Photocatalytic Activity and Antibacterial Efficacy of UVA-Treated Titanium Oxides

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Statement of Purpose: Recent studies have shown UVA irradiation of titanium oxides may lead to photocatalytic activation (PCA) and produce reactive oxygen species (ROS). ROS have shown a bactericidal effect via damage to DNA or RNA or the rupture of cell membranes. Titanium oxide PCA has also been shown to accelerate the degradation of organic dyes, such as methylene blue (MB). Recent studies have attempted to use organic dye degradation generated through PCA as a predictor for bactericidal activity.[1] However, there is still some disagreement on which titanium oxide phase or phase combination produces the greatest PCA. The objectives of the current study were to compare the PCA generated MB degradation for a variety of titanium oxide phase combinations and compare the results of antibacterial efficacy against *Escherichia coli (E. coli)*.

Materials and Methods: 12.7-mm diameter commercially pure titanium grade 4 (CPTi) bar stock (Fort Wayne Metals, Fort Wayne, IN) was cut into 2-mm thick discs and anodized in three electrolytes as listed in Table I.

Table I – Anodization Electrolytes

Electrolyte	Sulfuric Acid	Phosphoric Acid	Oxalic Acid	Hydrogen Peroxide
A	3.5 M	0.19 M	0.25 M	0.75 M
B	5.6 M	-	-	-
C	1.4 M	0.03 M	-	0.75 M

A DC rectifier was used to anodize discs in 12 V, 10 s steps to a final forming voltage of 180 V for electrolytes A and C and 156 V for electrolyte B. Surface oxide phases were determined using thin-film X-ray diffraction (XRD). For the PCA and bactericidal studies, non-anodized titanium discs were used as controls. The PCA of each oxide (n = 8) was measured via degradation of 2 mL of a 0.001% MB solution under 20 W UVA (365 nm) LED light (~23 mW/cm^2) illumination for a 4-hour period. A one-way ANOVA ($\alpha = 0.05$) with post hoc Tukey analysis was used to determine significant differences in MB degradation between anodized surfaces and CPTi at selected times.

Bacterial cultures were prepared by inoculating tryptic soy broth (TSB) with isolated colonies of *E. coli* and incubating the cultures with aeration at 37°C until stationary phase (16-20 hours). Stationary cultures were then diluted 100-fold in TSB and grown to logarithmic phase to a concentration of approximately 10^8 colony-forming units per mL (CFU/mL). Ten-fold serial dilutions of the cultures were then prepared in TSB, and a concentration of 10^4 CFU/mL was used for inoculation. Discs were placed in a 24-well polystyrene cell culture plate and incubated with 1 mL of bacterial inoculum for 18 h. After incubation, the discs were washed twice with 1 mL of PBS and transferred to wells containing 1 mL of fresh PBS. Discs were exposed to UVA irradiation for 1 h. Additional samples were covered with foil and kept in the dark to serve as controls. Following irradiation, the discs were transferred to clean wells containing 1 mL of PBS to be sonicated and serially diluted for CFU counting.

Results: XRD (data not shown) revealed the anodized titanium oxide surfaces produced in each electrolyte to be primarily anatase phase (A180), rutile phase (B156), and an anatase/rutile mixture (C180). All anodized surfaces, showed accelerated MB degradation compared to non-anodized CPTi controls as illustrated in Figure 1.

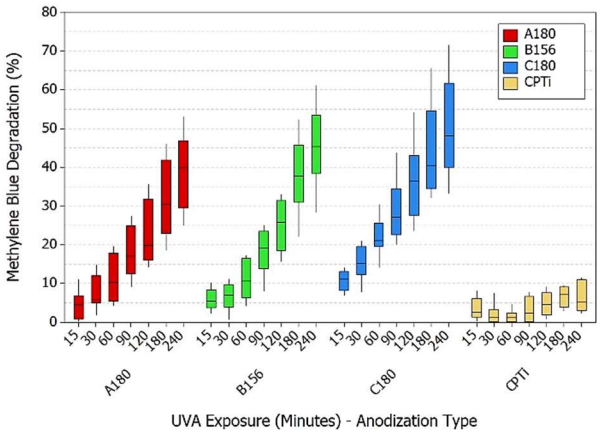

Figure 1. 20W LED MB Degradation

After 1 h of UVA irradiation, the surfaces achieved a bacterial reduction of 93% (CPTi), 95% (A180), 99% (B156), and 98% (C180) as shown in Figure 2.

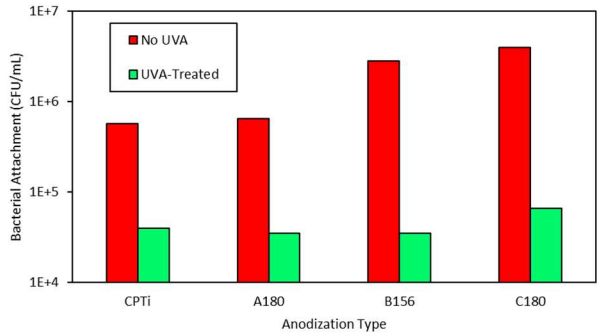

Figure 2. Bacterial Attachment Reduction

Conclusions: All anodized surfaces showed accelerated MB degradation compared to CPTi. After 1 h of exposure, the mixed phase surface of C180 showed the greatest degradation. All surfaces reduced bacterial attachment to approximately the same amount of CFU, however, B156 and C180 promoted more initial bacterial attachment, which resulted in higher killing efficiency. Further research is ongoing on the bacterial efficacy of the anodized coatings against commonly acquired bacterial species to determine the susceptibility to titanium oxide PCA.

References: [1](Pantaroto HN. Dent Mater. 2018;34(7):e182-e195.)