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FABRICATION OF TiO_2 NANOTUBES FOR BIOAPPLICATIONS

IZDELAVA TiO_2-NANOCEVK ZA BIOMEDICINSKO UPORABO

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Titanium (Ti), due its most promising biomaterial properties, can be used in the medical devices that interact with the body tissue, specifically to evaluate, treat, augment or replace any tissue, organ or function of the body. In the present work, we prepared titanium dioxide (TiO_2) nanotubes with different diameters in the same electrolyte by tailoring different parameters of electrochemical anodization. These structures have more nanorough regions and more surface area that can promote protein binding and cell adhesion in order to increase the lifetime of implants and other medical devices. As an example, we showed the differences in the protein binding of human acute-phase serum amyloid A (SAA) to the TiO_2 nanotubes of different diameters, specifically of 50 nm and 15 nm, as well as comparing it with the binding to the foil alone. We found that SAA binds most prevalently to the nanotubes 50 nm, as opposed to the nanotubes 15 nm or the foil.

Keywords: TiO_2 nanotubes, electrochemical anodization, protein binding

Zaradi dobre biokompatibilnosti se titan (Ti) uporablja pri izdelavi različnih implantatov in medicinskih merilnih priprav, ki so med merjenjem v neposrednem stiku s telesnim tkivom. V tem delu smo prikazali metodo izdelave površin, pokritih z nanocevkami iz titanovega dioksida (TiO_2), kjer lahko s spreminjanjem parametrov procesa anodizacije spreminjamo premere nastalih nanocevk. Izdelane površine, prekrite z nanocevkami, imajo veliko efektivno površino, kar lahko bistveno vpliva na vezavo proteinov in adhezijo celic, od česar pa je v končni fazi odvisna tudi trajnostna doba implantatov. V prikazanem delu smo kot primer prikazali vezavo proteina serumskega amiloida A (SAA) na TiO_2-nanocevke premerov 15 nm in 50 nm ter na gladko titanovo površino. Preliminarni rezultati meritev kažejo, da se protein SAA najmočneje veže na površino, prekrito s TiO_2-nanocevkami 50 nm.

Ključne besede: TiO_2-nanocevke, elektrokemijska anodizacija, vezava proteinov

1 INTRODUCTION

Titanium is considered to be the most biocompatible metal, due to its resistance to body-fluid effects, great tensile strength, flexibility and high corrosion resistance.[1–3] The combination of the strength and biocompatibility of titanium alloys[4] makes them suitable for medical applications.[5–8] Several *in vitro* studies[9–12] have demonstrated that the cells cultured on titanium nanotubular surfaces exhibited high adhesion, proliferation, alkaline phosphatase (ALP) activity and bone matrix deposition. The influence of the nanomorphological features of titanium nanotubes on the cellular response is particularly striking, especially the finding that there is a clear effect of the diameter and that the diameters of 15–20 nm are optimal for an increased cell adhesion and proliferation.[12] Furthermore, the size effect of titanium nanotubes was confirmed for several types of living cells, i.e., mesenchymal stem cells, haematopoietic stem cells, endothelial cells, osteoblasts and osteoclasts.[13,14] The size effect is explained by a specifically tailored nanotubular morphology because the integrin clustering in the cell membrane leads to a focal adhesion complex with a diameter of about 10 nm, which is a perfect fit for the nanotubes with the diameters of about 15 nm.[15] Irrespective of the location of an implant (a blood-contacting, orthopaedic or dental implant) the first step made after the implantation is the adsorption of proteins from the surrounding tissue or medium. Gongadze et al.[16–18] proposed a mechanism for the adhesion of the cells to a nanorough titanium implant surface with sharp edges, exhibiting more surface area and electric charge than a micro-sized surface. These surface characteristics of a contact surface affect the functional activity of cells. Cells are assumed to bind more strongly to the sharp convex edges or spikes of nanorough regions. Therefore, in order to improve the biological, chemical and mechanical properties and performance of a biomaterial, a surface-modification method such as electrochemical anodization[19] is used to obtain different diameters of nanotubes.

Serum amyloid A (SAA) is a major acute-phase protein in humans that can be elevated, in the circulation, up to 1000 fold during infections or injuries[20] and can represent an invaluable biomarker for inflammation. It has

been implicated in various diseases and pathological states, such as atherosclerosis, rheumatoid arthritis and cancer, among others.[21] When cleaved, SAA products can be deposited into amyloid plaques that can lead to amyloidosis. Due to its expedited and high responsiveness to external stimuli, SAA could be a useful diagnostic and prognostic marker, depending on the disease studied. In this work, we present a fabrication of titanium dioxide nanotubes with different diameters made with electrochemical anodization and an example of protein binding that could be bioapplicable.

2 EXPERIMENTAL WORK

2.1 Growth of titanium dioxide nanotubes with electrochemical anodization

For the fabrication of different titanium dioxide nanostructures, titanium foils of a thickness 0.1 mm and a purità 99.6 % are used. Before anodization, the titanium foils were degreased using successive ultrasonication in acetone, ethanol and deionized (DI) water for 5 min. Each sample was dried in a nitrogen stream. Ethylene glycol (EG)-based electrolytes were used for growing the nanostructures with specific amounts of water and specific concentrations of hydrofluoric acid (HF) for different nanostructures. The specifications of the electrolyte used and the anodization conditions for obtaining different diameters of nanotubes are listed in **Table 1**. All the anodization experiments were carried out at room temperature (≈ 20 °C) in a two-electrode system with a titanium foil as the working electrode and a platinum gauze as the counter electrode. Different anodization parameters, like the anodization time, the applied voltage, the concentration of chemicals, etc. need to be set to obtain the specific morphologies of the nanostructures.

The formed nanostructures were kept in ethanol for specific time periods to remove all the organic components from the electrolyte, washed with distilled water and dried in a nitrogen stream. The morphologies of the titanium nanotubes were observed with scanning electron microscopy (SEM).

Table 1: Anodization conditions for different TiO_2 nanotubular surfaces

Tabela 1: Spreminjanje premerov TiO_2-nanocevk v odvisnosti od razmer pri anodizaciji

Nanotube diameter	Electrolyte	Potential used (V)	Anodization time (h)
15 nm	EG + 8 M water + 0.2 M HF	10	2.5
50 nm	EG + 8 M water + 0.2 M HF	20	2.5

Immunofluorescence: Human recombinant serum amyloid A (hrSAA) protein (at a concentration of 1 μg/μL) was applied as a droplet of 20 μL onto the diameter 50 nm and 15 nm TiO_2 nanotubes and the foil (all 0.5 cm × 0.5 cm in size). The samples were incubated for 30 min, followed by washing 3-times with PBS for 5 min each. Blocking was performed with bovine serum albumin 1 % and milk 5 % in PBS for 30 min. The blocking buffer was replaced with a primary antibody (an anti-SAA mouse monoclonal antibody, a 1 : 100 dilution) and incubated overnight, followed by washing 3-times in PBS for 5 min. The secondary antibody (a goat anti-mouse IgG conjugated with FITC, a 1 : 800 dilution) was incubated for 30 min, followed by washing 3-times in PBS and immunofluorescent detection (Nikon Eclipse E400, a Nikon Digital Camera DXM1200F).

3 RESULTS AND DISCUSSION

3.1 Morphology of titanium dioxide nanotubes

By tailoring the anodization conditions (the applied voltage, the anodization time and concentrations of the chemicals) we obtained the nanotubes of diameters 15 nm and 50 nm. SEM images of the top surfaces of diffe-

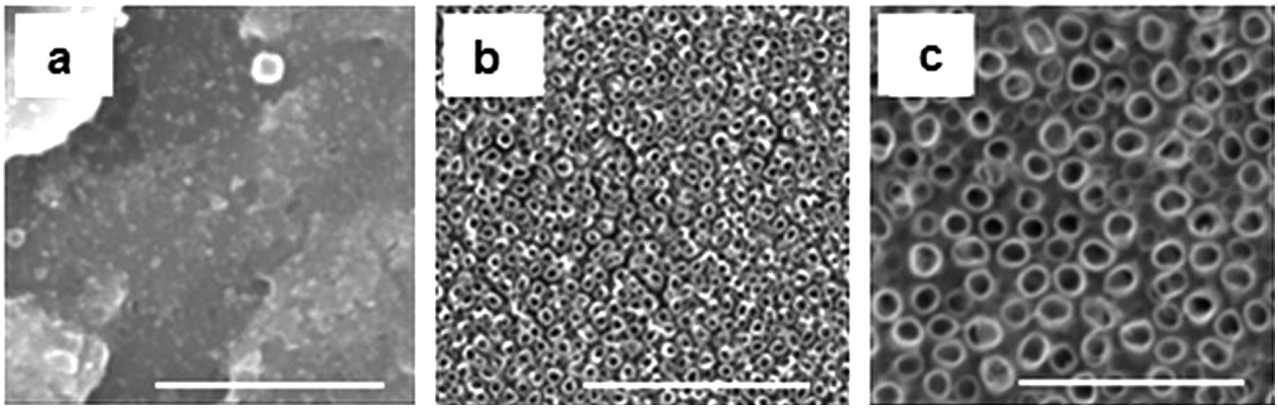

Figure 1: SEM images of the top surfaces of: a) Ti foil, b) diameter TiO_2 nanotubes 15 nm and c) diameter TiO_2 nanotubes 50 nm. The scale bar is 500 nm.

Slika 1: SEM-posnetki: a) površina titanove folije, b) površina s TiO_2-nanocevkami premera 15 nm in c) površina s TiO_2-nanocevkami premera 50 nm. Dolžina črte 500 nm.

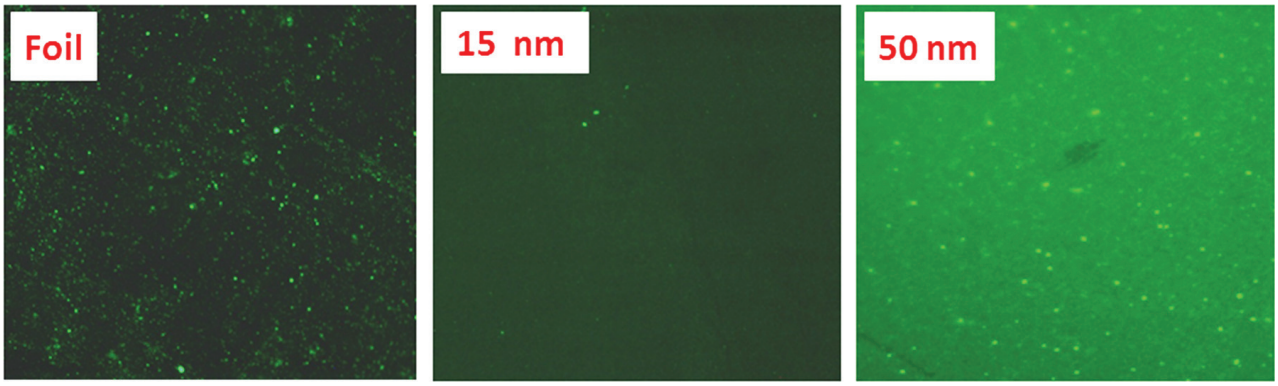

Figure 2: Immunofluorescent micrographs of hrSAA protein binding to the foil and TiO_2 nanotubes of diameters 15 nm and 50 nm – examples of protein binding

Slika 2: Imunofluorescenčni mikroposnetki vezave proteina hrSAA na površino folije in na površino z nanocevkami TiO_2 premera 15 nm in 50 nm; primeri vezave proteina

rent nanotube surfaces and the titanium foil are shown in **Figure 1**.

Immunofluorescence: The binding of human recombinant SAA protein to the TiO_2 nanotubes and the foil shows a more prevalent signal on the TiO_2 nanotubes 50 nm than on the nanotubes 15 nm or the foil under the same conditions as shown in **Figure 2**.

4 CONCLUSIONS

Different diameters of TiO_2 nanotubes were obtained with electrochemical anodization. Immunofluorescence studies involving SAA were carried out on the TiO_2 nanotubes and compared with the Ti foil. The protein binding to the non-coated titanium dioxide nanotubes, with SAA being the sample protein, is shown to be dependent on the nanotube diameter size, increasing with larger diameters.

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