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Spontaneous adsorption of silver nanoparticles on Ti/TiO$_2$ surfaces. Antibacterial effect on *Pseudomonas aeruginosa*

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ABSTRACT

Titanium is a corrosion-resistant and biocompatible material widely used in medical and dental implants. Titanium surfaces, however, are prone to bacterial colonization that could lead to infection, inflammation, and finally to implant failure. Silver nanoparticles (AgNPs) have demonstrated an excellent performance as biocides, and thus their integration to titanium surfaces is an attractive strategy to decrease the risk of implant failure. In this work a simple and efficient method is described to modify Ti/TiO$_2$ surfaces with citrate-capped AgNPs. These nanoparticles spontaneously adsorb on Ti/TiO$_2$, forming nanometer-sized aggregates consisting of individual AgNPs that homogeneously cover the surface. The modified AgNP–Ti/TiO$_2$ surface exhibits a good resistance to colonization by *Pseudomonas aeruginosa*, a model system for biofilm formation.

1. Introduction

Nanotechnology is nowadays having a high impact on almost all areas of biotechnology [1,2] and in medicine [2–5]. In fact, applications of nanotechnology-derived materials, especially of nanoparticles (NPs) of different materials, in areas related to diagnosis and therapeutics, such as pathogen detection [6], drug delivery [7–10], burn healing [11], disease diagnosis [12] and imaging [13,14], among others, are in continuous progress.

On the other hand, one of the most investigated issues in medicine is related to the development of implantable devices that help in the treatment of diverse diseases and in the replacement of parts of the body. The insertion of implants and medical devices has emerged as a common and often life-saving procedure. Titanium has a high strength-to-weight ratio, is resistant to corrosion, biocompatible and has the property to osseointegrate, and these are some of the reasons why it is widely used in orthopedics and dentistry [15,16]. Titanium-made implants have a high performance and durability: for instance, some dental implants can remain in place for over 30 years.

However, one of the most important risk factors of all invasive medical devices is that they predispose to infection [17] by damaging or invading epithelial or mucosal barriers, as well as by supporting the growth of microorganisms by serving as reservoirs. Invasive medical devices impair host defense mechanisms and, when contaminated, can result in resistant chronic infection or tissue necrosis, these being the major objections to the extended use of implant devices [18]. Thus, biomaterial implant-related infections remain as one of the main causes of implant failures [18,19].

Either as ionic species or colloidal particles, silver has been used as an antimicrobial agent for years [20], long before the appearance of antibiotics. Silver materials have some advantages with respect to antibiotics: unlike them they show antibacterial effect against all bacteria and they do not present the problem of developing resistance (as it does happen with antibiotics).

In particular, silver nanoparticles (AgNPs) have excellent biocidal properties [21–25], although their mechanism of action is not yet completely understood [26,27]. Several methods of synthesis, such as chemical reduction in aqueous and non aqueous solvents, photochemical reduction and sol–gel methods can be used to prepare stable AgNPs [28]. It has been demonstrated that silver nanoparticles retain their bactericidal properties [28–30] when included in coatings [31,32]; bandages for burn healing [33], and dressing materials for wound repair [34], among others. Therefore, they can be included as coating on orthopedic and other implantable devices [31,35,36].

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Since bacterial infections associated with titanium implants remain a major cause of their failure [37], considerable interest has arisen in the preparation and study of the properties (both physical–chemical and antibacterial) of Ti/TiO_2 surfaces modified with silver [38–41]. Several strategies have been used for their preparation, including the complexation of Ag^+ ions with mercaptan-terminated phosphonates [41], silver sputtering [38], adsorption of nanoparticles on amine-terminated silane monolayers [42], the use of silver and hydroxyapatite/TiO_2 slurries [43], and sol–gel procedures [40,44], among others. It has been reported that these modified surfaces are biologically compatible [37,45] and present antimicrobial activity [46]. Moreover, in some cases it has been demonstrated that they present visible light photocatalysis [47].

The aim of this paper is to report a simple and easy method to modify Ti/TiO_2 surfaces with citrate-capped AgNPs by spontaneous adsorption. To the best of our knowledge, this simple method – which does not need the preparation of a monolayer of organic molecules (phosphonate, silane, etc.) on the Ti/TiO_2 surface as a previous step, or any heating procedures – has not been previously reported in public literature. The antimicrobial effect of the modified surfaces has been tested against *Pseudomonas aeruginosa* (*P. aeruginosa*), an opportunistic microorganism that can cause severe, life-threatening infections and is primarily a nosocomial pathogen [48].

This easy method of adsorbing AgNPs (and other nanoparticles) from solution can be used to modify not only Ti/TiO_2 surfaces of implants but also other surfaces (as long as they have an oxide layer) that need to be in contact with a biologically active environment and which are also plausible of biofilm development. The strategy can also be applied to prepare catalysts such as those consisting of gold nanoparticles [49] (capped with carboxylic acids) on TiO_2 and other oxides [50], which have been found to be effective to catalyze important reactions. Finally, it may also be interesting to assess the possibility of using TiO_2 and other oxide surfaces to recover residual metallic nanoparticles from water, an important issue for environmental decontamination.

2. Experimental

2.1. Silver nanoparticle preparation

Silver nanoparticles in aqueous solution were prepared by reduction of $AgNO_3$ with $NaBH_4$ and stabilized by using trisodium citrate. All reagents used in the synthesis were of analytical grade and solutions were prepared with Milli Q water (18 MΩ cm). Briefly, 1 mL of a 5 mM aqueous $AgNO_3$ solution was added to 16 mL of a 1.06 mM aqueous sodium citrate solution under magnetic stirring in an ice/water bath at around 0 °C. Then, 100 μL of a freshly prepared 100 mM aqueous $NaBH_4$ solution were added dropwise over 5 min. The initially colorless solution became yellow and was stirred at around 0 °C for 1 h and 45 min. The final Ag concentration in the nanoparticle solution is 3.16×10^{-2} mg Ag/mL. The nanoparticles obtained hereby are stable for weeks. A picture of the final solution is shown in the inset in Fig. 1a.

2.2. Characterization of AgNPs

UV–vis spectra of the nanoparticles (1:4 dilutions of the original solutions, 7.9×10^{-3} mg Ag/mL) were acquired with a Lambda 35 double beam spectrophotometer from Perkin Elmer (Waltham, MA, USA).

As-prepared citrate-capped AgNPs were characterized by Transmission Electron Microscopy (TEM) using a Philips CM200 UT microscope operating at 200 kV and room temperature.

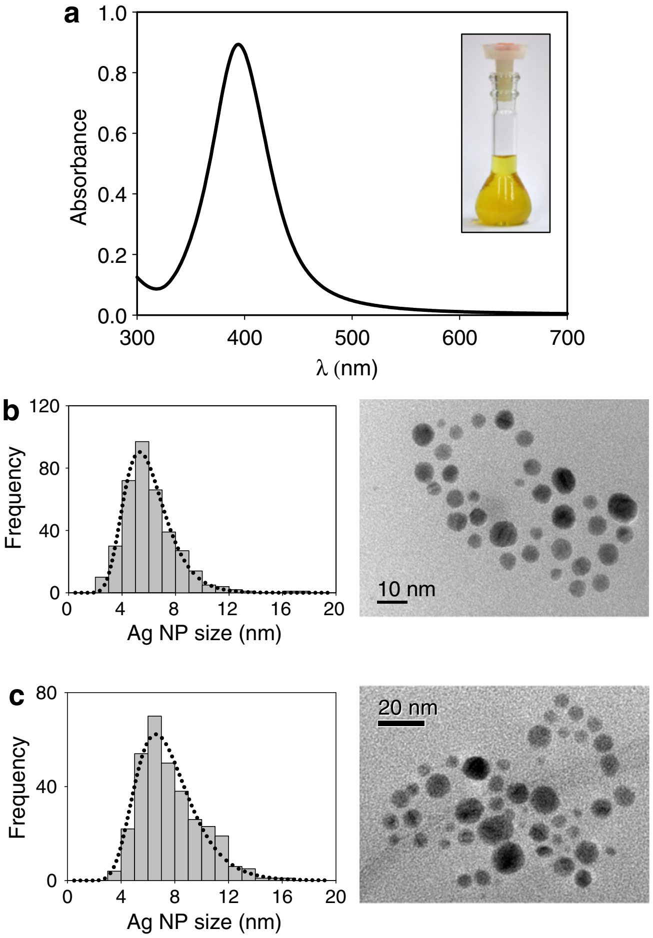

Fig. 1. (a) UV vis spectrum (1 in 4 dilution of the original solution). Inset: picture of the original AgNP solution. (b) and (c) TEM imaging of silver nanoparticles: (b) fresh nanoparticles: (left) histogram showing the size distribution of the AgNPs. The dotted line corresponds to the fitted log-normal distribution; (right) TEM image showing individual nanoparticles. (c) Nanoparticles three weeks after the synthesis: (left) histogram showing the size of the AgNPs. The dotted line corresponds to the fitted log-normal distribution; (right) TEM image showing individual nanoparticles.

2.3. Immobilization of AgNPs on Ti/TiO_2 surfaces

Titanium foils (99.7%, 0.25 mm thickness, obtained from Johnson-Mathey) were first polished with abrasive paper, sonicated for 45 min, and then polished at mirror grade with 1 μm diamond paste, further sonicated for 45 min, thoroughly rinsed and dried. The freshly polished Ti substrates were incubated in the as-prepared AgNP solutions (3.16×10^{-2} mg Ag/mL) in the dark at 4 °C for 24 h. The used solutions had been synthesized 1–3 weeks prior to their use for Ti substrate incubation, and therefore show some aging. For some AFM measurements the incubation was made for only 45 min in the same solutions.

To assess the role of the citrate capping some Ti foils were modified with AgNPs whose citrate capping had been replaced by polysorbates (Tween 20™) by a modification of a procedure reported for gold nanoparticles [51]. The concentration of the citrate and polysorbate-capped AgNPs solution used for the 24 h incubation was the same, and the modified substrates were analyzed by XPS.

2.4. Characterization of AgNPs on Ti/TiO_2

X-ray Photoelectron Spectroscopy (XPS) measurements of the silver nanoparticles immobilized on Ti/TiO_2 were performed using