



Bactericidal Effects of HVOF-Sprayed Nanostructured TiO₂ on *Pseudomonas aeruginosa*

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Titanium dioxide (TiO₂) has been shown to exhibit photocatalytic bactericidal activity. This preliminary study focused on examining the photocatalytic activity of high-velocity oxy-fuel (HVOF) sprayed nanostructured TiO₂ coatings to kill *Pseudomonas aeruginosa*. The surfaces of the nanostructured TiO₂ coatings were lightly polished before addition of the bacterial solution. Plates of *P. aeruginosa* were grown, and then suspended in a phosphate buffer saline (PBS) solution. The concentration of bacteria used was determined by a photo-spectrometer, which measured the amount of light absorbed by the bacteria-filled solution. This solution was diluted and pipetted onto the coating, which was exposed to white light in 30-min intervals, up to 120 min. It was found that on polished HVOF-sprayed coatings exposed to white light, 24% of the bacteria were killed after exposure for 120 min. On stainless steel controls, approximately 6% of the bacteria were not recovered. These preliminary results show that thermal-sprayed nanostructured TiO₂ coatings exhibited photocatalytic bactericidal activity with *P. aeruginosa*.

Keywords nanostructured TiO₂, photocatalytic bactericidal activity, *Pseudomonas aeruginosa*

1. Introduction

Extensive studies have been conducted on the photocatalytic activity of titania (TiO₂) (Ref 1-4). These studies have found that TiO₂ can be used in the photodegradation of inorganic materials (Ref 1), organic compounds (Ref 2, 3), and in the destruction of bacteria such as *Escherichia coli* (Ref 4). Photocatalysis is a process in which an illuminated substrate decomposes compounds by oxidation. Upon illumination by ultra-violet (UV) or white light radiation, semiconductor substrate materials such as TiO₂ can create electron-hole pairs, which can generate free hydroxyl (OH⁻) radicals from the oxidation

of water, which tend to adsorb onto the TiO₂ surfaces. These radicals participate in secondary reactions with organic compounds, resulting in decomposition of the organic compound. This phenomenon may increase the applicability of titania for use in the destruction of microorganisms, which consist primarily of organic-based compounds (Ref 4). It has been shown that thin TiO₂ films are capable of producing a photocatalytic bactericidal effect to destroy *E. coli* and *Pseudomonas aeruginosa* bacteria (Ref 4, 5).

Most studies have focused on the use of thin films of TiO₂ to degrade organic compounds such as stearic acid, solid octadecane, or 2-propanol (Ref 3, 6). The thin films were fabricated by a dip-coating process on glass, by the pyrosol process deposition (Ref 3), or by chemical vapor deposition (Ref 7). While decomposition of the organic compounds was typically high after UV illumination, these thin films may find limited application in areas where films with higher bond strength and anti-wear resistance levels are required.

Thermal-sprayed coatings generally adhere well to the underlying metallic substrates. They are typically better suited for applications in which surface stresses may be applied to the protective layer. Lima and Marple (Ref 8) have shown that the bond strength levels (on steel substrates) of high-velocity oxy-fuel (HVOF)-sprayed nanostructured TiO₂ coatings are 2.4 and 1.6 times higher than those of conventional TiO₂ coatings deposited via air plasma spray (APS) and HVOF, respectively. Nanostructured TiO₂ coatings also exhibited superior wear resistance. Yang et al. (Ref 9) have shown that HVOF-sprayed TiO₂ coatings possessed significant photocatalytic activity to degrade phenol after exposure to UV radiation. These studies have led to the hypothesis that HVOF-sprayed TiO₂ coatings should possess photocatalytic

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bactericidal activity sufficient to destroy microorganisms such as *P. aeruginosa* bacteria, while being able to resist stresses from constant, routine cleaning.

P. aeruginosa is an opportunistic pathogen that causes serious infections in cystic fibrosis, intensive care, and immunocompromised patients (e.g. cystic fibrosis, cancer, and AIDS) (Ref 10, 11). These bacteria bind easily to stainless steel surfaces (Ref 12) to form highly organized communities known as biofilms (Ref 13). Given that stainless steel is widely used in the food sector, in hospitals, and in medical devices, it can serve as an effective reservoir and growth medium for colonies of *P. aeruginosa* that will cause serious infections.

It is important to point out that these stainless steel surfaces previously described, such as those used to fabricate tables, counters, and hoods, are being constantly cleaned by different types of cleaning tools (e.g., brushes), and continuously exposed to wear. Therefore, HVOF-sprayed nanostructured TiO₂ coatings with higher adhesion strength and anti-wear resistance levels should withstand the stresses of constant cleaning.

The objective of this study was to conduct a preliminary study focused on examining the photocatalytic activity of HVOF-sprayed nanostructured TiO₂ coatings to kill *P. aeruginosa*.

2. Experimental Method

2.1 Thermal Spraying and Particle Diagnostics

Nanostructured titania (TiO₂) (TiCP2, Altair Nanotechnologies, Reno, NV, USA) with a particle size distribution from $d_{10}=18\ \mu\text{m}$ to $d_{90}=38\ \mu\text{m}$ was deposited by using an oxy-propylene HVOF torch (DJ 2700-hybrid, Sulzer Metco, Westbury, NY, USA). The particle size distribution was determined by using a laser diffraction particle size analyzer (LS 13320, Beckman Coulter, Miami, FL, USA).

The coatings were deposited on grit-blasted 304 stainless steel circular coupons, each with 6 mm diameter and 5 mm height. The final coating thickness was approximately 100 μm . The stand-off distance (distance between the torch and the substrate) was 18 cm. The sensor of an in-flight particle diagnostic tool was positioned at this distance to measure the temperatures and velocities of the particles. At this position, and measuring approximately 3000 particles, the average particle temperature and velocity values were $2070 \pm 190\ ^\circ\text{C}$ and $900 \pm 90\ \text{m/s}$, respectively.

X-ray diffraction (XRD), based on Cu K α radiation, was used to determine the phases present in the final HVOF-sprayed coatings. A 2θ diffraction angle ranging from 20 to 60 $^\circ$, with a step size of 0.05 $^\circ$ and a step time of 2.5 s, was used.

2.2 Tests with *P. aeruginosa*

P. aeruginosa was grown on brain-heart-infusion (BHI) 1.5% agar plates at room temperature. The bacteria were

suspended in a phosphate buffer saline (PBS) solution to facilitate application on the nanostructured TiO₂ coating surface. A photo-spectrometer was used to measure the concentration of bacteria in the PBS solution. The absorbency was 0.10, which represents approximately 4 million bacterial cells per milliliter (mL) of solution. The samples were diluted to approximately 400 bacterial cells per 50 μL of solution.

The TiO₂ coating samples were mildly polished with 600 grit paper to remove the rough top layer. The samples were washed with water and 70% methanol, after which they were heated in an autoclave (SG-120, Steris Corp., Mentor, OH, USA) at 120 $^\circ\text{C}$ for 20 min and vacuum dried at 10 mmHg for 5 min to remove residual methanol and sterilize the surface. In total, 50 μL of the bacterial cell solution was pipetted on the surface. Furthermore, 50 μL of the bacterial cell solution was also pipetted on to polished 304 stainless steel shim stock samples (0.08 mm thick), which were used as controls. After contamination, the surfaces were exposed to cool, white fluorescent light (wavelength, $\lambda=380\text{-}750\ \text{nm}$) in 30-min intervals, up to 120 min. Some samples of the contaminated stainless steel controls were exposed to short wavelength UV illumination ($\lambda=260\ \text{nm}$) for 60 min. The bacterial solutions were removed after illumination and plated on to BHI 1.5% agar plates. The recovered bacterial cells in the solution were allowed to grow overnight in an incubator set at 37 $^\circ\text{C}$. Cell forming units (CFUs) were counted to record the growth of bacteria. It was assumed that each CFU originated from a single bacterial cell in the illuminated solution.

3. Results and Discussion

3.1 White Light Illumination

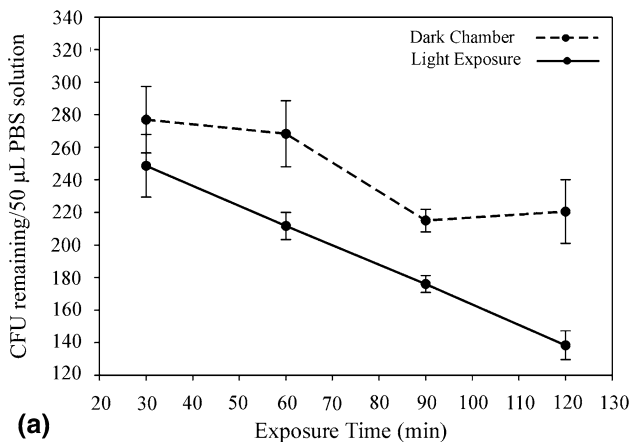
P. aeruginosa bacterial solution was pipetted onto the polished surfaces of the HVOF-sprayed nanostructured TiO₂ coatings and 304 stainless steel to study the photocatalytic bactericidal activity of the coating. Given the preliminary nature of the study, it was necessary to determine the appropriate source of illumination that would generate photocatalytic activity sufficient to destroy the bacterial cells.

VanHaecke et al. (Ref 12) and Giltner et al. (Ref 14) have shown that *P. aeruginosa* readily binds to 304 stainless steel and grows to form biofilm communities. In the present study, samples of the contaminated stainless steel controls were exposed to short wavelength UV illumination ($\lambda=260\ \text{nm}$) for 60 min. After this exposure time, all the bacterial cells were destroyed, and no growth was observed when the exposed bacterial solution was plated on to BHI 1.5% agar plates. The experiments were extended to solutions of 4,000,000 cells per 50 μL PBS solution. After exposure to short wavelength UV illumination for 60 min, 3 ± 0.1 cells remained. For these experiments, 7 samples ($n=7$) were studied. The standard error of the mean, calculated by dividing the standard deviation by the square-root of the number of

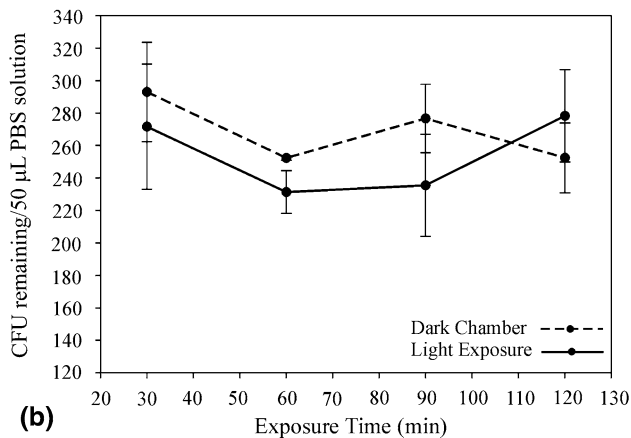
samples (Ref 15), is shown with the average. The standard errors of the mean will be reported with the averages of all other parameters mentioned in this study. Even though stainless steel surfaces have been shown to promote the growth of *P. aeruginosa* (Ref 12, 14), the high-energy short wavelength UV illumination killed a majority of the cells on the surface.

White light, with longer wavelengths (380-750 nm) and lower energy, was chosen as the source of illumination. Some photocatalytic activity was expected since the absorbance range of TiO₂ is about 388-415 nm for band gap values of 3.0 and 3.2 eV for the rutile and anatase phases, respectively (Ref 1, 2). Samples of the bacterial solution with an average concentration of 350 ± 0.5 cells ($n=8$) per 50 μL PBS solution were pipetted on to mildly polished nanostructured TiO₂ coatings and polished 304 stainless steel. The samples were exposed to 30-min intervals of cool, white fluorescent light. Figure 1 shows the average number of CFUs recovered from the surfaces after exposure. It was assumed that each CFU was equivalent to a bacterial cell. Error bars depicting the standard error of the mean are shown ($n=3$ for each point).

The results presented in Fig. 1(a) show that as the exposure time increases, the number of cells recovered



(a)



(b)

Fig. 1 Bacterial cells recovered from (a) nanostructured TiO₂ coatings and (b) 304 stainless steel control samples

from the nanostructured TiO₂ coating surfaces decreases. After 30 min of exposure, approximately 250 ± 4 cells remained on the surface; after 120 min of exposure, approximately 140 ± 2 cells remained on the surface. On the 304 stainless steel control surfaces, a slight decrease in the number of cells recovered from the surface was observed as the exposure time increased (Fig. 1b). However, in all the time intervals, the number of cells recovered from the steel surface was greater than 220 cells per 50 μL PBS solution. As shown by VanHaecke et al. (Ref 12), *P. aeruginosa* readily binds to and grows on 304 stainless steel surfaces. So, decreases in the number of bacterial cells recovered from the steel surfaces could probably be due to strong binding of the cells to the steel surfaces. The large error bars on the curve for the 304 stainless steel in Fig. 1(b) support this observation since it was difficult to remove the bacterial cells from the steel surfaces.

3.2 Dark Chamber Experiments

Estimation of the number of bacterial cells that remain permanently bound to the surfaces will enable a more accurate determination of the number of cells that were destroyed by the photocatalytic activity of the nanostructured TiO₂ coating surfaces. Figure 1 shows the average number of CFUs recovered from the nanostructured TiO₂ coating and 304 stainless steel surfaces after being held in a dark chamber, without any type of light exposure. On all the samples, the number of cells recovered was at least 200 cells per 50 μL PBS solution. With the exception of the 90-min exposure time point, the number of cells recovered on both the nanostructured TiO₂ coating and 304 stainless steel surfaces was close, to within 15% or less. The large deviation observed at the 90-min exposure time point was probably due to evaporation of the PBS solution on the TiO₂ surface and difficulty recollecting the solution for further testing. Other sources of error are also possible.

Since limited bacterial death occurred on the samples held in the dark chamber, the results shown in Fig. 1 suggest that about a third of the bacterial cells adhered to the nanostructured TiO₂ coating surfaces and were not recollected for growth on BHI 1.5% agar plates. Tables 1 and 2 show the approximate number of cells that adhered permanently to the nanostructured TiO₂ coating and 304 stainless steel surfaces, respectively. Each value is the difference between the original number of cells in the 50 μL PBS solution and the number of cells recovered, as shown in Fig. 1. The results shown in Table 1 suggest that as the residence time of the bacteria on the nanostructured TiO₂ coating increased, the number of cells bound to the

Table 1 Approximate number of cells typically bound to the nanostructured TiO₂ coating surfaces

Time, min	Original CFU, per 50 μL	CFU recovered, per 50 μL	CFU attached, per 50 μL
30	348	277	71
60	348	268	80
90	348	215	133
120	348	221	127

Table 2 Approximate number of cells typically bound to the 304 stainless steel surfaces

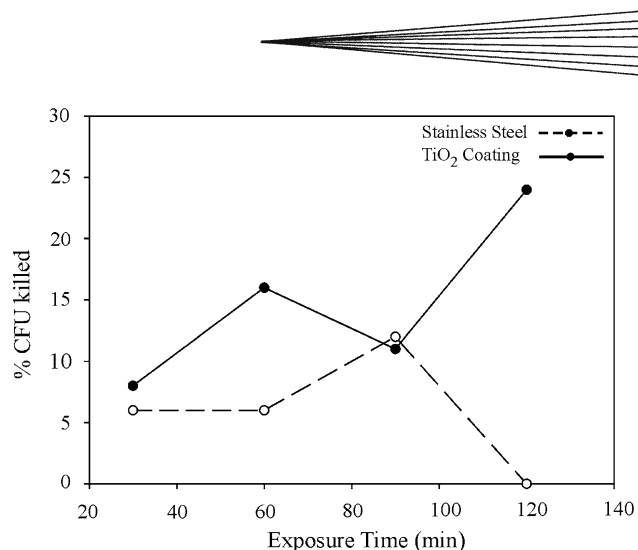
Time, min	Original CFU, per 50 μ L	CFU recovered, per 50 μ L	CFU attached, per 50 μ L
30	348	293	55
60	348	252	96
90	348	277	71
120	348	253	95

surfaces also increased. Though some changes occurred on the 304 stainless steel surfaces, greater adhesion occurred on the nanostructured TiO₂ coating surfaces (see Table 1 and 2). It should be noted that the calculated number of cells attached to the surfaces is an estimate since cells will grow and proliferate on all surfaces regardless of exposure conditions.

3.3 Photocatalytic Activity Versus the Formation of Biofilms

A comparison of the curves presented in Fig. 1(b) for the 304 stainless steel surfaces shows that the amount of cells recovered after light exposure ranges from 230 to 280 cells per 50 μ L PBS solution, while for the case of no light exposure, the range was 250-295 cells per 50 μ L PBS solution. These ranges were close since bacterial killing was assumed to be limited on the 304 stainless steel surfaces. This assumption is justified since 304 stainless steel surfaces do not exhibit significant photocatalytic activity and have not been shown to kill PAK bacterial cells. Therefore, these results suggest that the primary reason for a reduction in the number of recovered bacterial cells for stainless steel was due to adhesion and biofilm formation on the surface.

For the nanostructured TiO₂ coating surfaces, Fig. 1(a) shows that the amount of cells recovered after light exposure ranged from 140 to 250 cells per 50 μ L PBS solution, while for the case of no light exposure, the range was higher and narrower at 215-275 cells per 50 μ L PBS solution. These results, coupled with those from Table 1, indicate that even though cell adhesion probably occurred on the nanostructured TiO₂ coating surfaces, bacterial cell death also occurred due to photocatalytic activity. Figure 2 shows the approximate percentage of bacterial cells that were killed by the nanostructured TiO₂ coating surfaces after different periods of white light exposure. These estimates were obtained by subtracting the number of CFUs recovered and the number of CFUs bound to the surfaces from the original number of CFUs, and then dividing by the original number of CFUs. The figure also shows that as the exposure time increases, the percentage of cells killed by the photocatalytic activity of nanostructured titania increases to about 24% after 120 min of exposure to white light. As mentioned previously, the large deviation observed at the 90-min exposure time point was probably due to evaporation of the PBS solution on the TiO₂ surface and difficulty recollecting the solution for further testing. On the stainless steel surfaces, the percentage of bacterial cells that are killed is much lower,

**Fig. 2** Percentage of bacterial cells killed after exposure to white light

and is about 6% for most of the exposure times studied. The cell death observed on stainless steel was most likely due to natural causes, rather than chemical or photocatalytic activity.

Kikuchi et al. (Ref 4) have shown that TiO₂ thin films can kill 100% of *E. coli* bacteria after exposure to UV radiation. While the high-energy UV radiation will be more effective to kill bacteria, the simplicity of *E. coli* bacteria also reduces their resistance to the photocatalytic effect of TiO₂. The low percentage of killing (Fig. 2) of *P. aeruginosa* bacteria after 120 min of exposure to white light is probably due to the ability of these bacteria to form and embed itself in a biofilm matrix. This matrix consists of protein, polysaccharide, and nucleic acid (Ref 16), which may provide protection against photocatalytic destruction.

3.4 Coating Phases

It is well known that the photocatalytic properties of TiO₂ are due to the presence of its crystalline phases, such as the anatase and rutile phases (Ref 2, 9). The anatase phase is also known to increase the photocatalytic performance of TiO₂ (Ref 2). Figure 3 shows typical XRD patterns of nanostructured TiO₂ powder (Fig. 3a), unpolished (Fig. 3b) and polished (Fig. 3c) HVOF-sprayed nanostructured TiO₂ coating. The powder and the coatings are highly crystalline. As shown in the figure, anatase and rutile are the phases that are present in the samples. No brookite or titanium sub-oxide phases are present. The volume percentage of anatase (C_A) in the powder and coatings was determined according to the equation developed by Berger-Keller et al. (Ref 17):

$$C_A = \frac{8I_A}{(13I_R + 8I_A)} \times 100 \quad (\text{Eq 1})$$

where I_A and I_R are the x-ray intensities of the anatase (101) and the rutile (110) peaks, respectively. The volume percentages of anatase and rutile were approximately 100 and 0% in the TiO₂ powder, 20 and 80% in the unpolished

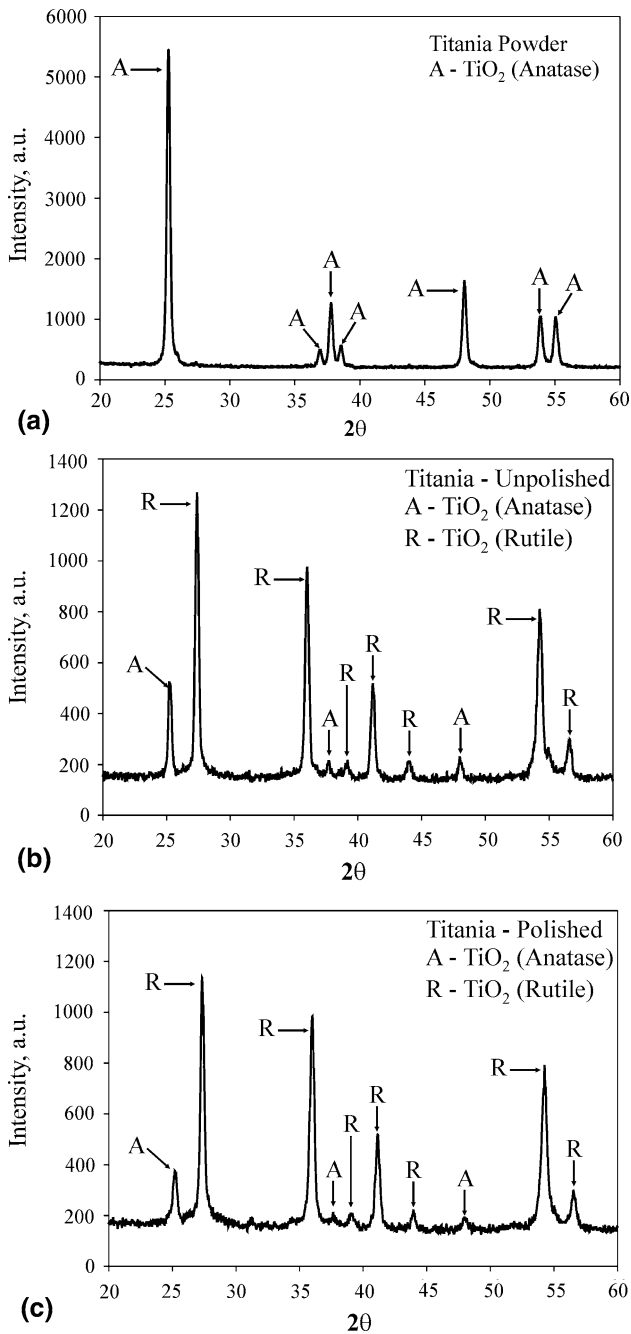


Fig. 3 Typical XRD pattern of (a) nanostructured TiO₂ powder, (b) as-sprayed (unpolished), and (c) polished HVOF-sprayed nanostructured TiO₂ coating

TiO₂ coating, and 17 and 83% in the polished TiO₂ coating, respectively. The volume percentages of the phases in the unpolished and polished coatings were approximately equal. Any difference that may occur in the photocatalytic bactericidal activity of these two coatings will probably not be due to the TiO₂ phases. The low percentage of anatase phase in the coatings and the lower photocatalytic activity of the rutile phase may have also contributed to the lower kill rates observed.

3.5 Future Work

An extension of this preliminary study to increase the kill rates of PAK on nanostructured TiO₂ coatings will be needed. Work is being conducted to use other thermal spraying methods such as flame spraying with air cooling to deposit coatings with increased anatase phase in the microstructure. Doping the nanostructured coating with metals such as silver may also show improved performance. Confirmation of the impact of the PAK biofilm on the kill rate will also be needed. A future study will include data on the bactericidal performance of nanostructured TiO₂ coatings to kill mutated PAK cells that are unable to produce biofilms. Finally, the authors were not able to gather statistically significant information on the bacterial killing rates on as-sprayed (unpolished) samples to report in this present work. These results will be reported in a future publication.

4. Conclusion

A preliminary study was conducted to examine the photocatalytic activity of HVOF-sprayed nanostructured TiO₂ coatings to kill *P. aeruginosa*. In total, 50 μL samples of phosphate buffer saline solution containing approximately 350 bacterial cells were pipetted on to nanostructured TiO₂ coating and 304 stainless steel surfaces. It was found that increasing the exposure time of the coating to white light increased the percentage of bacterial cells that were killed. The presence of the anatase and rutile phases in the TiO₂ coatings provided additional evidence that the photocatalytic properties of TiO₂ were responsible for the observed bacterial cell destruction.

The results of this study have shown that 24% of *P. aeruginosa* cells were killed by the nanostructured TiO₂ coatings after 120 min of exposure to white light. This was probably due to the ability of these bacteria to form and embed itself in a protective biofilm matrix. Further investigations will be necessary to determine the exposure times needed to achieve 100% killing on thermal-sprayed nanostructured TiO₂ coatings.

These preliminary results (proof of concept) are considered promising. However, some degree of uncertainty still exists regarding the amount and ability of *P. aeruginosa* to bind to nanostructured TiO₂ coating surfaces. Therefore, a detailed microbiological study of the binding and receptor mechanism of *P. aeruginosa* to TiO₂ will be needed. Both conventional, fused-and-crushed TiO₂ and nanostructured TiO₂ will be used for these future studies.

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