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Non-covalent derivatization of aminosilanized titanium alloy implants Silver-enhanced coating of antibacterial organics

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ABSTRACT

The Ti6Al4V surface, commonly employed in biomedical prostheses, has been chemically functionalized by non-covalent binding to obtain improved antibacterial responses. The methodology combines the well-known antibacterial activity of silver(I) ions with those of other common antimicrobials such as carboxylic acids, *trans*-cinnamaldehyde, farnesol, and the broad-range antibiotic chloramphenicol. To this end, on previously aminosilanized Ti6Al4V surfaces (Material A) were attached via saline bonds (involving both ionic and dipole interactions) acetic, propanoic, benzoic, and sorbic acids (Material B). Likewise silver(I) acetate undergoes non-covalent cross-linking with the aminosiloxane-coated surface (Material C) after incubation at 60 °C for 48 h, thus resulting in a density of *ca.* 4.11×10^{-8} mol cm^{-2}. The coordinating ability of Ag(I) ions enables further attachment of the above-mentioned carboxylic acids onto the surface (Material D) and other neutral antimicrobials (Material E). Surface characterization has been accomplished using infrared spectroscopy and X-ray photoelectron spectroscopy, which support non-covalent binding. In particular, the existence of ammonium and carboxylate groups together with other intact functional groups (carbonyl, hydroxyl and amino) reveals that aminosiloxane-coated surfaces adhere via both ionic and weak interactions. As a proof of concept, adhesion and viability tests of *Staphylococcus aureus* on the functionalized surfaces show the adequate performance of the coverage at short and medium contact times with the surrounding cells.

1. Introduction

An emerging and far-reaching domain of applied research involves the creation of functional brushes on biomedical surfaces, usually based on titanium alloys and ceramic materials [1–7]. Chemical functionalization aims to prevent formation of bacterial biofilms [8], which are closely related to numerous chronic infections and common opportunistic pathogens such as *Staphylococci* [9,10]. Typical synthetic strategies on titanium surfaces bear on aminosilanization followed by covalent attachment of antibiotics (e.g. vancomycin) [11,12] or cell-binding peptides [13–15], although a plethora of antimicrobials and antifouling agents can be employed. Probably, silver ions constitute the most widely commercialized broad-spectrum antimicrobial agent [16–22] suitable for both medical devices and household goods. The biocidal action can also be exerted by silver nanoparticles (AgNPs) [23–28], and although it has long been conjectured that Ag(I) released from nanoparticles should be the actual toxicant responsible for cell lysis, the point has remained elusive for more than one decade. A recent study definitively rules out any particle-specific toxicity, showing the lack of antimicrobial action under anaerobic conditions that hamper Ag oxidation [29].

During the course of research aimed at engineering titanium surfaces with organic materials, we explored the ability of basic NH_2 groups, easily created by aminosilanization of medical titanium surfaces, to attach non-covalently organic bioactive compounds, which could also be in equilibrium with the surrounding physiological fluid. To this aim, we came across a facile and reproducible chemical functionalization by both carboxylic acids and assisted by Ag(I) ions. In short, ligand-based reactions occur between adjacent coordination sites on the metal center, which results in addition to an enhanced and prolonged antibacterial activity. While this protocol harnesses obviously the well-known coordinating ability of Ag(I) ions, its application to drug immobilization and release appears to be essentially unprecedented. Herein we discuss this surface chemistry by developing a relatively simple operational procedure to functionalize a typical medical alloy (Ti6Al4V) with carboxylic acids and other bioactive ligands for which covalent attachment is no longer required, and utilizing analytical methods to

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assess the interfacial properties and bioactivity of silver-enriched titanium organocomposites.

Pristine Ti6Al4V was first subjected to piranha oxidation and then aminosilanization followed by thermal cross-linking. An improved stepwise procedure has been recently developed to create a high density of amino groups on the surface [30]. As depicted in Scheme 1, the resulting material (A) was derivatized by reaction with carboxylic acids (thus affording ammonium salts, B), such as sorbic, benzoic and propanoic acids, which are effective against common bacteria [31–34]. The antimicrobial effect has been attributed to both ionic and non-ionic forms at different pH values, although the pH dependence is not directly related to acid dissociation [35]. Alternatively, the use of silver acetate gets access to the corresponding carboxylates (C) involved in non-covalent interactions with the underlying aminosiloxane layers. This material can now undergo ligand-exchange reactions with other broad-spectrum biocides or the aforementioned acids giving rise to multifunctional metal complexes (E and D, respectively) that inhibit bacterial adhesion and proliferation. As a proof of concept, we have assessed the behavior of the above functionalization against *Staphylococcus aureus*, responsible of a large number of infections occurring after implant surgery.

2. Materials and methods

2.1. Materials

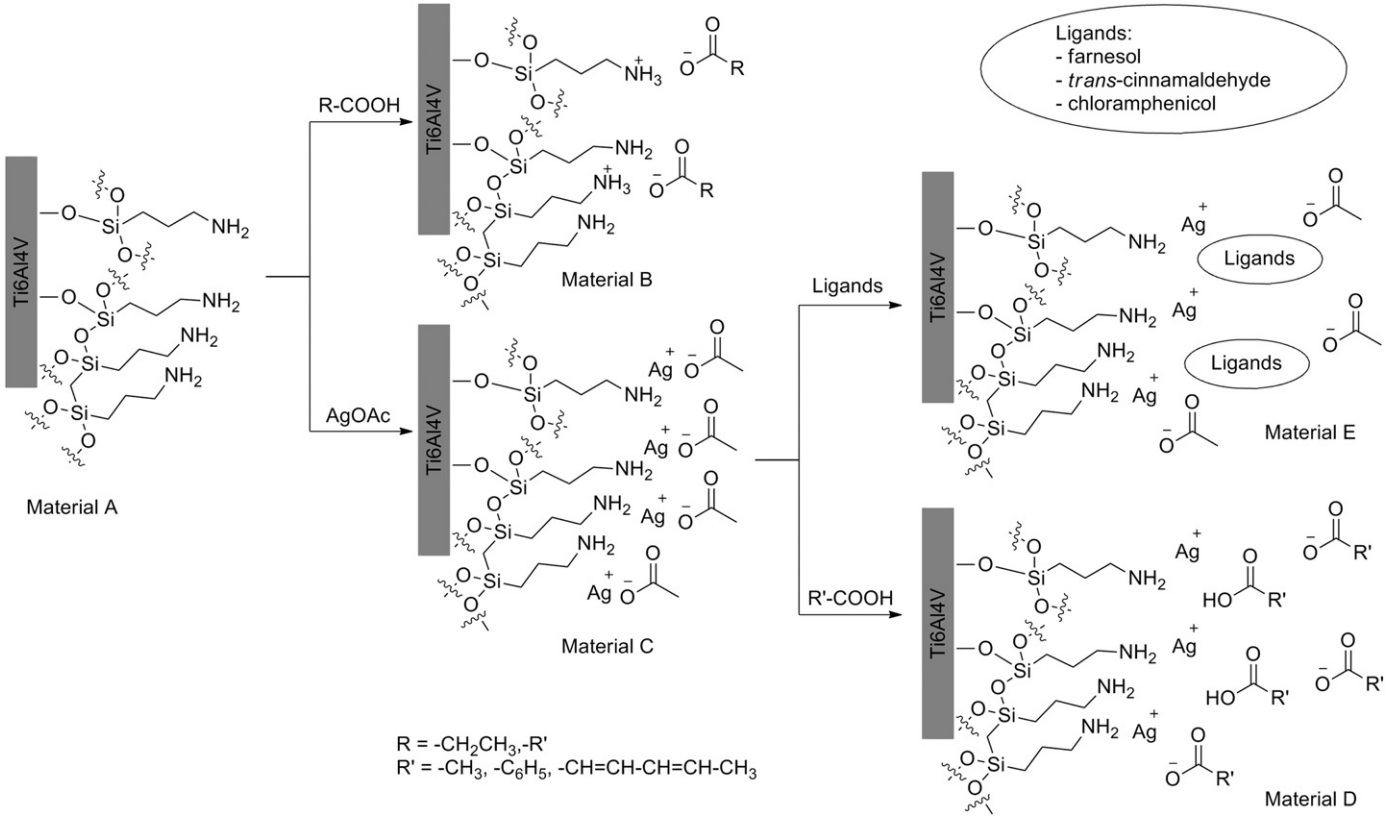
3-Aminopropyltrimethoxysilane (APTMS), sorbic acid, silver acetate, *trans*-cinnamaldehyde, chloramphenicol and other reagents were purchased from commercial suppliers and used as received. Anhydrous toluene was obtained following standard procedures and stored on Na wires. All aqueous protocols were performed with in-house distilled water. Ti6Al4V disks (ELI grade 23), 25 mm in diameter and 2 mm in thickness were obtained from DKSH Ltd (SWZ). For the sake of clarity, all chemical protocols refer to modification of only one side of the Ti alloy disks (i.e. the upper side exposed to the liquid, with a surface area of 4.91 cm^2).

2.2. Structural analyses

FT-IR spectroscopy (ATR) was used to check the modified layers. The spectrophotometer was equipped with a Smart Orbit™ specular plate ATR accessory (Thermo Scientific). A gold mirror was used to collect a background spectrum. X-ray photoelectron spectroscopy (XPS) was recorded in a Thermo Scientific spectrophotometer (Kα system) with monochromatized Al Kα radiation at 12 kV. Measurements were taken at a take-off angle of 90° with respect to the sample surface. The analyzed area is typically 0.16 mm^2. Survey scans over a binding energy range of 0–1350 eV were taken for each sample. High-resolution XPS data were obtained for nitrogen (1s), oxygen (1s), carbon (1s), silver (3d), chlorine (2p) and silicon (2s) atoms. Background subtraction, peak integration, and fitting were carried out with XPS Peak 4.1 software. Peaks were fitted after Shirley-type background [36].

2.3. Surface preparation

The disks were polished to a mirror finish by a three step protocol on a Buehler Beta Grinder–Polisher, and then cleaned in an ultrasonic bath with water, acetone and ethanol. The polished disks were immersed in piranha solution (1:1, 98% H_2SO_4, 30% H_2O_2), cleaned with water and ethanol, and placed on a crystallizer containing 1 M solution of APTMS in wet toluene for at least 6 h. The disks were sonicated in anhydrous



Scheme 1. Chemical derivatization of biomedical titanium alloys (Ti6Al4V) via aminosilanization. Grafting of broad-spectrum acid antimicrobials takes place by either direct formation of ammonium salts (B) or Ag-templated ligand exchange (D and E) from a silver-coated aminopolysiloxane precursor (C).