Colloids and Surfaces B: Biointerfaces 145 (2016) 597–606

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Cytocompatibility and antibacterial activity of titania nanotubes incorporated with gold nanoparticles

Tingting Yang [a,b], Shi Qian [a], Yuqing Qiao [a], Xuanyong Liu [a,*]

[a] State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, PR China
[b] University of Chinese Academy of Science, Beijing 100049, PR China

ARTICLE INFO

Article history:
Received 22 March 2016
Received in revised form 18 May 2016
Accepted 25 May 2016
Available online 26 May 2016

Keywords:
TiO_2 nanotubes
Gold nanoparticles
3-aminopropyltrimethoxysilane
Cell adhesion
Antibacterial effect

ABSTRACT

TiO_2 nanotubes prepared by electrochemical anodization have received considerable attention in the biomedical field. In this work, different amounts of gold nanoparticles were immobilized onto TiO_2 nanotubes using 3-aminopropyltrimethoxysilane as coupling agent. Field emission scanning electron microscopy and X-ray photoelectron spectroscopy were used to investigate the surface morphology and composition. Photoluminescence spectra and surface zeta potential were also measured. The obtained results indicate that the surface modified gold nanoparticles can significantly enhance the electron storage capability and reduce the surface zeta potential compared to pristine TiO_2 nanotubes. Moreover, the surface modified gold nanoparticles can stimulate initial adhesion and spreading of rat bone mesenchymal stem cells as well as proliferation, while the osteogenous performance of TiO_2 nanotubes will not be reduced. The gold-modified surface presents moderate antibacterial effect on both *Staphylococcus aureus* and *Escherichia coli*. It should be noted that the surface modified fewer gold nanoparticles has better antibacterial effect compared to the surface of substantial modification of gold nanoparticles. Our study illustrates a composite surface with favorable cytocompatibility and antibacterial effect and provides a promising candidate for orthopedic and dental implant.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Recently, TiO_2 nanotubes have drawn increasing attention, as they are currently being used in the biomedical field because of their good biocompatibility and bioactivity in physiological environment [1]. The geometry of the self-organized TiO_2 nanotubes fabricated by electrochemical anodization can be easily controlled [2,3]. Research has shown that TiO_2 nanotubes can promote hydroxyapatite nucleation and formation in the simulated body fluid compared to the flat TiO_2 surface [4,5]. Enhancement of adhesion, differentiation, collagen secretion and ECM mineralization of rat bone mesenchymal stem cell on the TiO_2 nanotubes surface has been reported [6].

The antibacterial ability of TiO_2 nanotubes is generally attributed to their semiconducting activity which determines they have good photocatalytic performance. TiO_2 is normally excited by ultraviolet light and generates electron/hole pairs which can react with the bacterial membrane and cause the bacteria in contact dead [7–9]. However, when TiO_2 is implanted into the body, where the actual situation was oblivious of any light, its antibacterial ability will become weaken or even disappear [10]. To solve this problem, noble metal doping is an effective strategy. When the noble metal is in contact with TiO_2, a Schottky barrier is established due to the alignment of Fermi level at the noble metal/TiO_2 interface [11,12]. Because of the Schottky barrier, electrons will accumulate at the noble metal side which has higher Fermi level and the holes diffuse to the TiO_2 side. In this way, when the doped TiO_2 is normally excited by ultraviolet light, recombination of electron/hole pairs was avoided, and the holes which are detrimental to bacterial cells were preserved [13]. In Cao's study, a composite structure of Ag/TiO_2 coating was produced by plasma immersion ion implantation method and it possesses long-term antibacterial ability [14].

The unique physical and chemical properties of gold nanoparticles make them widely used in the field of catalysis, sensing and biology [15,16]. In the biomedical field, it is reported that gold nanoparticles are very attractive materials, as they have been used in the imaging, diagnostics and therapies application due to their distinctive properties such as good stability, biocompatibility and flexibility in functionalization [17,18]. Studies have shown that thiol and amine could react with gold nanoparticles to form

* Corresponding author.
E-mail address: xyliu@mail.sic.ac.cn (X. Liu).

http://dx.doi.org/10.1016/j.colsurfb.2016.05.073
0927-7765/© 2016 Elsevier B.V. All rights reserved.

Au—S and Au—N bonds, therefore gold nanoparticles have a strong affinity with protein and amino acids [19–21]. In Yi's report, gold nanoparticles of 20 nm in diameter were uniformly dispersed in the cytoplasm and nucleus which promote the differentiation of bMSC into osteoblasts through p38 MAPK pathway [22]. Also, it was confirmed that gold nanoparticles can enhance the proliferation and mineralization of MC3T3-E1 cells [23]. Moreover, gold nanoparticles have antibacterial activity against Gram-positive and Gram-negative bacteria due to the interaction with the functional groups of bacteria [24].

In this study, we propose a novel way to introduce gold nanoparticles onto the anodized TiO_2 nanotubes by using 3-aminopropyltrimethoxysilane (APS) as the coupling agent. In this method, different amounts of gold nanoparticles can be immobilized by modifying the immersion time in a sonic bath. The physical and chemical properties of the composite surface were measured. The cytocompatibility of gold nanoparticles incorporated TiO_2 nanotubes with rat bone mesenchymal stem cells was investigated. Meanwhile, we further tested the antibacterial property of the composite surfaces against both *Staphylococcus aureus* and *Escherichia coli*.

2. Materials and methods

2.1. Specimen fabrication

2.1.1. Preparation of TiO_2 nanotubes

Commercial pure titanium plates (Cp Ti; TA1, purity >99.85%), with dimension of $10 \times 10 \times 1\,mm^3$ and $20 \times 10 \times 1\,mm^3$ (for the test of zeta-potential) were used. The plates were then immersed in a mixed acid solution (volume ratio of $HF:HNO_3:H_2O$ is 1:5:34) for 5 min twice to remove the thin oxide layer. Prior to electrochemical anodization, the plates were ultrasonically cleaned successively in ethanol and DI water several times and dried in the presence of air. TiO_2 nanotubes were fabricated in a two-electrode system where a graphite electrode was served as cathode and the pretreated Ti plate was used as anode. The nanotubes were formed on the titanium plate in an electrolyte solution of 1 wt% HF for 30 min at a DC constant voltage of 20 V. The samples were then washed with DI water and dried in air for further use.

2.1.2. Preparation of gold nanoparticles

Gold nanoparticles were prepared by the sodium citrate reduction of tetrachloroauric acid ($HAuCl_4$) by the Frens method [25]. Prior to synthesis, all the glassware used in the following process was rinsed in aqua regia (volume ratio of $HCl:HNO_3$ is 3:1) and washed with DI water thoroughly. In Erlenmeyer flask, 100 ml of 0.01 wt% $HAuCl_4$ solution was heated to boiling temperature under magnetically stirring. Then 5 ml of 1 wt% sodium citrate solution was added and kept boiling for further 15 min with heating and stirring successively. After cooling to room temperature, the product was stored at 4 °C in darkness. The monodisperse spherical gold nanoparticles covered with citrate shell were named as GNP in this article.

2.1.3. Self-assembling of GNP/TiO_2 nanotubes composite surface

The composite surface was prepared by assembling APS to link between gold nanoparticles and TiO_2 nanotubes through the two steps. The first step is the formation of positively charged layer on the TiO_2. The as-treated TiO_2 nanotubes were immersed in 5 vol.% APS solution and sonicated in the sonic bath. Then the samples were rinsed with DI water and dried in air. After the self-assembling process, a APS monolayer was deposited onto the TiO_2 nanotubes through the reaction between CH_3O—Si— (from APS) and the hydroxyl group (—OH) on the wall of nanotubes. Therefore, the nanotubes were introduced a positive charged layer terminated with NH_3^+ group (from APS). The APS-treated nanotubes were immersed in the gold colloid for 24 h. Thus, the negative gold nanoparticles were anchored to the inner and outer wall of TiO_2 nanotubes resulting from electrostatic attraction. Besides, because of the repulsive force between the nanoparticles, gold nanoparticles could uniformly disperse on the surface without any aggregation. Finally, the assembly plates were annealed at 450 °C in air for 2 h (the heating rate is 1 °C/min), and then furnace cooling to room temperature. The APS layer can be removed via aforementioned heat treatment and those assembling nanoparticles retained [26]. In this article, the experiment groups were labeled as GNP-1 and GNP-3 corresponding to the 1 h and 3 h of ultrasonic time respectively and the heat treated TiO_2 nanotubes without assembling was marked as TNT.

2.2. Surface characterization

The surface and cross-section views of TiO_2 nanotubes before and after GNP assembling were characterized by scanning electron microscopy (SEM; S-4800, Hitachi, Japan). The phase composition of samples was detected by a thin-film X-ray diffraction (XRD; D/MAX-2550, Rigaku, Japan). The elemental composition and chemical state of surfaces were determined by X-ray photoelectron spectroscopy (XPS; Physical Electronics PHI 5802). The range of charge transfer and separation on the surface could be analyzed using photoluminescence (PL; FluoroMax-4).

2.3. Surface zeta potential measurements

The surface zeta potential of the sample was measured by Surpass electroki-netic analyzer (Anton Parr, Austria). Two plates with the dimension of $20 \times 10 \times 1\,mm^3$ were arranged parallel on the sample holders. 0.001 M KCl solution was used as the electrolyte and the pH value was adjusted by 0.05 M HCl and 0.05 M NaOH. In this measurement, the potentials resulting from the motion of ions in the diffusion layer were measured according to Helmholtz-Smoluchowski equation,

$$\zeta = \frac{dI}{dP} \times \frac{\eta}{\varepsilon \times \varepsilon_0} \times \frac{L}{A} \qquad (1)$$

in which ξ is the zeta-potential, dI/dP represents the slope of the streaming current versus pressure difference, η, ε_0 and ε denote the viscosity, vacuum permittivity and dielectric constant of the electrolyte solution, L and A are the length and cross-section of the streaming channel, respectively.

2.4. Au release

The GNP-modified samples were soaked in 5 ml ultrapure water at 37 °C for 30 days without stirring. At the end of incubation, the leaching liquid was collected, and the amount of Au released from the composite surface was analyzed by inductively-coupled plasma atomic emission spectrometry (ICP-AES; JY2000-2, France).

2.5. Protein adsorption assay

Protein solution was prepared via dissolving bovine serum albumin (BSA; Sigma, USA) powder in phosphate buffer saline (PBS; pH 7.4, Hyclone, USA) to obtain the following used BSA solution with the concentration of 250 μg/ml. The prepared BSA solution of 1 ml was seeded on the specimens in 24-well plates. After 24 h of incubation at 37 °C, the samples were removed from the plates and the concentration of residual solution was quantified using BCA protein assay kits (Cat. No. 23227, Pierce Biotechnology, USA). The amount of adsorbed BSA on the sample surface could be calculated via the

Download English Version:

https://daneshyari.com/en/article/598985

Download Persian Version:

https://daneshyari.com/article/598985

Daneshyari.com