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Effect of UV-photofunctionalization on oral bacterial attachment and biofilm formation to titanium implant material

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ABSTRACT

Bacterial biofilm infections remain prevalent reasons for implant failure. Dental implant placement occurs in the oral environment, which harbors a plethora of biofilm-forming bacteria. Due to its transmucosal placement, part of the implant structure is exposed to oral cavity and there is no effective measure to prevent bacterial attachment to implant materials. Here, we demonstrated that UV treatment of titanium immediately prior to use (photofunctionalization) affects the ability of human polymicrobial oral biofilm communities to colonize in the presence of salivary and blood components. UV-treatment of machined titanium transformed the surface from hydrophobic to superhydrophilic. UV-treated surfaces exhibited a significant reduction in bacterial attachment as well as subsequent biofilm formation compared to untreated ones, even though overall bacterial viability was not affected. The function of reducing bacterial colonization was maintained on UV-treated titanium that had been stored in a liquid environment before use. Denaturing gradient gel-electrophoresis (DGGE) and DNA sequencing analyses revealed that while bacterial community profiles appeared different between UV-treated and untreated titanium in the initial attachment phase, this difference vanished as biofilm formation progressed. Our findings confirm that UV-photofunctionalization of titanium has a strong potential to improve outcome of implant placement by creating and maintaining antimicrobial surfaces.

1. Introduction

Dental implants have become a popular restorative choice with an initial success rate of up to 98% [1]. Depending on implant type, this success rate declines over time and ranges between 90.1 and 95.4% after 5 years, with a further reduction to about 89% and 83% after 10 and 16 years, respectively – the longest observation period reported so far [2]. Older patients, those with systemic conditions, smoking status [3,4] or prior periodontal disease [5–7] are affected by an overall higher failure rate. Most complications can be attributed to lack of sufficient osseointegration and infection. Hence, complete and infection-free establishment of bone-implant integration has become a persistent challenge in oral rehabilitation. The major causes for implant-related infections and inflammatory responses are microbial biofilms, which can form on all currently employed implant materials [8–10]. Biofilm formation is a multi-step process that starts with the bacterial attachment to natural or artificial surfaces. This initial interaction between bacteria and surface can occur directly via charged groups (e.g. phosphoryl-, carboxyl-, and amino-groups) present on their complex cell surface layer [11]. Since the bacterial cell surface is in direct contact with the environment, their charged cell surface layer groups are able to interact with ions or charged molecules present on the implant material surfaces [12]. In addition to this direct interaction, microorganisms can exploit other molecules including host proteins that adhere to the implant material to achieve surface colonization [13]. In the oral environment relevant for dental implant dentistry, molecules derived from saliva such as the proteins involved in

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pellicle formation that provide additional bacterial adhesion sites as well as blood components, can attach to the implant material and change certain surface characteristics [14–16]. Therefore, implant surface characteristics and the molecules from relevant bodily fluids that can attach to the implant material are important determinants in the amount and composition of bacterial biofilm to be formed.

Recent approaches to address the challenge of implant failure include ultraviolet (UV)-mediated photofunctionalization of titanium (Ti) [17–19], a popular implant material due to its excellent biocompatibility, corrosion resistance and its ability to promote osseointegration [20–23]. UV irradiation leads to the modification of titanium implant surfaces from a hydrophobic to a superhydrophilic state and removes hydrocarbon contamination [17,24,25]. These extreme changes in surface properties have been studied extensively for their effect on enhancing osteoblast attachment and proliferation, which leads to greatly improved osseointegration of titanium implants [26–29]. Despite this very encouraging extensive research regarding bone-implant integration, very little is known to date about the effect of photofuntionalization on bacterial attachment and biofilm formation on titanium surfaces despite its importance for lasting implant success [30]. A recent report demonstrated that UV treatment of Ti surfaces can reduce attachment and monospecies biofilm formation of *Staphylococcus aureus* and *Streptococcus pyogenes* [30], the major pathogens for orthopedic implant infections. While this is a very promising observation, dental implants are exposed to a more challenging environment: the microbiota of the oral cavity. Extensive 16S rRNA gene sequencing and microbiome studies revealed that over 600 different oral microbial taxa colonize the various surfaces present in the mouth [31]. The bacterial species implicated in dental implant-associated diseases such as peri-mucositis and peri-implantitis are generally very similar to those associated with periodontal diseases [32,33]. Many of these bacteria are able to readily attach to surfaces including titanium implants and employ saliva and/or blood-derived proteins for enhanced attachment [16,34]. This ability to exploit host fluids for surface colonization presents an additional challenge in biofilm prevention, especially during implant placement, when the sterile surface becomes exposed to the oral environment and the surgical wound site.

In this study, we investigated if UV-treatment of titanium surfaces has an effect on the attachment and biofilm formation of complex oral microbial communities during time periods that are relevant for the initial implant placement and wound healing directly post-surgery. UV-irradiation-induced titanium surface properties were evaluated and bacterial biomass accumulation at different time points reflecting initial attachment and early biofilm formation events were determined in the presence of salivary and blood components. Community profiles of the attached microorganisms were compared between UV-treated and untreated titanium surfaces.

2. Materials and methods

2.1. Titanium disc preparation, surface analysis and UV treatment

Titanium (Ti) discs (20 mm in diameter and 1.5 mm in thickness) were prepared by machining commercially pure titanium (Grade 2). Titanium disks were autoclaved and stored in the dark for 4 weeks to standardize the age of the titanium, since titanium age is known to affect its biological and osteoconductive capabilities [35,36]. Titanium disks were treated with UV light for 12 min with a photo device (TheraBeam Super Osseo, Ushio Inc., Tokyo, Japan) immediately prior to use [37,38], while control discs were left untreated. The surface morphology of the discs was examined using scanning electron microscopy (XL30, Philips, Eindhoven, Netherlands) [26]. The hydrophilic and hydrophobic properties of the titanium discs were evaluated by measuring the contact angle of 10 μl ddH_2O [19].

2.2. Oral microbial community and culture conditions

We used a previously described cultivable microbial community representative of the complex oral microbiome as model system for bacterial attachment and biofilm formation [39,40]. The microbial community was grown anaerobically (80% N_2, 10% H_2, and 10% CO_2) at 37 °C in a modified rich medium (SHI-FSMS) developed to support a high number of oral taxa from human saliva samples (50% SHI medium [40], 25% filtered saliva, 0.5% mannose, 0.5% sucrose). Initial attachment of cells and biofilm formation were evaluated after 3 and 16 h incubation, respectively. Overnight oral microbial community culture was adjusted in fresh SHI-FSMS medium to an optical density (OD) at 600 nm of 1, for evaluation of attachment and to an OD_{600nm} of 0.1 for measurement of biofilm formation. For both types of experiments, one ml of the oral microbial community at the respective concentration was placed onto titanium discs immediately after UV-photofunctionalization or directly onto untreated discs in sterilized 12-well polystyrene culture plates (Fisher Scientific). Oral microbial community cultures at the two relevant concentrations used in this study were also inoculated directly into the polystyrene plate to serve as positive controls for bacterial attachment and biofilm formation. Additionally, UV-treated and untreated titanium discs as well as wells without discs were incubated with sterile medium to serve as background controls. Samples were statically incubated at 37 °C under anaerobic conditions for 3 or 16 h corresponding to the respective experiments. To evaluate the continuous effect of UV-treatment, UV-treated and untreated titanium discs were immersed in fresh SHI-FSMS medium for 24 h at 37 °C, under anaerobic conditions prior to inoculation of bacteria. After this pre-incubation period, the medium was removed and overnight oral community culture diluted to an OD_{600nm} of 0.1 was added to disc for analysis of biofilm formation after 16 h incubation. Concurrently, the diluted community culture was placed on UV-treated and untreated titanium discs surfaces that had not undergone pre-immersion in medium for comparison. For all experiments, medium was removed at the end of the incubation period; the discs were gently transferred into 6-well polystyrene culture plates and washed three times with 5 ml sterile phosphate-buffered saline (PBS) prior to further processing.

2.3. Crystal violet assay

A 0.5% crystal violet solution was used to determine biomass accumulation onto the titanium discs surface and control wells. The PBS-washed titanium discs were placed into a 12-well plate, submerged in one ml crystal violet solution and incubated at room temperature for 20 min. The discs were then carefully transferred to a 6-well plate and washed four times with 5 ml PBS to remove excess crystal violet. The plates were gently shaken for 5 min during the last two PBS washes to ensure complete removal of residual dye. After the final PBS wash, the discs were transferred to a new 12-well plate. One ml of 95% ethanol was added and the plate was incubated at room temperature on a rotatory shaker (VWR rocking double platform shaker model 200) at 250 rpm for 15 min. The ethanol solution containing the crystal violet stain retained by the biofilms was transferred into 1.5 ml cuvettes (USA Scientific) and the optical density at 595 nm was determined for total biomass evaluation. All experiments were performed in triplicate for each time point and repeated three times to ensure technical and