JJMIE

Volume 14, Number 3, September. 2020
ISSN 1995-6665
Pages 289 - 294

Jordan Journal of Mechanical and Industrial Engineering

The Antimicrobial Effect of UV-Activated Titanium Dioxide Material Deposited on Dental Implants

Abdul Hai Alami[1,4,*], M Adel Moufti[2], Mohammad Hamad[3,c], Kamilia Aokal[4], Abdullah Abu Hawili[5], Rawand Naji[2], Mohammad Faraj[4]

[1]Sustainable and Renewable Energy Engineering Department, University of Sharjah, P.O.Box 27272, Sharjah, United Arab Emirates
[2]College of Dental Medicine, University of Sharjah, P.O.Box 27272, Sharjah, United Arab Emirates
[3]College of Health Sciences, University of Sharjah, P.O.Box 27272, Sharjah, United Arab Emirates
[4]Center for Advanced Materials Research, Research Institute of Science and Technology, University of Sharjah, P.O.Box 27272, Sharjah, United Arab Emirates
[5]Sharjah Research Academy, P.O.Box 60999, Sharjah, United Arab Emirates

Received December 9 2019 Accepted July 28 2020

Abstract

This work presents a pilot study on the photoelectric effect of anatase titanium dioxide (TiO_2) deposited on grade 4 titanium discs on their antimicrobial properties. The focus will be especially directed towards dental implants applications. This study details specimen preparation and microstructural characterization by scanning electron microscopy, X-ray diffraction and Raman spectroscopy to ensure a homogenous coverage of the TiO_2 material on the discs. The samples were further tested to highlight the photoelectric response of titanium dioxide to ultraviolet radiation in the form of electrical current within the discs. Six discs (three bare Ti, and three coated with TiO_2) were seeded with a 5 µl of Escherichia coli culture. One disc of each group was subjected to the same UV light source used for the opto-electrical analysis for 0, 1 or 5 minutes. Bacteria on the discs were then harvested and incubated to examine number of viable cells. The obtained electrical properties confirmed that the surface-coating provides simultaneous oxidation-reduction driven reactions under the photoinduced catalytic activity. This activity proves the benefits of incorporating a TiO_2 layer in mitigating the number of active E-Coli bacteria in a microbial setup by as much as 21% after 5 minutes of UV exposure. This photoelectrical effect has a profound impact on the development of an in-situ oral disinfectant material deposited on titanium-based dental implants. It is expected that the approach will promote facile antimicrobial treatment for patients that is non-invasive and at the same time very effective.

© 2020 Jordan Journal of Mechanical and Industrial Engineering. All rights reserved

Keywords: Energy; titanium dioxide; photoelectric response; dental implants; surface modification; E-coli; anti-microbial; peri-implantitis;

1. Introduction

When teeth are lost, dental implants (titanium inserts) are placed into the jawbone in order to retain dental prostheses. In other words, "false teeth" are replacing missing teeth (Tagliareni and Clarkson 2015). Despite high costs associated with dental implants, the emergence and success of these implants have created a revolution in the dental profession and has greatly improved the quality of life for patients. It is estimated that *ca.* 300,000 dental implants were placed each year in the United States (Puleo and Thomas 2006). A trend of a significant and continuous increase in number of dental implant placement has been observed and is predicted. The dental industry continues to see a huge interest in the lucrative business of making titanium implants (Elani et al. 2018). Just like their natural predecessors, the integrity and survival of dental implants in the mouth is often compromised by bacteria adhering to the implant surface, secreting toxic by-products of their metabolic processes and causing inflammation and tissue loss around the implant. This eventually leads to the loss of implant retention in the bone, and ultimately its dislodgement and detachment. This phenomenon is known as peri-implantitis, and is reported to affect up to 20% of patients during 5–10 years after implant placement (Salvi et al. 2017).

Numerous procedures have been devised to mitigate bacterial colonization upon the implant surface. These include (i) coating the implant surface with antibiotic-releasing agents such as Ag and Zn, which are known to possess anti-microbial properties and (ii) coating with materials with known photocatalytic effects that become bactericidal under ultraviolet radiation (Norowski and Bumgardner 2009). To date, no surface treatment strategy has been completely successful in providing satisfactory antibacterial effect, and thus the quest for the ideal surface modification continues.

Despite its established antimicrobial effect, UV-activated TiO_2 has not been utilized in clinical dentistry

* Corresponding author e-mail: aalalami@sharjah.ac.ae

(Alami et al. 2020). This is due to two practical challenges for its use in the mouth: first, the difficulty to deliver UV to all aspects/surfaces of the implant/prosthesis (Norowski and Bumgardner 2009). These areas lie between teeth (interdental) or between the tooth and overlaying gum and already inaccessible for cleaning by the patient, and therefore may not benefit from the UV beam. Second, the very long UV exposure time required exposure duration to UV radiation to gain bactericidal effect is too long for most modern dental procedures (Norowski and Bumgardner 2009), and can cause a significant damage to DNA of the human cells.

This being said, various researchers were able to report on benefits of using TiO_2 material for their anti-microbial effects in the *invitro* or *invivo* animal settings. For example, Suketa et al. (2005) reported a substantial suppression in viability of two microbial species "*Actinobacillus actimmycetcmcomilans"* and *"Fusobacterium nucleatum"* to less than 1% under UV-A illumination for 120 minutes. More recently, Pantaroto *et al.* (2018) examined the antimicrobial effect of titanium discs coated with different types of TiO_2 (anatese and rutile) and concluded that when these were subjected to UV-A for one hour. The authors found that A-TiO_2 (anatase) and M-TiO_2 (mixture of anatase and rutile) exhibited an antibacterial action by 99.9% and 99% respectively, whereas R-TiO_2 (rutile) did not exhibit significant bacterial reduction. The tested microorganism was a biofilm of *Streptococcus sanguinis*, *Actinomyces naeslundii* and *Fusobacterium nucleatum*). There is a consensus amongst the researchers that a minimum of one-hour exposure to UV light is required to bring significant antimicrobial effect (Norowski and Bumgardner 2009; Suketa et al. 2005; Pantaroto et al. 2018; Choi et al. 2009 and Grischke et al. 2016). This renders the application of this technique impractical in clinical dentistry as it will be too difficult to keep the patient mouth open for one hour while shining the UV on the target. In addition, there is a concern over subjecting the live tissues to UV radiation to a focussed UV beam for this period of time.

The above studies contradict with other experiments that reported significant eradication of bacterial cells in much shorter time (Pleskova et al. 2016; Joost et al. 2015; Shiraishi et al. 2009; Zaborowska et al. 2015). In fact, one of these studies argued that the 60-minute exposure to UV light was sufficient to kill the bacteria regardless of the substrate they were cultured upon by employing the effect of the UV radiation on its own (Zaborowska et al. 2015). These studies have also used UV-illuminated TiO_2-coated titanium substrates and tested different stings of bacteria. Shiraishi et al. (2009) reported on the viability of suppressing of *Staphylococcus aureus* by 93% after 30 minutes of exposure to UV-A illumination. Zaborowska et al. (2015) reported elimination of 42% of *Staphylococcus aureus* after 15 minutes, while Joost el al. (2015) found a 75% reduction of the *Escherichia coli*'s viability after 5 minutes only of exposure to UV-A rays.

The varying results may be explained by the varying experimental methodology and procedures, as well as different bacterium species under investigation, not to mention the physiochemical characteristics of the anatase TiO_2. For example, Singh et al. (2011) examined the effect of various TiO_2 microstructural characteristics (pore size, surface area, aspect ratio, …etc.) on the bacterial cell adhesion, and concluded that the these characteristics significantly influence bacterial adhesion, with surface roughness being the major factor in enhancing this adhesion.

This study investigates the photoelectric generation of TiO_2-coated titanium discs under the influence of UV-A radiation, and the associated microbicidal effect against *E coli* microbes seeded over coated discs. The implants surface will be thoroughly cleaned and TiO_2 will be deposited upon it, and then subjected to UV radiation to amplify the possible photoelectric response that would be detrimental for microbial cells, thus acting as an antimicrobial agent. Titanium dioxide (TiO_2), with its high chemical stability, is a widely used photocatalyst in many environmental applications. With a bandgap of 3.2 eV, the photo-induced activity of anatase TiO_2 under UV radiation provide it with antibacterial properties that drive a chemical reaction (photocatalysis) and allow for the production of Reactive Oxygen Species (ROS). This in turn propagates the photodegradation of organic compounds, namely bacterial (Maness et al. 1999; Carp et al. 2004). In addition to this effect, the generated current could also be beneficial for the hard-to-reach areas of teeth and thus enhance the level of dental protection achieved.

2. Materials and Methods

2.1 Specimens (disc substrate and coating):

The prepared sample consists of three sets of three grade 4 titanium discs (nine in total), 10 mm in diameter and 2.5 mm in thickness (Shaanxi Yunzhong Industry Development Co., Ltd, China, sourced through Alibaba.com). The composition of the discs was examined using X-ray fluorescence (XRF) technique and confirmed 99.7% purity of the received discs (traces of aluminium (0.26%) and iron (0.04%) are found). The TiO_2 coating material was obtained from a suspension of 0.5g of TiO_2 crystalline nano-powder particles (size 20-25 nm purchased from Sigmaaldrich.com) in a solution of 0.25g ethyl cellulose and 1.75g of terpinol dispersed in 5 ml of ethanol. The mixture was sonicated to form a homogenous slurry and then deposited on the Ti discs via spin coating (WS-650Mz-23NPPB Spin Processor) at 5000 rpm for 30 sec. The discs were annealed at 450 ºC on the hotplate for 30 min.

2.2 Microstructural characterization:

Topographical inspection of the materials deposited on the titanium discs was done using a VEGA3 TESCAN SEM, operating at 30 kV acceleration voltage. The associated energy-dispersive X-ray spectrometer (EDS) was used for elemental analysis in two-dimensional mapping mode. Raman spectroscopy was carried out at room temperature in the back-scattering geometry using an inVia Raman microscope from Renishaw with its 514 nm laser running from 30 to 800 cm^{-1} with an exposure time of 30s and a laser intensity of 10%. X-Ray diffraction (XRD) measurements are taken with a Bruker D8 Advance DaVinci X-ray diffractometer with Cu Kα radiation operating at $\lambda = 1.5406$ Å

2.3 Opto-electrical characterization:

The specimens were subjected to UV-electronic characterization, the source of which was a Mineralight Multiband handheld long-wave UV lamp operating at 6 watts (115 VAC/60 Hz) and at 365 nm wavelength. The 1 cm discs with an exposed area of 0.4 cm^2 were kept at a constant distance of 5 cm from the UV light source subjecting the disc to a potential input of 4.57 W/cm^2 for the whole duration (~10 minutes) of the experiment. This optical characterization of the TiO_2 on titanium discs provides evidence that the electrical effect stems from the electromagnetic excitation.

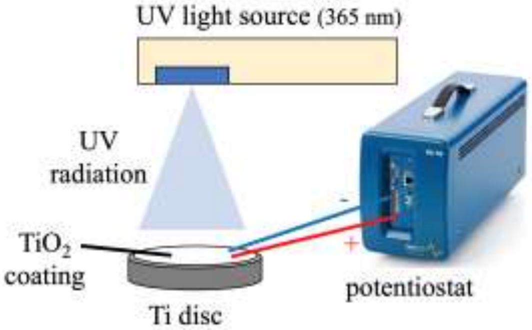

Figure 1. Optical characterization setup

The current was recorded with and without incident light using a Bio-Logic SP200 potentiostat in a 2-electrode setup on which two measurements have been conducted. One allowing for the control of the supplied voltage (0V) that is left constant for the duration of the experiment while the other applies a linear voltage sweep at 1mV/s from -0.1 V to 0.1 V. In the former, a 1-minute resting period is applied prior to current measurement. The experiment lasted for 10 minutes with a potential window of 50μV resolution. The latter engaged in no resting period and lasted for 200 seconds. The tests were also conducted on bare titanium discs as a reference.

2.4 Microbial testing:

The Gram-negative bacterium Escherichia coli (ATCC 25922) was selected for the current pilot study to evaluate antibacterial activity of TiO_2 disc in response to UV exposure. Escherichia coli culture was grown at 37ºC to log phase in Mueller Hinton broth with shaking at 150 rpm. The culture was then washed three times with sterile phosphate-buffered saline (PBS) and adjusted to a density of 0.5 McFarland in PBS. Finally, 5 µl of 0.5 McFarland E. coli suspension was spotted onto 6 discs; three of which were bare Ti discs (group 1), while the other were TiO_2-coated (group 2). One disc of each group was either subjected to UV ray for 5 minutes, or for 1 minute, or not subjected to UV light at all (negative control) to try to single out the best exposure time in this pilot study. The UV light (long wavelength) was the same UV light source used for the opto-electrical analysis, and wad placed at a distance of 1.2 cm. Each of the 6 discs was moved into a falcon tube containing 5 ml of sterile PBS, vortexed for 30 seconds and serially diluted. 5 µl of each dilution was spotted onto Mueller Hinton Agar, allowed to dry and plates were incubated at 37ºC for 24 hours. Following incubation colonies were counted to number of viable cells.

3. Results and discussion

3.1 Microstructural characterization:

The microstructural morphology of the deposited titanium dioxide film is shown in Figure 2 (a), with the energy dispersive X-ray spectroscopy (EDS) patterns shown in Figure 2(b) and (c) showing the homogenous distribution of the oxygen and the titanium. The surface of the discs shows the roughness inherent from the cutting process that was conducted to bring the discs to size. These patterns are coincidentally similar to those expected on the surface of maulers in teeth. This makes the results more relevant to the intended application.

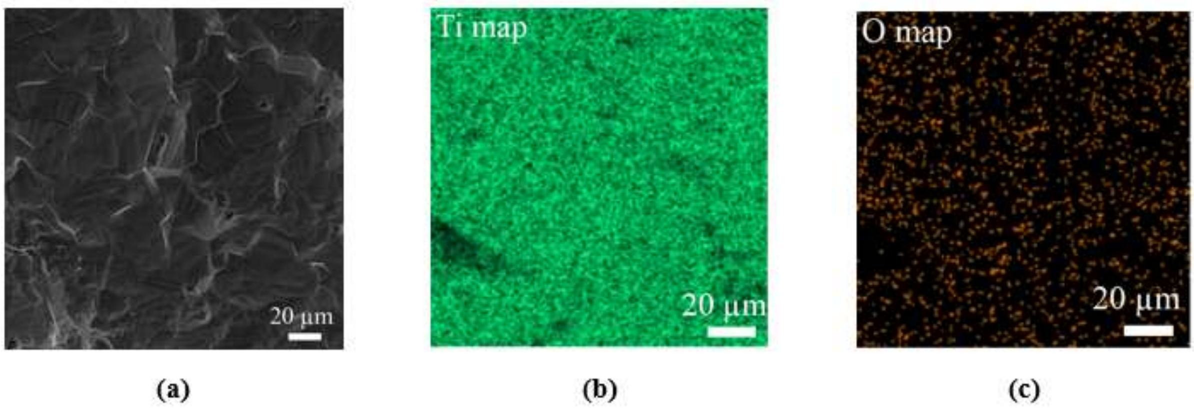

Figure 2. (a) SEM micrograph of the deposited titanium dioxide layer on the Ti disc with the EDS map of (b) titanium and (c) oxygen

 © 2020 Jordan Journal of Mechanical and Industrial Engineering. All rights reserved - Volume 14, Number 3 (ISSN 1995-6665)

The X-ray diffraction (XRD) patterns of the untreated discs with small traces of anatase titanium dioxide at 38.5º and 74.4º. The patterns are shown in Figure 3(a).

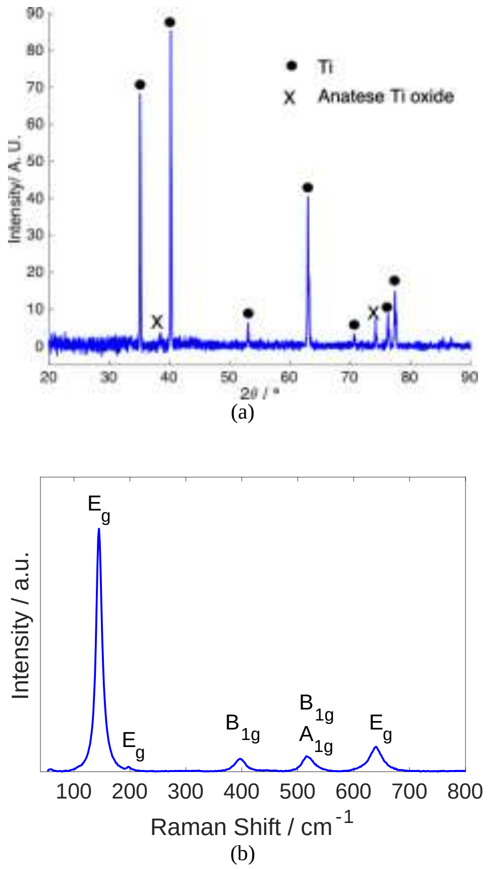

Figure 3. (a) X-ray diffraction patterns of bare Ti discs and (b) Raman spectra of anatase TiO_2 coating

The Raman spectra shown in Fgure 3(b) (Alami et al. 2020) shows six vibration modes that are characteristic of TiO_2 in the anatase phase. They correspond to: E_g (144 cm^{-1}), E_g (197 cm^{-1}), B_{1g} (399cm^{-1}), A_{1g} (513 cm^{-1}), B_{1g} (519 cm^{-1}), and E_g (639 cm^{-1}) (Ohsaka et al. 1978). The Raman test was also done for the bare discs, and no anatase TiO_2 peaks were detected.

3.2 Opto-electrical characterization:

The specimens were exposed to UV radiation while measuring the voltage and current generated due to this electromagnetic stimulation. The addition of TiO_2 is expected to generate enough charge via the interaction with UV radiation to produce the antimicrobial effects. The results show consistently low values of current readings with no externally applied excitation (either by UV radiation or voltage) shown as a black line in Figure 5 (a). Once the discs are illuminated via the UV source, the generated current, shown as a blue line in Figure 5(a), is seen to significantly increase compared with the discs in the dark up to the fifth minute (the ~320 s mark), where the measured current response for the illuminated condition is seen to take a sharp dip, most likely due to the natural threshold limit of the TiO_2 absorption of UV light. A cubical function curve fit was added to the data, shown as dotted blue and black lines in Figure 5(a), to assist in understanding the general trend of the photoelectric reaction to be essentially different, and it appears to be in favour of the UV illuminated discs (the more negative the current the higher the photogeneration). The aperiodic peaks of current that appear in Figure 5(a) are due to the expected recombination that occurs once the electrons are generated and are not transported through a hole-transport material (HTM) into a counter electrode that is absent in the current setup. Similar observation can be seen in the work of (Wang et al. 2014).

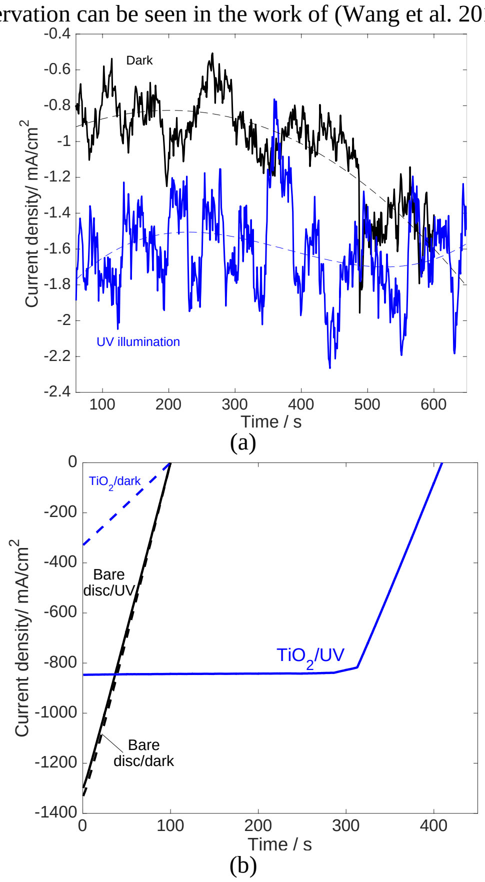

Figure 5. Current density vs. time over a) 0V supplied voltage and (b) a sweep voltage of -0.1 to 0.1V

On the other hand, with a linear change in voltage, a more prominent variation is seen with respect to dark and light conditions for discs with and without TiO_2 deposition. The highest obtained response is recorded for the discs with TiO_2 deposition are shown in Figure 5(b), where the area under the curve depicts the enhancement of the generated/stored charge within the active TiO_2 material. In general, the titanium dioxide layer exhibits a significant increase in excitation with longer UV exposure (see Figure 5a), apparently due to the prolonged residence time of the radiation that allows more photocurrent to be generated.

3.3 The antimicrobial effect:

The results for the bacterial culture for the tested discs are shown in Figure 6 for both coated and bare samples under longwave UV illumination. It is interesting to note that the surface with titanium dioxide has shown a decrease in the number of cells by around 38% under no UV illumination. Also, the UV illumination of the discs for 1 minute is seen to provide a photo-excitation that is mild enough for bacteria to flourish, as their numbers are seen to increase. On the other hand, the opposite effect of UV radiation and TiO_2 deposition is seen quite clearly for samples that received UV soaking time of five minutes. The titanium discs that has TiO_2 deposits exhibit the least count of E-coli cells compared with any other disc set, and also around 20% less colonies than the bare disc set that was also exposed to UV for five minutes.

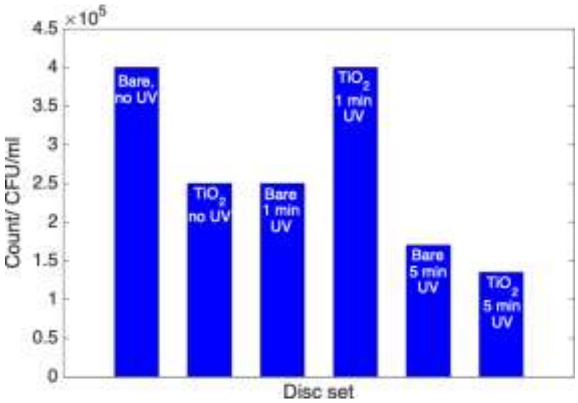

Figure 6. Bacterial cell count under various surface and illumination conditions

The results can be correlated with the photo-electric activity seen in Figure 5 where the reaction time of the discs covered in TiO_2 appear to benefit from an "activation time" that is around 5 minutes before appreciable changes to the bacterium cell count can take place. The bulge that the cubic fit exhibits (the blue dotted line of Figure 5(a)) indicates that the average current generation is increased with longer UV soak time.

4. Conclusions

This paper reports on the utilization of a thin titanium dioxide coating for antibacterial effect on dental implants. These implants are usually manufactured from a special alloy of titanium metal, and thus the TiO_2 layer would be compatible with the implant material and also biocompatible with living tissue. The TiO_2 layer is known to have a photoelectric effect under ultraviolet (UV) radiation, which is an added advantage as UV light sources are available at dental clinics for various applications such as photocuring of dental cement. The results obtained highlight the benefits of the added TiO_2 layer in reducing the numbers of active E-Coli bacteria in a microbial setup by as much as 21% after 5 minutes of UV exposure. This photoelectrical effect has a profound impact on the development of an in-situ oral disinfectant material deposited on titanium-based dental implants. Future work will include a more comprehensive microbial study protocol that includes different UV soak time, alternate microbial species as well as structural and compositional modifications of the deposited TiO_2 layer.

References

[1] Alami, A H et al. (2020). The Photoelectric Behavior and Antimicrobial Effect of Titanium Dioxide Deposition for Dental Implants. Key Engineering Materials, 865, pp.97–103.

[2] Alami, A H et al. (2018). Assessment of Calotropis natural dye extracts on the efficiency of dye-sensitized solar cells. *Agronomy Research*, 16(4), pp.1569–1579. [online]. Available from: https://doi.org/10.15159/AR.18.166 [Accessed April 1, 2019].

[3] Alami, Abdul Hai et al. (2018). Assessment of Photoelectrode Material Based on (TiO_2)1-x / (Al65Cu24Fe11)x in Dye-Sensitized Solar Cell Applications. *Materials Science Forum*, 928, pp.94–99. [online]. Available from: https://www.scientific.net/MSF.928.94 [Accessed April 1, 2019].

[4] Carp, O., Huisman, C.L. and Reller, A. (2004). Photoinduced reactivity of titanium dioxide. *Progress in Solid State Chemistry*, 32(1–2), pp.33–177. [online]. Available from: https://www.sciencedirect.com/science/article/pii/S0079678604000123?via%3Dihub [Accessed July 6, 2019].

[5] Choi, J.-Y. et al. (2009). Photocatalytic Antibacterial Effect of TiO 2 Film of TiAg on Streptococcus mutans. *The Angle Orthodontist*, 79(3), pp.528–532.

[6] Grischke J, Eberhard J, Stiesch M. Antimicrobial dental implant functionalization strategies -A systematic review. Dent Mater J. 2016;35(4):545-558. doi:10.4012/dmj.2015-314

[7] Elani, H.W. et al. (2018). Trends in Dental Implant Use in the U.S., 1999–2016, and Projections to 2026. *Journal of Dental Research*, 97(13), pp.1424–1430.

[8] Joost, Urmas et al. (2015). Photocatalytic antibacterial activity of nano-TiO_2 (anatase)-based thin films: Effects on Escherichia coli cells and fatty acids. Journal of Photochemistry and Photobiology B: Biology, 142, pp.178–185. [online]. Available from: http://www.sciencedirect.com/science/article/pii/S101113441400373X.

[9] Maness, P.C. et al. (1999). Bactericidal activity of photocatalytic TiO_2 reaction: Toward an understanding of its killing mechanism. *Applied and Environmental Microbiology*.

[10] Norowski, P.A. and Bumgardner, J.D. (2009). Biomaterial and antibiotic strategies for peri-implantitis: A review. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 88B(2), pp.530–543.

[11] Ohsaka, T., Izumi, F. and Fujiki, Y. (1978). Raman spectrum of anatase, TiO_2. *Journal of Raman Spectroscopy*, 7(6), pp.321–324. [online]. Available from: http://doi.wiley.com/10.1002/jrs.1250070606 [Accessed June 26, 2019].

[12] Pantaroto, H.N. et al. (2018). Antibacterial photocatalytic activity of different crystalline TiO2 phases in oral multispecies biofilm. *Dental Materials*.

[13] Pleskova, S.N., Golubeva, I.S. and Verevkin, Y.K. (2016). Bactericidal activity of titanium dioxide ultraviolet-induced films. Materials Science and Engineering: C, 59, pp.807–817. [online]. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0928493115304537.

[14] Puleo, D.A. and Thomas, M. V. (2006). Implant Surfaces. Dental Clinics of North America, 50, pp.323–338.

[15] Salvi, G.E., Cosgarea, R. and Sculean, A. (2017). Prevalence and Mechanisms of Peri-implant Diseases. Journal of Dental Research, 96(1), pp.31–37.

[16] Shiraishi, K. et al. (2009). Antibacterial metal implant with a TiO_2-conferred photocatalytic bactericidal effect against Staphylococcus aureus. Surface and Interface Analysis.

[17] Singh, A.V. et al. (2011). Quantitative characterization of the influence of the nanoscale morphology of nanostructured surfaces on bacterial adhesion and biofilm formation. PLoS ONE.

[18] Suketa, N. et al. (2005). An Antibacterial Surface on Dental Implants, Based on the Photocatalytic Bactericidal Effect. Clinical Implant Dentistry and Related Research, 7(2), pp.105–111.

[19] Tagliareni, J.M. and Clarkson, E. (2015). Basic Concepts and Techniques of Dental Implants. Dental Clinics of North America, 59, pp.255–264.

[20] Wang, Y. et al. (2014). A novel self-cleaning, non-enzymatic glucose sensor working under a very low applied potential based on a Pt nanoparticle-decorated TiO_2 nanotube array electrode. Electrochimica *Acta*, 115, pp.269–276.

[21] Zaborowska, M. et al. (2015). Bacteria-material surface interactions: methodological development for the assessment of implant surface induced antibacterial effects. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 103(1), pp.179–187. [online]. Available from: http://doi.wiley.com/10.1002/jbm.b.33179.

[22] Zhang, D. et al. (2018). Efficiency and high-temperature response of dye-sensitized solar cells using natural dyes extracted from Calotropis. In *5th International Conference on Renewable Energy: Generation and Application, ICREGA 2018*.