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The effect of different titanium nitride coatings on the adhesion of *Candida albicans* to titanium

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ARTICLE INFO

Article history:
Accepted 22 July 2013

Keywords:
TiN
Candida albicans
Adhesion
Surface property
Titanium implant

ABSTRACT

Objectives: The aim of the present study was an in vitro evaluation of the effects of different titanium nitride (TiN_x) coatings on *Candida albicans* (*C. albicans*) adhesion to titanium and to correlate these findings to differences in specific surface characteristics (surface topography, roughness, chemical component, and surface free energy).

Methods: TiN_x coatings were prepared by physical vapour deposition (PVD), a plasma nitriding process or a dual nitriding process. Surface properties were analysed by the optical stereoscopic microscopy, scanning electron microscopy, roughmeter, and drop shape methods. Quantity comparisons of *C. albicans* on the four surfaces were assessed by cell count and XTT reduction assays. Types of adhesive *C. albicans* were explored by SEM and confocal laser scanning microscope.

Results: The nitrided modifications were found to influence the surface properties and fungal susceptivity of flat titanium. Compared to flat titanium, fewer adhered *C. albicans* in yeast form were observed on the TiN-coated surface, whereas the plasma nitrided surface did not show any reduced potential to adhere *C. albicans* in hyphal or yeast form. The dual nitrided coating showed anti-fungal characteristics, although a small quantity of hyphae were identified. Our findings indicate that the Ti_2N phase is prone to *C. albicans* hyphae, while the TiN phase inhibits their adhesion.

Conclusions: Different TiN_x phases could influence the characteristics of *C. albicans* adhesion. TiN coating by PVD could be a potential modification to inhibit *C. albicans*.

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1. Introduction

Device-related infections can be traced to the adhesion of pathogenic microorganisms on surfaces of biomaterials, such as pacemakers, prostheses and implants.[1,2] Biomaterial provides an ideal interface for microorganism colonisation and aggregation, leading to the interface being covered with a pathogenic biofilm.[3,4] *Candida* species are the most common fungi associated with biofilm-related infections.[5]

Candida albicans (*C. albicans*) is the most prevalent fungus in the oral cavity, and its occurrence is strongly associated with denture-related stomatitis.[6–8] This particular fungal infection has been found in 60–65% of prosthesis carriers with diffuse

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0003–9969/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.archoralbio.2013.07.012

clinical manifestations. If asymptomatic subjects are taken into account, the percentage increases to 75%.[8] The pathogenesis of *C. albicans* infection includes both local factors (such as trauma, saliva, pH, permeability of dentures, and microbial plaques) and systemic factors (such as diabetes, kidneys problems, Xerostomia, and immunodeficiency) related to the host and to the *Candida* capability to adhere and proliferate in host epithelial tissues.[7] Dental prostheses and implants, which may rarely be removed for disinfection, have to be regarded as sources with a high potential for (re)infection for oral candidosis. Failing dental implants are associated with a microbial biofilm traditionally associated with periodontitis. Interestingly, *Candida* species not usually associated with periodontitis are commonly isolated from peri-implantitis lesions.[9–11] Yeast can colonise implant surfaces after implantation and play an important role in forming Candid-bacterial polymicrobial biofilms (the source of implant-related infections).[12] The major characteristics contributing to the virulence of *C. albicans* is its flexibility in attaching to biomaterial surfaces and forming biofilms on them when they are in contact with the mucosa.[13] Therefore, for both therapy and prevention, a more effective treatment to control microbial biofilm adhesion to dental materials is needed. However, many studies report that *C. albicans* can easily colonise and aggregate on titanium prostheses and implants.[1,14,15]

Although titanium is widely used in medical fields, it has some problems (physical abrasion, corrosion and aesthetic complains) when used in the oral environment.[16–18] Titanium nitride (TiN_x) coatings have recently been introduced into the dental field to solve these problems. Researchers have found that TiN_x performs excellently when used to cover titanium denture bases due to its high hardness, remarkable resistance to wear and corrosion, and intrinsic biocompatibility.[18,19] It also has aesthetic appeal (a characteristic golden colour due to the formation of an N-enriched layer[20]) when covering titanium abutments and fixed dentures, which allows the implant to be better camouflaged under the gingival tissue.[21] Annunziata et al.[22] recently reported that TiN coatings showed a dual function in promoting bone formation and reducing bacterial adhesion. Piscanec et al.[18] reported that nitridation of titanium implant surfaces under physiological conditions can promote the in vivo formation of bone-like material. These findings reflect a previously undetected degree of bioactivity of TiN in vitro and in vivo, which could be of great importance in the process of osteointegration of TiN-coated implants. As a new modification used in oral field, TiN_x is considered to be a potential reservoir for infection with *C. albicans*. However, scientific knowledge on the fundamental interactions between fungal cells and titanium substrata is poor and, to our knowledge, few studies are available on the adhesion of fungi to TiN_x coatings.

To address these limitations, we sought to determine (1) whether and to what extent TiN_x coatings influence the biological behaviour of *C. albicans*, and (2) whether these findings are related to differences in specific surface characteristics (surface topography, roughness, chemical components, and surface free energy).

2. Materials and methods

2.1. Preparation of substrates

Commercial titanium substrates (10 mm × 10 mm × 1 mm) were directionally ground to a 1500 grit silicon carbide finish. After cleaning by ultrasonic agitation and drying in hot air, they were divided into four groups. Group 1: Physical vapour deposition (PVD) of TiN-coated titanium (PVD TiN). A pulsed vacuum arc deposition system (YBHn IIA-1-001, Belarus Information Institution, Belarus) was used to deposit PVD TiN coatings. The chamber pressure was 7×10^{-3} Pa with an initial substrate bias of 900 V. After sputter cleaning for 5 min, the voltage bias was decreased to 100 V, and nitrogen gas was bled into the chamber to 150.0×10^{-3} Pa. TiN was deposited under these conditions for 1 h. Group 2: Plasma nitrided titanium (Plasma nitrided). A plasma ion nitriding furnace system (LD–30A, Wuhan Shoufar surface engineering Co., China) was used to deposit nitriding films. The nitriding process was performed at 850 °C for 4 h using a 100% N_2 atmosphere at a pressure of 300–400 kPa. Group 3: Dual nitrided titanium (Dual nitrided). Plasma nitrided combined PVD TiN films were prepared following these steps: deposition of plasma nitrided coating, cleaning organic contaminants by ultrasonic agitation, and deposition of an additional PVD TiN coating. Group 4: Flat pure titanium (Flat Ti). Well-polished, non-coating titanium served as a control. After ultrasonic cleaning, the substrates were sterilised by UV irradiation for 30 min.

2.2. Surface properties

OSM (Nikon SMZ1500) and SEM (Hitachi JSM-4800) were utilised to investigate the surface topography. A roughmeter (TR240, Beijing Shidai, China) was used to measure roughness. The around roughness average (R_a), the root mean square roughness (R_q), and the mean roughness depth (R_z) were collected. A drop shape analysis system (EasyDrop Standard, KRUSS, Germany) was used to evaluate the contact angles at 20 °C using ultra-pure water and glycerol. Eight measurements in different areas of each sample were obtained, and the average values of contact angles and the free energy (SFE) were calculated.

2.3. Preparation of C. albicans adhesion models

Standard *C. albicans* cells subcultured from ATCC 90028 strains and saliva collected from a healthy adult volunteer were prepared as described by Zhou et al.[23] The concentration of standard cells, 1×10^7 cfu/ml, was optimal for the adhesive study. One millilitre of sterile saliva was pipetted into each well of a 24-well plate containing eight substrates from each group. After 2 h at 37 °C, substrates were washed with PBS and transferred to a new plate. One millilitre of standard cells was added to each well and shaken on a rocker table (75 rpm) for 90 min at 37 °C. Each sample was gently washed in PBS to remove non-adhesive cells and placed in a new plate. Two millilitres of yeast nitrogen base medium (YNB) supplemented with 100 mM glucose was then added. After cultivating at 37 °C

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