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Antibacterial photocatalytic activity of different crystalline TiO_2 phases in oral multispecies biofilm

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ABSTRACT

Objective. Titanium dioxide (TiO_2) incorporation in biomaterials is a promising technology due to its photocatalytic and antibacterial activities. However, the antibacterial potential of different TiO_2 crystalline structures on a multispecies oral biofilm remains unknown. We hypothesized that the different crystalline TiO_2 phases present different photocatalytic and antibacterial activities.

Methods. Three crystalline TiO_2 films were deposited by magnetron sputtering on commercially pure titanium (cpTi), in order to obtain four groups: (1) machined cpTi (control); (2) A-TiO_2 (anatase); (3) M-TiO_2 (mixture of anatase and rutile); (4) R-TiO_2 (rutile). The morphology, crystalline phase, chemical composition, hardness, elastic modulus and surface free energy of the surfaces were evaluated. The photocatalytic potential was assessed by methylene blue degradation assay. The antibacterial activity was evaluated on relevant oral bacteria, by using a multispecies biofilm (*Streptococcus sanguinis*, *Actinomyces naeslundii* and *Fusobacterium nucleatum*) formed on the treated titanium surfaces (16.5 h) followed by UV-A light exposure (1 h) to generate reactive oxygen species production.

Results. All TiO_2 films presented around 300 nm thickness and improved the hardness and elastic modulus of cpTi surfaces ($p < 0.05$). A-TiO_2 and M-TiO_2 films presented superior

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photocatalytic activity than R-TiO_2 ($p < 0.05$). M-TiO_2 revealed the greatest antibacterial activity followed by A-TiO_2 (≈99.9% and 99% of bacterial reduction, respectively) ($p < 0.001$ vs. control). R-TiO_2 had no antibacterial activity ($p > 0.05$ vs. control).

Significance. This study brings new insights on the development of extra oral protocols for the photocatalytic activity of TiO_2 in oral biofilm-associated disease. Anatase and mixture-TiO_2 showed antibacterial activity on this oral bacterial biofilm, being promising surface coatings for dental implant components.

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1. Introduction

Despite the evidence of excellent dental implant therapy results, peri-implant mucositis and peri-implantitis disease can still occur if pathogenic bacteria accumulate on the implant surface and its components, such as abutments [1]. While peri-implant mucositis is characterized by inflammatory soft tissue infiltrate around the implant, peri-implantitis presents signs of inflammation combined with bone loss around osseointegrated implants [2]. According to a recent meta-analysis study, the overall prevalence of peri-implant mucosistis can be as high as 43%, while peri-implantitis prevalence is around 22% [3]. Therefore, the importance of preventing biofilm formation on implant structures and dental implant components is highlighted, as mucositis can potentially progressing into peri-implantitis if left untreated.

Once dental implant component, such as abutments are exposed in the oral cavity, their surfaces are immediately covered with an acquired pellicle and instantly subjected to bacterial colonization [4]. The genera *Actinomyces* and *Streptococcus* are the main initial colonizers in oral cavity and a common secondary colonizer associated with peri-implantitis is *Fusobacterium* spp. [5,6]. This bacterial colonization is directly influenced by the materials surface properties including chemical composition, surface roughness and surface free energy [7]. Hence, the development of films onto dental implant abutments and dental implant surfaces have been investigated as a possible approach to make their surfaces less prone to biofilm colonization, helping the long-term success of implant therapy [8].

For this reason, in order to reduce bacterial colonization to dental implants and their components, some photocatalytic compounds, such as titanium dioxide (TiO_2), have been incorporated to their surfaces [9–13]. When photocatalyzed TiO_2 produces reactive oxygen species (ROS) [14] that promotes the degradation of bacterial membranes, therefore presenting an antibacterial effect [15]. However, this process depends on the band gap of the materials, which can be different, depending on the crystalline form. The TiO_2 occurs in two main crystalline forms: anatase and rutile [16]. The band gap of TiO_2 corresponds to about 3.2 eV for anatase and around 3.0 eV for rutile and it can only absorb ultraviolet light (UV) (≤400 nm) [17,18]. Among UV light sources, the UV-A ($\lambda = 315–400$ nm) has been used in some studies [15,19–21] as the longer wavelength is less harmful to the host cells [15,22,23].

Several methods are used for TiO_2 deposition on biomaterials such as sol–gel [24,25], spin-coating [12,26], anodization [9,10,27], atomic layer deposition [28] and magnetron sputtering [11,13,29–31]. Magnetron sputtering is a extensively used method as it produces films with greater adhesion, hardness and hydrophilicity [19,30,32,33], and it is also able to generate isolated phases of anatase and rutile [34]. Therefore, it would be of great value aggregate the qualities of sputtered films with the promising antibacterial effect of TiO_2.

Even though the antibacterial effect of TiO_2 has been investigated in some previous studies using different deposition methods [8,11,12,35,36] it is difficult to perform comparisons between them [35] and, consequently, obtain consistent conclusions about the TiO_2 application. This is due to the high variability in the test conditions used for different studies. In one hand, studies reported that TiO_2 has an antibacterial effect [8–10,27,28,37–40], while on the other hand, different studies revealed no influence on early biofilm formation [9,36], highlighting the need for further studies.

Furthermore, there is no study that has investigated the TiO_2 photocatalytic and antibacterial activities on a multispecies biofilm composed by 3 peri-implantitis specific microorganisms, while simulating the oral environment in the implant abutment area, with a previous acquired saliva pellicle, which is a strong point about our study, since it resembles the *in vivo* situation. In addition, no study has correlated the bacterial adhesion on different crystalline phases of TiO_2 films during the light exposure, which is critically important, since different TiO_2 phases present different effects.

Therefore, in this study we developed TiO_2 films with different crystalline phases (anatase, rutile and a mixture of both) onto commercially pure titanium (cpTi) surfaces using magnetron sputtering, aiming to evaluate which crystalline phase would be able to produce more ROS after UV-A light activation, and then leading to greater biofilm reduction. Our hypothesis is that each crystalline phase will have a different antibacterial effect against the *in vitro* biofilm tested. For this, the physical–chemical, photocatalytic degradation and antibacterial properties of these titanium coatings were analyzed by using a periimplantitis-associated oral multispecies biofilm model composed of *Streptococcus sanguinis*, *Actinomyces naeslundii* and *Fusobacterium nucleatum*.

2. Materials and methods

2.1. Experimental design

CpTi discs (grade II, American Society for Testing of Material) (MacMaster Carr), 10 mm diameter and 2 mm thickness, were

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