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Brief communication

Micropatterned TiO_2 nanotube surfaces for site-selective nucleation of hydroxyapatite from simulated body fluid

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ABSTRACT

TiO_2 nanotube layers can provide greatly enhanced kinetics for hydroxyapatite formation from simulated body fluid compared with smooth, compact TiO_2 surfaces. In the present work we show how this contrast in reactivity can be used to create highly defined lateral microstructures where bone-like hydroxyapatite can be deposited with very high selectivity. For this we used a photolithographic approach to produce micropatterned TiO_2 nanotube layers surrounded by compact oxide that were then immersed in a simulated body fluid (SBF) solution. Not only the tubular vs. flat geometry but also the finding that compact oxides created in phosphate electrolytes in particular suppress apatite deposition are crucial for a very high reactivity contrast. Overall the results show the feasibility of stimulating hydroxyapatite deposition at surface locations where needed or desired. This provides a valuable tool for biomedical device design.

1. Introduction

Starting in the 1950s [1], titanium and its alloys have been widely used in medical applications, e.g. as dental or orthopedic implants, due to their bioinert properties [2]. Various techniques have been developed to further increase the biocompatibility of the material [3], such as alkali [4,5] or anodization treatments [6,7]. Typically, immersion tests in simulated body fluid are conducted with these surfaces, as the extent and rate of bone-like hydroxyapatite ($Ca_5(PO_4)_3OH$) deposition from solution is generally accepted as an indicator of the biocompatibility of a surface [8]. In recent years it has been shown that one promising surface modification is to coat titanium with layers of highly ordered TiO_2 nanotubes, which can be created employing a simple but optimized electrochemical treatment. The height, diameter and crystal phase of the nanotube layers can be adjusted, enabling the formation of highly controlled regular nano-topographies [9–13]. Previous work has shown that nanotube layers, especially when crystallized by annealing, enhance the deposition of bone-like hydroxyapatite from simulated body fluid compared with flat, compact titanium oxide [14,15]. Kodama et al. investigated different nanotube diameters and found the highest apatite deposition rate on 100 nm diameter nanotubes [16]. These findings show that a considerable contrast in hydroxyapatite formation exists on different topographies.

In the present work we explore the feasibility of exploiting these differences in hydroxyapatite formation rate on different TiO_2 surfaces to achieve site-selective and pattern matched deposition. For this we use a classic photolithographic micropatterning process to establish a morphological contrast of tubular vs. flat TiO_2, and expose the surface to simulated body fluid (SBF). Such micropatterned surfaces have been widely discussed as substrates for cell studies, biosensors and cellular microdevices, as outlined in El-Ali et al. [17] and Folch and Toner [18]. For example, micropatterns of electrosprayed apatite have been shown to influence the differentiation and orientation of osteoblast-like cells [19]. Another study attested to a better protein adsorption ability of biomimetic apatite, i.e. apatite nucleated from simulated body fluid, than of plasma sprayed layers [20]. A rather indirect process for apatite patterning was developed by Yao et al., who deposited apatite from simulated body fluid onto previously deposited bioglass particles, which consequently acted as heterogeneous nucleation sites for apatite formation [21–24].

The apatite micropatterning process presented here provides a method for creating highly defined micropatterns without applying a nucleating agent or any sort of chemical contrast, as the TiO_2 nano-topography itself triggers the selective formation of apatite.

2. Materials and methods

2.1. Anodization and pattern formation

The formation of TiO_2 nanotube patterns was achieved using a three step process (first anodization, photolithographic patterning,

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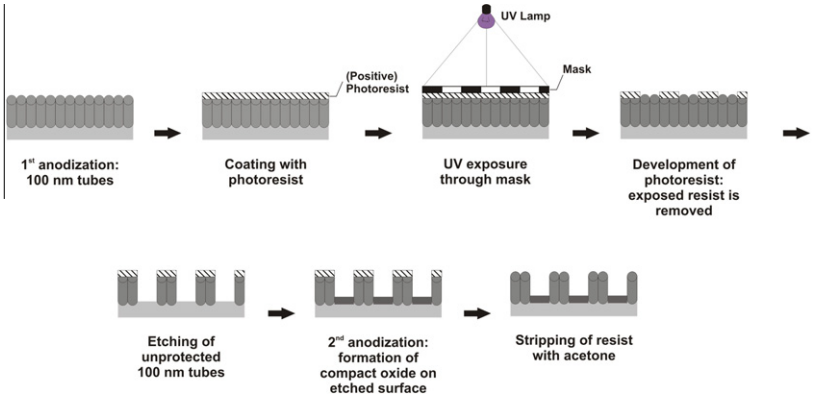

Fig. 1. Patterning process (schematic).

second anodization). As the substrate we used Ti foils (99.6% purity, Advent Ltd.) with a thickness of 0.1 mm. Prior to the patterning process the foils were ultrasonically cleaned in ethanol and deionized (DI) water and dried in a nitrogen gas stream. Then the samples were anodized using an electrochemical cell with a three electrode configuration. Platinum gauze served as the counter-electrode and a Haber-Luggin capillary with Ag/AgCl (1 M KCl) was used as the reference electrode. To create nanotube layers the samples were anodized in 1 M NaH_2PO_4/0.12 M HF or 1 M Na_2SO_4/0.12 M HF using a high voltage potentiostat (Jaissle IMP

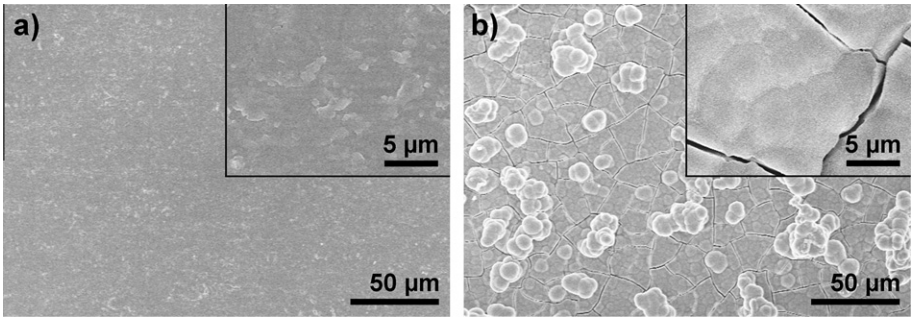

Fig. 2. Comparison of apatite forming ability after 48 h immersion in SBF. (a) Compact oxide produced by anodization in 1 M NaH_2PO_4. (b) One hundred nanometer nanotube surface produced by anodization in NaH_2PO_4/HF. The insets show the same surfaces at higher magnification.

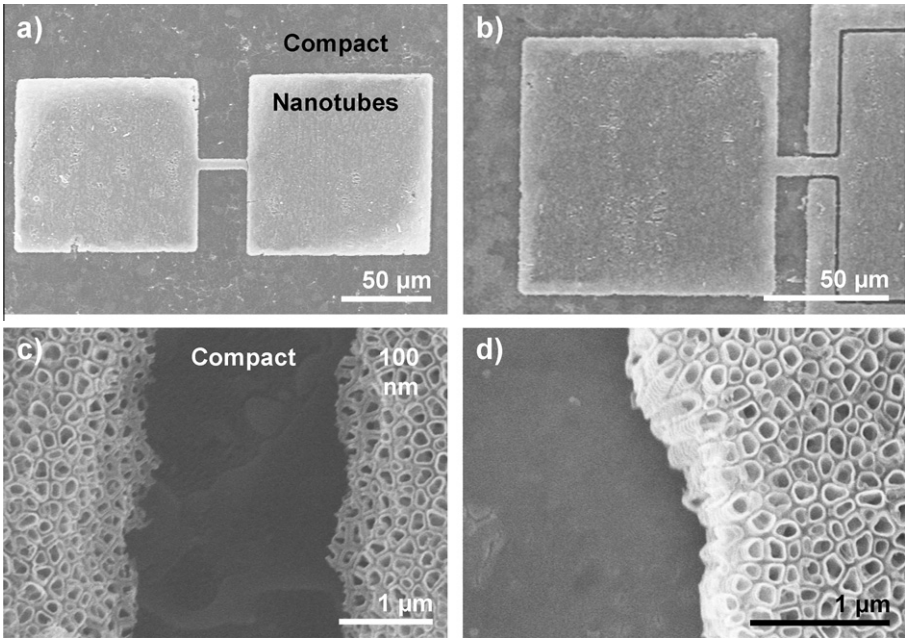

Fig. 3. Patterns of 100 nm nanotubes and compact TiO_2. (a and b) FE-SEM micrographs of patterned surfaces ready for SBF immersion. (c) FE-SEM micrograph of small compact oxide line in 100 nm nanotube matrix. (d) FE-SEM micrograph showing the borderline between compact oxide and nanotubes in detail.

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