

NANOSTRUCTURED TITANIA DOPED WITH SILVER NANOPARTICLES FOR PHOTOCATALYTIC WATER DISINFECTION

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Abstract

The research is focused on water disinfection via new technological concept based on smart combination of nanostructured TiO₂ layer and silver nanoparticles. The effective TiO₂/Ag NPs photocatalytic system active in visible range was created via anodization of Ti thin film and UV-assisted and electrochemical pulse deposition of Ag NPs. Its efficiency was evaluated on complete inhibition of yeast *Candida glabrata*. A morphology of TiO₂/Ag NPs system was studied via scanning electron microscopy (SEM).

Keywords: TiO₂ nanotubes, anodic oxidation, electrodeposition, UV-assisted deposition, Ag NPs, *C. glabrata*

1. INTRODUCTION

Microbial contamination of water, especially drinking water, is a major problem in developing but also developed countries [1-3]. Fungi are usually removed using filtration and chemical coagulation [4] or can be inactivated by UV radiation [2], chlorination or ozonisation. But these methods might be insufficient to remove all fungi cells [2]. The list of significant microbial pollutants in water include various bacteria, viruses, parasites, fungi and yeast [1-5].

The advanced oxidation processes (AOPs) represent innovative technologies for water purification. One of the most effective AOPs is heterogeneous photocatalysis. This technology can be used to decompose any organic pollutant and microorganism even at their very low concentration [6-12]. Titanium dioxide (TiO₂) is known as a superior photocatalyst mainly due to its chemical, mechanical and thermal stability and non-toxicity [12]. Nowadays, there is a trend to avoid using TiO₂ in a suspension form and to replace it with thin photocatalytic layers. In comparison with flat surfaces, the nanostructured TiO₂ layers strongly increase the active surface area, and thus oxidation abilities, improve electron transport, and last but not least upgrade their physical and chemical properties [13].

One of the most convenient method to produce nanostructured TiO₂ is anodic oxidation (AO) [14-16]. Till now the researchers have focused on fabrication of nanostructured titania using thick Ti foil [16-19]. Therefore the achievement of TiO₂ nanostructures on thin Ti coatings (under 500 nm) remains very challenging [20]. The thickness of Ti film is a key parameter regarding the economic question, feasibility of its deposition and the transparency of final TiO₂ layer after annealing, which is demanding for intended photocatalytic applications [13].

TiO₂ has a relatively wide band gap with 3.2 eV for anatase phase and 3.0 eV for rutile phase [21, 22]. Therefore, the ultraviolet irradiation is required for its photoactivation. However, UV represents only 5% of solar spectrum. Thus the shift of TiO₂ absorption into the visible range of spectrum can strongly enhance the effectivity of photocatalytic process. For this purpose, the titania can be decorated with noble metals such as silver [23-25].

Silver nanoparticles (Ag NPs) have attracted a great attention due to their remarkable physical and chemical properties and significant antimicrobial features [24, 26]. According to the last studies, the mechanism of photocatalytic effect of TiO₂/Ag NPs photocatalytic system can be explained by shift of TiO₂ absorption into visible region through localized surface plasmon resonance of Ag NPs [23, 24, 27].

In our work, the basic substrate for fabrication of nanostructured TiO₂ surface in the form of nanotubes or nanopores was thin titanium film. Nanotubular TiO₂ (TNTs) was prepared in optimized electrolyte containing ethylene glycol, ammonium fluoride and water. In general, anatase exhibits higher photocatalytic activities than rutile. Therefore, the crystallization from amorphous TiO₂ was achieved by annealing in vacuum at temperatures between 400-600 °C. As prepared nanostructured TiO₂ was decorated with Ag NPs via pulse electrochemical deposition of Ag NPs and UV-assisted chemical reduction of silver nitrate.

This work deals with inhibition of *Candida glabrata* using TiO₂/Ag NPs photocatalytic system fabricated as mentioned above. *C. glabrata* is considered as one of the most significant cause of mucosal and systemic infections [28]. These infections are difficult to treat and are often resistant to many azole antifungal agents. Consequently, *C. glabrata* infections have a high mortality rate in compromised, at-risk hospitalized patients.

2. EXPERIMENTAL

2.1. Materials

Titanium (99.99 %, Porex, CZ), ethylene glycol (C₂H₆O₂, p.a., Penta, CZ), ammonium fluoride (NH₄F, Sigma Aldrich, DE), potassium dicyanoargentate (K[Ag(CN)₂], p., Sigma Aldrich, DE), potassium cyanide (KCN, p., Merck, DE), silver nitrate (AgNO₃, p.a., Penta, CZ), ethanol (C₂H₆O, p.a., Penta, CZ), *Candida glabrata* (CCY 26-20-21, SVK), yeast malt broth (HiMedia Laboratories, SVK), acridine orange (research grade, Serva, DE). Deionized water was obtained from Millipore RG system MilliQ (Millipore Corp., USA). All listed materials were used as purchased without any further purification.

2.2. Preparation of Ti film

Titanium thin film with thickness of 500 nm was deposited onto 4-inch p-type silicon wafer (100) covered by 1 μm of thermal silicon dioxide [29]. The three-grid radio frequency-induced coupled plasma Kaufman ion beam source (Kaufman & Robinson-KRI®, Inc., USA) was employed to deposit Ti thin films. The energy of bombarding ions was set to be of 300 eV with corresponding deposition rate of 0.16 Å s⁻¹. The process pressure was 1.2x 10⁻² Pa. Root mean square roughness of deposited layers was determined as ±0.76 nm by atomic force microscopy method using Dimension Icon (Bruker, Germany) in ScanAsyst® mode.

2.3. Synthesis of TNTs

The electrolyte solution used for AO of Ti film was prepared from ethylene glycol containing ammonium fluoride (1.8 wt%) and deionized water (2 vol%). Before AO the prepared Ti film was consecutively immersed into acetone, isopropanol and rinsed with deionized water to remove impurities and then dried by compressed nitrogen. The size of anodized sample with titanium thin film was 1 cm × 1 cm. The sample was placed in the two-electrode configuration and anodized using power supply controlled by LabView program. The substrate operated as the working electrode and the stainless-steel mesh as the counter electrode. The course of AO process was set with a voltage ramp from 0 V to the potential of 20 V with a sweep rate of 1 V/s. After the first 20 s, the potential was kept constant at the value of 20 V. The anodized samples were rinsed with deionized water and dried by compressed nitrogen. To induce crystallization, samples were annealed in vacuum oven at 450 °C for 3 h. The heating rate (ramp) was set to 3 °C/min. The morphology of all samples were examined using scanning electron microscopy (FE Tescan Mira II LMU).

2.4. Pulse electrodeposition of Ag NPs

In order to achieve higher and photoinduced antimicrobial activity, the fabricated TNTs were further decorated by Ag NPs via pulse electrodeposition technique. Ag NPs were deposited from aqueous electrolyte containing 7.5 g/L K[Ag(CN)₂] and 80 g/L KCN under the constant current of 8 mA. Similarly like in synthesis of TNTs, the pulse deposition of Ag NPs was carried out in two-electrode configuration with gold counter electrode employing device with controlled circulation of electrolyte.

2.5. UV-assisted deposition of Ag NPs

Nanostructured TiO₂ was decorated with Ag NPs also via UV-assisted in situ synthesis. 0.1 M aqueous solution of silver nitrate was reduced by absolute ethanol acting as an electron donor. 2.5 μL of silver nitrate solution was drop-coated on the TiO₂ nanostructures and subsequently 2.5 μL of ethanol was added. Such prepared sample was placed under UV radiation for 30 min, which was provided by UV lamp with wavelength of 366 nm and radiance intensity of 1.8 mW/cm² placed in a distance of 1 cm above the sample. After reaction, TiO₂ substrates decorated with Ag NPs were rinsed with deionized water and dried with compressed nitrogen. As-prepared samples were used for photocatalytic tests without further modification.

2.6. Antimicrobial activity of Ag NPs/TiO₂ system

Tests of antimicrobial activity were performed under four fluorescent UV lamps Sylvania Lynx-S 11W with radiance intensity of 5 mW/cm². The reaction vessel consisted of Petri dish with filtration paper and 5 mL of deionized water. The Petri dish was covered by quartz glass to avoid dehydration of samples. 15 μL of tenth diluted (10⁻²) *C. glabrata* suspension was dropped on TiO₂/Ag NPs substrate. The samples were irradiated for four different times, namely 0, 5, 15, and 30 min. The acridine orange dye was used for observation of cell viability using NIKON Eclipse E 200 microscope with mercury bulb (NIKON, C-SHG1) and CCD camera (PixeLINK PL-A662). This dye is able to bind to DNA in dead cell and so in epi-fluorescence microscope this complex emits red light. The LUCIA Net software was used for microscope image analysis. The number of live and dead cells was calculated and expressed as the survival ratio (SR), i.e. number of live cells divided by total number of cells.

3. RESULTS AND DISCUSSION

3.1. Synthesis of TNTs

The observation of anodization curve during the TNTs formation enabled us to follow the whole electrochemical reaction in ethylene glycol based electrolyte with optimized composition in order to achieve homogeneous nanotube ordering and complete removal of initial oxide barrier layer. The homogeneity and ordering of TNTs are strongly dependent on the quality of the sputter-deposited Ti thin film.

Figure 1 shows the current vs time characteristics during AO of Ti thin film on silicon substrate. Three phases were observed as described further. The current increased due to the formation of initial barrier layer on the surface of Ti layer during the first phase (I.). The thickness of this first layer can be decreased

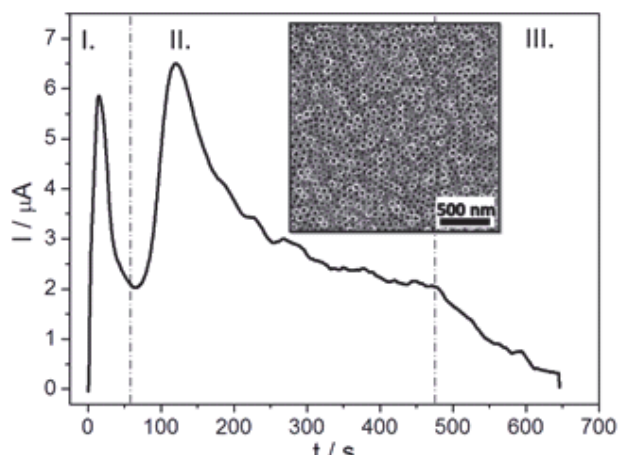


Figure 1 Current voltage transient during anodization of thin Ti film. The top view of the TNTs are shown in the inset image

by using the voltage ramp in the first seconds of AO. The nanotubular growth region on graph was visible on the second phase (II.) of AO and the drop of current on the third phase (III.) was assigned to reaching the interface between Ti/SiO₂ layers. The inset image shows the regularly ordered TNTs fabricated under the optimal conditions. The average maximal height of TNTs was about 400 nm and their diameter of about 50 nm.

3.2. Pulse electrodeposition of Ag NPs

For deposition of Ag NPs we used two types of TNTs, pristine and annealed. **Figure 2** shows the various structures of Ag NPs on both of these substrates. There can be seen from **Figure 2a** the nanoparticle aggregates with the size of about 100-200 nm appeared on the pristine TNTs. The aggregation of nanoparticles into cluster might be caused by high resistance of the non-annealed TNTs. Annealed TNTs were successfully decorated with Ag NPs with homogenous distribution and uniform size of about 50 nm (see **Figure 2b**). As can be seen in **Figure 2c**, UV-assisted deposition of Ag NPs on nanostructured TiO₂ substrate resulted in almost homogeneous TiO₂ surface coverage with Ag NPs. The size of Ag NPs varied from 10 nm to 40 nm. Shape of as prepared Ag NPs was spherical.

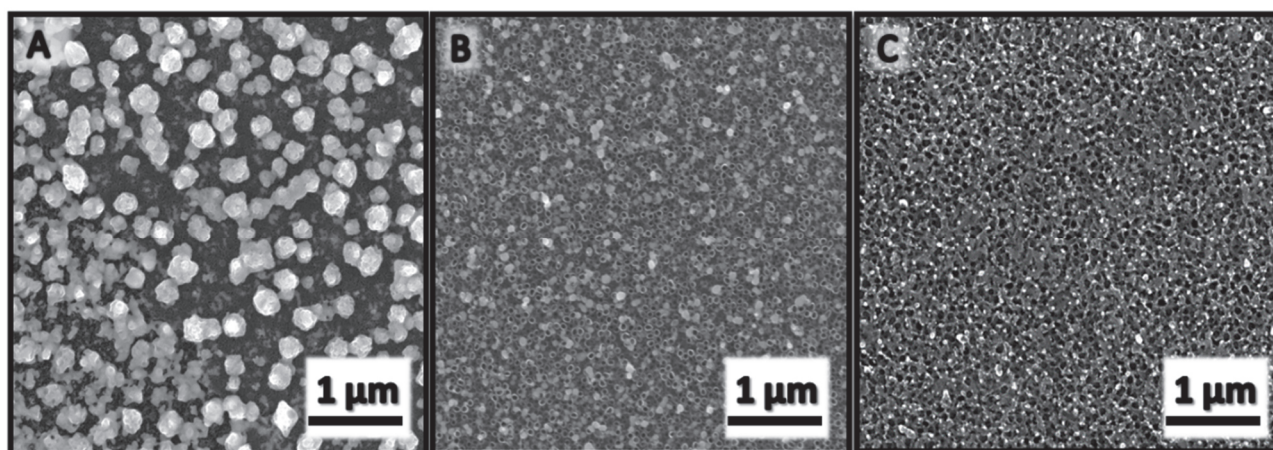


Figure 2 SEM images of Ag NPs electrodeposited on A) pristine and B) annealed TNTs and C) deposited by UV-assisted method on pristine nanoporous TiO₂ surface

3.3. Antimicrobial activity of Ag NPs/TiO₂ system

Photocatalytic antimicrobial activity of TiO₂/ Ag NPs system was investigated on degradation of *C. glabrata* yeast. **Figure 3** shows the results of three different samples; pristine TNTs, TNTs with electrodeposited Ag NPs and UV deposited Ag NPs. The irradiation time was set as follows: 0, 5, 15 and 30 min. The pristine TNTs exhibited the lowest antimicrobial activity in comparison with other tested samples, i. e. only 25 % of yeast were killed after 30 min. The sample of nanostructured titania with electrodeposited Ag NPs evinced almost 2 times higher antimicrobial activity than pristine TNTs, namely 45 % of cells were inhibited in 30 min. The highest antimicrobial activity was found for nanostructured titania with UV deposited Ag NPs. The mortality of cells rapidly increased to 91 % after 30 min. It is obvious that antimicrobial activity of as prepared system is highly time-dependent. In case of UV irradiation period of 5 min, the highest antimicrobial activity was observed for the system with TiO₂ and UV deposited Ag NPs, however after 15 min of UV radiation, the highest value of antimicrobial activity was calculated for TiO₂ with electrodeposited Ag NPs. Although antimicrobial activity of TiO₂/electrodeposited Ag NPs increased after 30 min, the increment was not as significant as in case of the sample with TiO₂/ UV deposited Ag NPs.

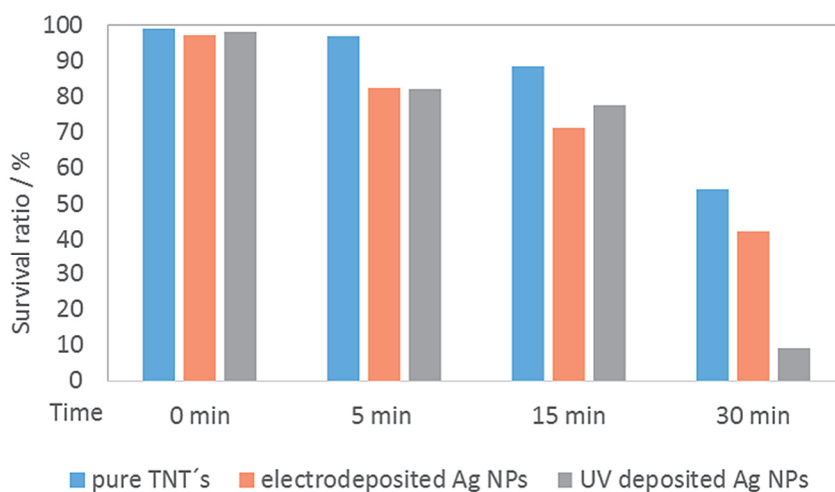


Figure 3 Survival ratio of *C. glabrata* after UV irradiation on different TiO₂ nanostructured surfaces

4. CONCLUSION

We created active photocatalytic system based on nanostructured TiO₂ and Ag NPs. According to the recent literature survey, such thin nanostructured photocatalytic layers prepared via AO have not been achieved yet. We conclude the doping of titania with noble metals such as silver nanoparticles can significantly enhance both photocatalytic and antimicrobial activity. The lowest antimicrobial photocatalytic activity was achieved for pristine titania, while the nanostructured titania decorated with Ag NPs using both immobilization methods improved the achievement of better results. The maximal antimicrobial photocatalytic activity was observed for TiO₂ with UV deposited Ag NPs with mortality of *C. glabrata* yeast cells of almost 91 %.

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