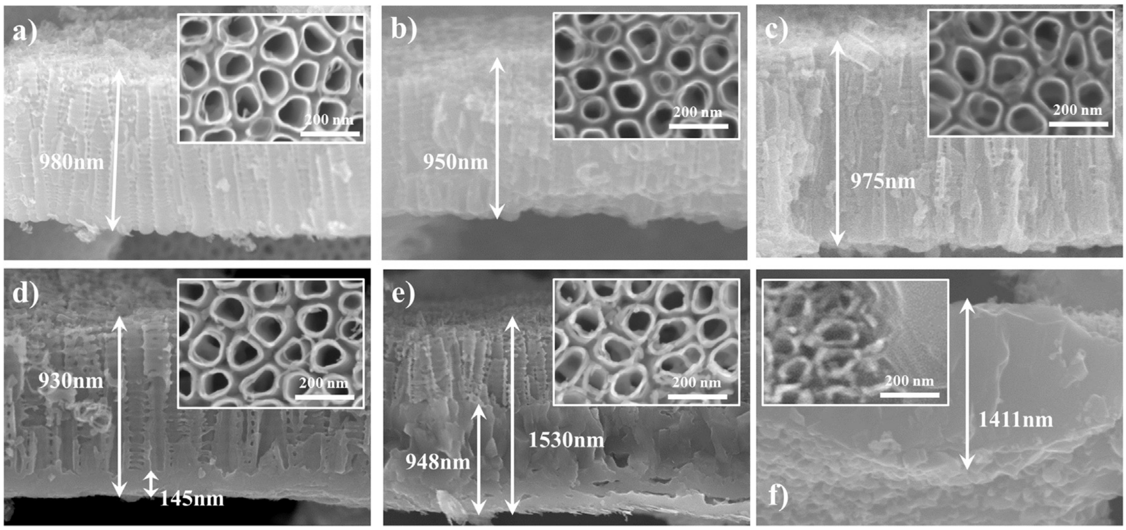

Fig. 1. Cross-section and top-view (inset) SEM images of TiO_2 nanotubes: a) amorphous and annealed at a temperature of: (b) 350 °C; (c) 450 °C; (d) 550 °C; (e) 650 °C; (f) 750 °C.

to increased photoelectrochemical properties, corrosion resistance, biocompatibility etc. [3].

In this approach, the present work consists in the elaboration of nanotubes via electrochemical anodic oxidation of Ti foil and followed by annealing at different temperatures, in order to obtain different crystallinity contents (anatase, mixture of anatase/rutile and predominantly a rutile phase). The morphology, chemical and phase composition of as-formed and annealed TiO_2 nanotubes were evaluated and the influence of the annealing treatment was correlated with the electrochemical stability. Furthermore, the comparison was extended for antibacterial inhibition activity as well as for haemocompatibility, as important aspects in regard of bioapplications.

2. Experimental

2.1. Nanotubes growth

TiO_2 nanotubes were obtained on Ti substrate (0.1 mm thickness, 99.6% purity) by electrochemical anodization in a 62:38 vol.% Glycerol: H_2O + 0.5 wt.%NH_4F electrolyte, using a two-electrode configuration with platinum as cathode and Ti substrate as anode. The exposed surface of the Ti sample was 1 cm^2. The cell voltage was sweeped from 0 V to 20 V with 0.1 V/s, and then kept constant at 20 V for 2 h. The as-grown TiO_2 nanotubes were then annealed in a furnace at different temperatures (350, 450, 550, 650 and 750 °C) for 2 h.

2.2. Morphology and composition characterization

A field-emission scanning electron microscope (Hitachi FE-SEM S4800) equipped with an energy dispersive X-ray (EDX) analyzer was used to investigate the morphology and composition of the as-formed and annealed samples. Cross-sections images were obtained from mechanically scratched samples.

2.3. Crystal structure characterization

The crystallinity of the prepared samples was investigated by X-ray diffraction analysis (XRD), using a X' PERT Philips PMD with a Panalytical X'celerator detector (using a CuKα radiation, $\lambda = 1.5418$ Å).

2.4. Fourier transform infrared spectroscopy analysis

Infrared spectra were acquired by Fourier transform infrared spectroscopy (FTIR) using a Spectrum 100 PerkinElmer equipment by diamond ATR techniques. The spectra were registered between 4000 cm^{-1} and 600 cm^{-1} (resolution of 4 cm^{-1}).

2.5. Electrochemical behavior characterization

Electrochemical behavior characterization was performed in a three-electrode cell, with the sample as working electrode, platinum as a counter electrode and as reference a Ag/AgCl electrode (Metrohm, Ag/AgCl, 3 M KCL). Measurements were performed with Autolab PGSTAT N301 and the exposed surface of the sample was of 1 cm^2. All experiments were performed in Hank's physiological solution with the composition (g/L) of NaCl—8.00, KCl—0.40, $CaCl_2$—0.14, $NaHCO_3$—0.35, $Na_2HPO_4 \cdot 2H_2O$—0.06, KH_2PO_4—0.60, $MgSO_4 \cdot 7H_2O$—0.06, $MgCl_2 \cdot 6H_2O$—0.1, Glucose—1.00 [19,20]. Measurements were performed after 20 min of immersion in Hank's physiological solution. Polarization curves (Tafel plots) were performed between ±200 mV vs. Ag/AgCl and free potential—at a scan rate of 2 mV/s (corrosion parameters such as j_{corr}—current density, E_{corr}—corrosion potential and β_c—cathodic Tafel slope were evaluated by Tafel extrapolation).

2.6. Static contact angle and surface energy

The wettability studies were performed with a contact angle video system Optical Contact Angle Meter—CAM 100 instrument. An equal volume of distilled water (3 μl) was placed on every sample by means of a micropipette, forming a drop or spreading on the surface. Measurements were done in triplicate.

The surface energy (ζ) of a biomaterial can be calculated from the contact angle using the following equation [21]:

$$\zeta = \gamma \cos\theta \quad (1)$$

where γ is the surface energy between water and air at 20 ± 2 °C (72.8 mJ/m^2) [22] for pure water and θ is the static contact angle.

2.7. Antimicrobial activity

In order to establish the antibacterial activity of the materias, *Staphyloccoccus aureus* (ATCC 25923) and *Pseudomonas aeruginosa*

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