7-1-2017

Photocatalytic TiO2 coating of plastic cutting board to prevent microbial cross-contamination

Veerachandra K. Yemmireddy
The University of Texas Rio Grande Valley

Yen-Con Hung

Recommended Citation

Title: Photocatalytic TiO$_2$ Coating of Plastic Cutting Board to Prevent Microbial Cross-Contamination

Author names and affiliations.

Veerachandra K. Yemmireddy (kranti@uga.edu), Yen-Con Hung (yhung@uga.edu)

Department of Food Science and Technology, University of Georgia, 1109 Experiment Street, Griffin, GA 30223-1797, USA.

Corresponding author.

Yen-Con Hung

Professor, Department of Food Science and Technology, University of Georgia,

1109 Experiment Street, Griffin, GA 30223-1797, USA.

Phone: 770-412-4739, Fax: 770-412-4748, E-mail: yhung@uga.edu

Present/permanent address.

Department of Food Science and Technology, University of Georgia, 1109 Experiment Street, Griffin, GA 30223-1797, USA.

Abstract

Kitchen cutting boards are one common source of microbial cross-contamination in foods. In this study, a method was developed to create an antimicrobial coating on HDPE cutting board using UV-activated TiO_2 nanoparticles (NPs). The antimicrobial efficacy of the developed coatings was tested against *E.coli* O157: H7 for 3h at 0.5 ± 0.05 mW/cm^2 UVA light intensity. In addition, the effect of NP loading (0.0125, 0.0625, and 0.125 mg/cm^2), and surface treatment of coatings by oxygen plasma for 1 to 15 min on the bactericidal efficacy was investigated. Further, the bactericidal efficacy of the TiO_2 coated cutting board on repeated use (i.e. 1, 2, 3 and 5 times) was also evaluated. The results showed that by increasing the NP loading from 0 to 0.125 mg/cm^2 has increased the log reduction from 0.37 to 1.18 CFU/cm^2. However, no significant difference (P>0.05) in the reduction was observed between NP loadings at 0.0625 and 0.125 mg/cm^2. Oxygen plasma treatment of the coated surfaces for 5 to 15 min significantly increased (P≤0.05) the log reduction compared to control sample without plasma treatment. Under the tested conditions, TiO_2 coating with 0.0625 mg/cm^2 NP loading followed by oxygen plasma treatment for 5 min was found to achieve the greatest reduction up to 2.67 log CFU/cm^2. Also, the coated-surfaces were found to retain the original bactericidal property even after up to 5 times washing treatment. The developed TiO_2 coating on cutting board showed promise to mitigate the risk of microbial cross-contamination by providing a stable antimicrobial activity for extended use. Plasma treatment further enhanced the bactericidal property of the developed coatings without affecting physical stability.

Keywords: Cutting board; TiO_2; Antimicrobial coating; Plasma treatment; *E.coli* O157:H7.

1. Introduction

Microbial cross-contamination of foods from food contact and non-food contact surfaces is a challenge for both consumers and processors alike. Especially, cross-contamination from food contact surfaces such as utensils, knives, cutting boards, conveyor belts, packaging materials, and other equipment surfaces is a widely reported issue. According to U.S. Food and Drug Administration (FDA), contaminated surfaces are among the top 5 risk factors contributing to several foodborne outbreaks (FDA, 2009). Among several food contact surfaces, cutting boards are notorious for their potential to cross-contaminate foods with spoilage and/or disease causing microorganisms (Carpentier, 1997; Monnin et al, 2012; Moretro et al, 2011; Todd et al, 2009). It is required to thoroughly wash and sanitize cutting boards after each use to reduce or avoid the potential risk of cross-contamination.

Several studies have been conducted in the past investigating appropriate cleaning procedures for plastic and wooden cutting boards as well as other surfaces which come in contact with contaminated foodstuff in a food preparation environment (Barker et al, 2003; Cogan et al, 2002; Kusumaningrum et al, 2003). Previous studies show that cleaning with disinfectants such as hypochlorite and quaternary ammonium significantly reduce the number of viable bacteria on contaminated kitchen surfaces and dishcloths, whereas cleaning with detergent and hot water was much less effective (Thormar and Hilmarsson, 2012). Antimicrobial surfaces such as cutting boards consisting of antimicrobial agents like triclosan and silver NPs were already available commercially. However, these antimicrobial cutting boards were found to be not very effective. Moretro *et al* (2011) tested the antibacterial activity of triclosan-containing cutting boards and found that these cutting boards only work under low humidity, long exposure and clean conditions. Also, the antimicrobial activity of these cutting boards reduced after repeated

washing. Similar results were also observed for silver containing plastic cutting boards (Moretro et al, 2012; Berrang et al 2010). Moreover, the antimicrobial agents and/or their ionized forms tested in the above studies need to be released from the surface to show their potential biocidal activity. It may lead to eventual loss of efficacy as the antimicrobial agents worn out of the cutting board on regular usage. Alternatively, antimicrobial agents that can function only under direct contact of microbial cells can be immobilized on the surface to provide long-lasting antimicrobial property. In this case, the antimicrobial agent will not be transferred to the food. Semiconductor metal oxides popularly known as photocatalyst NPs were one example in this category.

Of the various metal oxides tested to date, titanium dioxide (TiO_2) has been recognized as the most promising photocatalyst because of its unique electronic structure, photostability, chemical inertness, commercial availability, low cost, and non-toxicity (Lan et al, 2013). In addition, TiO_2 shown to exhibit excellent photocatalytic antimicrobial activity over a wide range of pathogens (Foster et al, 2011). Antimicrobial properties of TiO_2 mainly attributed to the high redox potential of the reactive oxygen species (ROS) such as hydroxyl ($^\bullet OH$), superoxide (O_2^-) radicals and hydrogen peroxide (H_2O_2) formed by photo-excitation in the presence electron donors and acceptors such as environmental oxygen and water. TiO_2 has been approved by the FDA for use in human food, drugs, cosmetics, and food contact materials (FDA, 2014). Antimicrobial activity of TiO_2 coatings on various materials such as stainless steel (Yemmireddy and Hung, 2015a; Sobczyk-Guzenda et al, 2013), glass (Muranyi et al, 2009), polymer (Wei et al, 2014), and ceramic tiles (Hasmaliza et al, 2016) were reported. However, as per our knowledge, no study has been reported so far on the development of antimicrobial cutting board using photocatalyst TiO_2 NPs. Cutting boards coated or incorporated with photocatalyst metal oxides would help to

minimize the risk of potential microbial cross-contamination during food processing or preparation. In addition, our preliminary studies indicate that oxygen plasma etching of the coated surface may help to enhance the photocatalytic efficiency further. Hence, the overall goal of this study is to develop a physically stable antimicrobial coating on plastic cutting board using TiO_2 NPs and determine its bactericidal efficacy upon repeated use. Specific objectives include to: (i). Develop a method to create a TiO_2 coating on HDPE cutting board, (ii). Determine the effect of NP loading and the plasma treatment time on the antimicrobial property of TiO_2 coating and (iii). Determine the antimicrobial efficacy of coating after repeated use.

2. Materials & Methods

2.1. Selection of materials

TiO$_2$ (Aeroxide® P25, Sigma-Aldrich, St. Louis, MO, USA) NPs with an approximate particle size of 21 nm and specific surface area of 50 m^2 g^{-1} as per suppliers specification were used in this study. Suspensions of TiO_2 in ethanol solvent were prepared at different NP concentrations (i.e. 1, 5, and 10 mg/mL) by using a 20 kHz Ultrasonic Processor (Model CPX 500, Cole Parmer Instruments, Vernon Hills, IL, USA). All the suspensions were sonicated for 15 min to obtain a homogenous mixture of NPs in ethanol. The prepared suspensions were then used for coating on a plastic cutting board. A high-density polyethylene (HDPE) cutting board (96" x 48" x 0.75") of black in color was purchased from a commercial supplier (CuttingBoard.com, Redmond, WA, USA) and cut into 4 x 4 cm^2 size coupons. The black colored cutting board was selected to provide better contrast and visual observation of white colored TiO_2 coating. Before coating, all the coupons were thoroughly cleaned with soap and water then air dried at room temperature.

2.2. Development of coating

Several preliminary studies were conducted to develop a method to create a physically stable TiO$_2$ coating on the plastic cutting board. A hot-press method first investigated for this purpose. Initially, suspensions of TiO$_2$ in ethanol at different NP concentrations (1, 5, and 10 mg/mL) were sprayed on a pre-heated (85±5 °C) stainless steel (SS) plate (4 x 4 cm^2) using an airbrush sprayer (Badger®, Model #200, Franklin Park, IL) by keeping the flow rate, distance from the spray head to the substrate, and the spray time as constant. Later, a plastic cutting board coupon (4 x 4 cm ^{2}) that has to be coated with TiO$_2$ was placed against TiO$_2$ coated SS plate of 85±5 °C. The whole assembly was then placed under a Carver® press (Model-M, Fred S. Carver Inc., Menominee Falls, WI, USA) and compressed at a pressure ranging from 5000 to 15000 psi for about 1 to 2 min in order to transfer the TiO$_2$ coating from the SS plate onto the plastic cutting board. Afterwards, the created coatings on the cutting board were dusted using a Crayola paint brush to remove loosely adhered NPs and the gain in weight of the coupons were measured using a calibrated balance. Multiple coatings of TiO$_2$ (i.e. single and double) were created in a similar fashion. The coatings were then tested for their photocatalytic activity by measuring the methylene blue (MB) decay rate under UVA light of 1 mW/cm^2 intensity as described in Yemmireddy and Hung (2015b) with modification. The results showed that the TiO$_2$ coating with 10 mg/mL NP concentration had highest photodecay rate followed by 5 and 1 mg/mL, respectively (data not shown). No significant difference in the decay rate observed between single and double coating. Hence, TiO$_2$ single coatings at 10 mg/mL were further tested for their photocatalytic bactericidal efficacy against *E.coli* O157: H7 (5-strain). The coatings on the cutting board have shown a reduction of 1.15, 2.19 and 5.71 log CFU/cm^2 after exposed to UV-A light for 1, 2, and 3 h, respectively. However, upon reusing the same coupons for multiple times, the bactericidal efficacy of the coatings for 3 h UV-A light exposure has reduced to 3.01 and

1.87 log CFU/cm^2 after 2 and 3 times, respectively. This gradually reduced photocatalytic bacterial property after repeated use could be attributed to a possible loss of NPs from the coating and the lack of physical stability. To address this issue, the coating procedure was further modified to achieve physically stable coating.

In the next stage of experiments, an exact amount of NPs evenly applied onto the cutting board followed by precise heat treatment to bond the NPs to the plastic surface in order to form a stable coating. Briefly, a 200 µL of the prepared TiO$_2$ suspensions in ethanol (i.e. 1, 5, and 10 mg/mL) were pipetted and uniformly spread on the HDPE coupons using a wire loop in order to achieve a 0.0125, 0.0625, and 0.125 mg/cm^2 TiO$_2$ loadings on each coupon, respectively. These coupons were then placed under an infrared (IR) light at a distance of 45 cm from the lamp for about 2 min to evaporate the solvent and leaving behind an unstable and uniform layer of TiO$_2$ NPs on the HDPE surface. Later, these coupons were either: (i) simply subjected to heat treatment in a hot air oven (Lindberg/Blue M mechanical oven, Asheville, NC, USA) at a temperatures ranging from 80 to 140°C for a period of 30 to 60 min, or (ii) compressed under a Carver® press with 5000 PSI pressure for 2 min following each heat treatment. The purpose of the heat treatment is to soften the surface of plastic enough to make a strong adhesive bond with the NPs, and the pressing step helps to further push the NPs into the plastic to achieve physically stable coating. Multiple coatings of TiO$_2$ (i.e. single or double) were created in a similar fashion. Subjective analysis of these coated samples under a digital light microscope 20x-200x (Celestron LLC, Model# 44308, Torrance, CA, USA) after washing coupons vigorously under running water for multiple times did not show a significant difference between just heat treated and/or heat treatment plus Carver pressed coatings. Hence pressing step was eliminated from the final

coating protocol. The heat treatment at 140ºC for 60 min was found to be optimum to achieve physically stable coating as well as maintaining the structural integrity of the cutting board.

2.3. Surface characterization and oxygen plasma treatment of the coatings

The surface of TiO_2 coated cutting boards were subjected to oxygen plasma treatment using a plasma cleaner (Model PDC-001, Harrick Plasma, Ithaca, NY, USA) at high RF power setting for 1, 5, and 15 min, respectively. For the surface morphological characterization of the plasma treated and untreated surfaces, top-view and cross-sectional examinations of coated coupons were carried out with a scanning electron microscope (FEI Teneo, Hillsboro, OR, USA) operating at 10 kV. Local elemental information of the coatings obtained with energy dispersive spectroscopy (EDS) (Oxford Instruments, Concord, MA, USA).

2.4.Bacterial cultures and Inoculum preparation

Five strains of *E. coli* O157: H7 isolated from different sources: E009 (beef), EO932 (cattle), O157-1 (beef), O157-4 (human), and O157-5 (human) were used in this study. All bacterial strains were stored at -70 $^{\circ}$C in tryptic soy broth (TSB) (Difco Laboratories, Sparks, MD, USA) containing 20 % glycerol and revived when necessary for experimental procedures. Prior to the experiment, cultures were activated at least twice by growing them overnight in 10 mL of TSB at 37 $^{\circ}$C. Later, one loop full of each bacterial strain was cultured separately in 10 mL of TSB and incubated on a shaker at 37°C and 230 rpm for 16 h. Following the incubation, the cells were harvested by sedimenting at 4000 x g for 12 min. The supernatant was decanted and the pellet re-suspended in 10 mL of sterile phosphate-buffered saline (PBS, pH 7.2). An equal volume (i.e. 2 mL) of each strain suspension was combined to obtain a 10 mL of a five-strain cocktail containing approximately 10^7 CFU/mL. Cell concentration was adjusted by measuring the absorbance of bacterial suspension at 600 nm using a UV/Vis spectrophotometer (Model DU

520, Beckman Coulter Inc., Brea, CA, USA) and confirmed by plating 100 μL portions of the appropriate serial dilution on tryptic soy agar (TSA) (Difco Laboratories) plates incubated at 37 °C for 24 h.

2.5. Testing photocatalytic bactericidal property

Prior to each experiment, the test samples were sanitized first by dipping them in a 70% ethanol for about 1 min and placed in a sterile petridish with a Whatman® No.2 filter paper at the bottom. The petri dishes containing sanitized coupons were then sterilized under germicidal UV light (254 nm) for 30 min in a biosafety cabinet. Samplings of these coupons after UV light treatment confirmed the absence of any bacteria. An aliquot of 200 μL of cell suspension was inoculated on each coupon as 20 drops of 10 μL each, and an equal size (4 x 4 cm^2) of transparent, sterile polyethylene film was placed on top of the inoculum as a coverslip to make a uniform contact with the coating. The filter paper at the bottom of the petri dish moistened with a 2 mL of sterile DI water to maintain humidity during the photocatalytic disinfection treatment. Later, the inoculated samples were illuminated with a UVA light system with four 40 W lamps (American DJ®, Model # UV Panel HP^{TM}, LL-UV P40, Los Angeles, CA , USA) at 0.5 ±0.05 mW/cm^2 for 3 h. The light intensity reaching on top of the sample was measured using a UV radiometer (UVP®, Upland, CA, USA) with a peak sensitivity of 365 nm. After the treatment, the survived cells were recovered both from the surface of the coupon and the plastic coverslip using a sterile swab in a 4 Oz Whirl-Pak® bag wetted with a solution containing 9.8 mL phosphate buffer saline (PBS) and 0.1% Tween 80, and stomached at medium speed for 1 min using a stomacher (Stomacher® 80). An uncoated coupon under UVA light was used as a control. Appropriate serial dilutions were prepared and plated on *E.coli* O157: H7 selective Sorbitol-

MacConkey agar (SMAC). The plates were incubated at 37°C for 24 h, and the log survival was reported as CFU/cm^2.

2.6. Testing bactericidal property of the coatings after repeated use

To determine the effect of repeated use on the bactericidal efficacy of TiO$_2$ coatings, the test samples were subjected to multiple use conditions by following the procedure described in Yemmireddy and Hung (2015a). After simulated use for 2, 3, and 5 times, the samples were then tested for their antibacterial property as per the procedure described in section 2.5.

2.7. Statistical analysis

All the tests were replicated three times. Analysis of variance (ANOVA) was used to determine the significant difference between the means. Least significant difference (LSD) of means tests was done for multiple comparisons. *P*-values of less than 0.05 were considered significant. Statistical testing was performed using Statistical Analysis System (SAS/STAT 9.3, 2011).

3. Results and discussion

3.1. Surface characteristics of the coatings

TiO$_2$ coatings on the cutting board at different NP loadings were shown in Fig 1. In general, the coatings on the cutting board were smooth and uniform in appearance. Also, increasing the TiO$_2$ loading from 0.0125 (Fig. 1A) to 0.125 mg/cm^2 (Fig. 1C) has increased the brightness and opacity of the coatings. This can be explained due to the presence of more surface exposed NPs with increasing TiO$_2$ load. Similar results were noticed by Ratova and Mills (2015) when increasing TiO$_2$ loading from 5 to 30 wt% in a composite LDPE film. Also, at the same NP loading, no significant difference was noticed between single and multiple coatings (i.e. Fig. 1B vs 1D and Fig. 1C vs 1E) when observed under the light microscope. Moreover, all the coatings

10

on the cutting board were found to exhibit excellent physical stability upon rubbing with thumb as well as vigorous washing under running water.

Cross-sectional SEM analysis of the coatings revealed that the coatings have a mean thickness of 461 ± 15.6 (A), 505 ± 10 (B), and 551 ± 32 (C) μm for NP loading of 0.0125, 0.0625, and 0.125 mg/cm^2, respectively (Figs. 2A1, B1, and C1). Surface topography of the coatings by SEM (top-view) analysis indicates that by increasing TiO$_2$ loading from 0.0125 to 0.125 mg/cm^2, the distribution of NPs and the effective surface coverage of the coating has increased significantly (Figs. 2A2, B2, and C2). Further, EDS analysis of the SEM micrographs confirmed that the Ti content in the coating has increased with increasing NP loading. EDS results showed that the surface of the coatings in samples A, B, and C were composed of 11.5, 46.2, and 61.3 wt % of elemental Ti relative to the uncovered area of elemental C, respectively (Figs. 2A3, B3, and C3). This indicates that within a given area of the coating, the presence of NPs were highest in sample C followed by sample B and sample A, respectively. Also, it should be noticed that the grain size of the NPs in the coating was increased with increasing TiO$_2$ loading and aggregated into several clusters. This can be partly attributed to the method of coating used in this study and also the patterned surface topography of the original HDPE cutting board which favors the coating suspension to form selective zones of deposition where NPs aggregate together as several clusters or layers.

3.2. Effect of oxygen plasma treatment on surface characteristics

Fig. 3 shows the SEM images of TiO$_2$ coatings at different NP loadings [0.0125 (A), 0.0625 (B), and 0.125 (C) mg/cm^2] which were oxygen plasma treated for 1 (P1-A, P1-B, P1-C), 5 (P5-A, P5-B, P5-C), and 15 (P15-A, P15-B, P15-C) min, respectively. In general, plasma treated coatings were found to be lighter and smoother in appearance with smaller grain size when

11

compared to untreated coatings (e.g. P5-A and P5-C vs A and C). EDS spectral analysis of the

respective coatings showed no significant difference ($P>0.05$) in the amount of Ti content (wt %)

in both plasma treated and untreated samples (Table 1). Also, relatively high standard deviations

of Ti content were observed for the coatings especially at higher NP loadings (e.g. 0.125

mg/cm^2). It can be mainly attributed to the two possible reasons: (i) inherent heterogeneity of the

coating method, and (ii) the plasma induced chemical transformation of TiO$_2$ coatings. A study

by Jung et al (2005) on plasma treated TiO$_2$ coatings reported that the coating density and grain

size were decreased at low power RF plasma (50W) and noticed an increase in the coating

density and surface smoothness as the plasma power increased up to 200 W. Further, XPS

analysis of the same coatings in their study revealed that the Ti$_{2p}$ peaks were decreased with

increasing plasma treatment indicating that the amount of Ti atoms in the film surface region was

lower than O atoms with plasma treatment. This explains in a way the possible reason for

visually lighter plasma treated TiO$_2$ coatings observed in this study. However, Kim et al (2008)

reported a contrasting view on the plasma treated coatings. The XPS spectra of plasma treated

and untreated TiO$_2$ films in their study revealed that the ratio of TiO$_2$ to Ti$_2$O$_3$+TiO increased

from 1.3 to 1.62 after plasma treatment suggesting that there is a reduction in number of oxygen

vacancies and increase in the amount of stoichiometric TiO$_2$ after plasma treatment. It is still

unclear the kind of chemical and structural transformations took place after plasma treatment of

TiO$_2$ coated cutting board developed in this study. Hence further in-depth characterization

studies need to be conducted to better understand this phenomenon.

3.3.Effect of TiO$_2$ loading and number of coats

The effect of TiO$_2$ loading on the bactericidal activity of the cutting board is shown in Table

2. Increasing the TiO$_2$ loading in the coating from 0 to 0.125 mg/cm^2 has increased the log

reduction from 0.37 to 1.18 CFU/cm^2. However, no significant difference ($P>0.05$) in the reduction was observed between the control sample with no TiO$_2$ (i.e. 0 mg/cm^2) and the sample loaded with TiO$_2$ at 0.0125 mg/cm^2. Whereas, increasing the TiO$_2$ loading five times from 0.0125 to 0.0625 mg/cm^2 showed a significant increase ($P≤0.05$) in the log reduction up to 1.26 log CFU/cm^2. Further, increasing the NP loading from 0.0625 to 0.125 mg/cm^2 did not show significant ($P>0.05$) increase in the reduction. These results are in agreement with our SEM-EDS results where the distribution and coverage of NPs significantly improved by increasing the NP loading. However, no significant difference in the TiO$_2$ amount on the coating observed between at 0.0625 and 0.125 mg/cm^2 loading (Table 1). This shows that under tested conditions, TiO$_2$ loading of 0.0125 mg/cm^2 in the coating is not sufficient to exhibit significant bactericidal activity when compared to the control. A minimum of 0.0625 mg/cm^2 TiO$_2$ loading is required to exhibit significant reduction.

Though coatings with 0.0625 or 0.125 mg/cm^2 TiO$_2$ loading showed highest log reductions, these reductions are less than 1 log when compared to control sample with only UVA treatment. These minimal reductions were partly attributed to the possible masking of surface active sites of the NPs when adhered to the plastic substrate during coating procedure involving heat treatment. Although, this condition helps to achieve physically stable and durable coating, but it greatly limits the photocatalytic bactericidal property. Since the photocatalyst mediated disinfection mechanism is a surface-active phenomenon; poor exposure of NPs on the surface may decrease the disinfection efficacy of the coating by avoiding direct contact between the TiO$_2$ NP and the bacterial cell as well as minimizing the generation of ROS needed for photocatalysis. Marugan et al (2008) reported that TiO$_2$ coatings on silica had shown significantly less bactericidal activity when compared with TiO$_2$ in suspension. They reported that in a coated supports, the contact

13

between TiO_2 and the microorganisms is limited to the TiO_2 crystal located on the external surface of the particles. This area represents only a small fraction of the semiconductor loading that is available for actual photo-killing effect resulting in minimal reductions. A similar phenomenon can be attributed to the observed results in the current study. In addition, if this small fraction of available TiO_2 sites is masked by plastic as might happen in this study, achieving higher log reductions is extremely difficult. Increasing the TiO_2 loading in the coating only helped a little to improve the surface coverage on the cutting board. Two strategies were followed in order to enhance the log reduction: (i) by using multiple coatings of TiO_2 to improve surface distribution, uniformity, and exposure of NPs, and (ii) by oxygen plasma etching of plastic to expose reactive facets of NPs on the surface.

The effect of multiple coatings on the log reduction is shown in Table 2. The goal was to achieve a constant NP loading using multiple coatings and compare the results of log reduction with corresponding single coatings of TiO_2. Two different multiple coatings systems: (i) A sample with 5 coatings of TiO_2 at 0.0125 mg/cm^2 to match with the total loading of 0.0625 mg/cm^2 (Fig. 1D) and (ii) A sample with 2 coatings of TiO_2 at 0.0625 mg/cm^2 to match with the total loading of 0.125 mg/cm^2 (Fig. 1E) were created and tested. The results showed no significant difference in the reduction between single and double coatings of TiO_2 for 0.125 mg/cm^2 loading. However, the sample with 5 layers of TiO_2 coating showed a higher reduction (1.87 log) compared to its corresponding single coated sample (1.26 log) at the same overall TiO_2 loading. Differences between single and multiple coatings are not well understood at the moment but are likely attributed to the increased coating uniformity and surface coverage by increasing number coats.

3.4.Effect of oxygen plasma treatment

14

TiO$_2$ coatings at different NP loadings (0.0125, 0.0625, and 0.125 mg/cm^2) were subjected to oxygen plasma treatment as described earlier. The purpose of plasma treatment was to help expose more TiO$_2$ NPs on the surface of the coating by selectively etching overlaid plastic layer. This condition helps to increase surface active sites of TiO$_2$ NPs in the coating and facilitate higher ROS generation for efficient photocatalytic disinfection. Jung et al (2005) found that plasma treated coatings showed 1.5 times higher photocatalytic activity in degradation of toluene when compared to untreated samples and is attributed to the increased catalytic surface area of TiO$_2$ NPs in the coating after plasma treatment.

The effect of plasma treatment time (1, 5, and 15 min) on the bactericidal activity of original coatings was shown in Table 3. Increasing the TiO$_2$ loading from 0.0125 to 0.125 mg/cm^2 increased the log reduction. Subjecting these original coatings to oxygen plasma treatment for 1min has shown an 81, 56, and 21 % increase in the reduction for 0.0125, 0.0625, and 0.125 mg/cm^2 NP loading, respectively. However, this increase was statistically not significant ($P>0.05$) compared to the untreated samples except for TiO$_2$ loading at 0.0625 mg/cm^2. This indicates that plasma treatment for 1 min may not be sufficient to etch the plastic covered on TiO$_2$ NPs or expose more reactive facets. Increasing the plasma treatment time to 5 min showed additional 43, 36, and 55 % increase in the reductions when compared to the respective samples that were plasma treated for 1 min. However, further increasing the plasma treatment time for 15 min showed no improvement in the log reductions. In fact, the reductions at 15 min plasma treatment were fallen back to the reduction levels which were observed at 1 min plasma treatment. It is likely that the lesser NP loading (0.0125 mg/cm^2) and plasma treatment time (1min) result in poor light absorption, while the higher NP loading (0.125 mg/cm^2) and plasma treatment time (15 min) result in greater light reflection. Both these conditions result in poor

photon conversion efficiency and subsequent ROS production on the surface of TiO_2 coating. Hence it is critical to identify optimum TiO_2 loading and plasma treatment time to achieve greater log reductions.

As per the tested conditions in this study, TiO_2 coatings at 0.625 mg/cm^2 NP loading has showed significantly higher reductions of 1.97, 2.67, and 2.01 log CFU/cm^2 at 1, 5, and 15 min plasma treatments, respectively when compared to the untreated sample. On the other hand, plasma treatment of 5 min has showed significant ($P<0.05$) increase in the reductions at 0.0125 (1.73 log), 0.0625 (2.67 log), and 0.125 (2.22 log) mg/cm^2, respectively when compared to the other plasma treatment times. Based on the results of the current study, plasma treatment for 5 min was found to be the optimum with a TiO_2 loading of 0.0625 mg/cm^2 to achieve highest log reductions.

3.5. Effect of repeated washing/cleaning

Physical stability and durable antimicrobial activity are the two most important considerations for the practical application of an antimicrobial cutting board. The effect of repeated washing/cleaning cycles for 1, 2, 3, and 5 times on the bactericidal efficacy of TiO_2 coated cutting board at different NP loadings (0.0125, 0.0625, and 0.0125 mg/cm^2), as well as different plasma treatment times (0, 1, 5, and 15 min) were shown in Table 4. In general, all the original coatings at different NP loadings without plasma treatment showed consistent bactericidal efficacy even after repeated use for up to 5 times. This shows that the TiO_2 coatings on the HDPE cutting board were physically very stable and retained their original bactericidal property after multiple uses. However, all these coatings showed a variable trend in reductions when subjecting them to plasma treatment for 1 to 15 min and washing for up to 5 times.

Overall, TiO$_2$ coatings at 0.0125 mg/cm^2 loading showed either gradual decrease or similar; while TiO$_2$ coatings at 0.0625 and 0.125 mg/cm^2 showed either increased or similar log reductions after repeated washing. For example, TiO$_2$ coatings at 0.0125 mg/cm^2 loading and plasma treatment for 1 min showed a reduction of 1.21 and 1.39 log CFU/cm^2 after 1st and 2nd use, respectively. The same samples showed significantly lesser reductions 0.63 and 0.64 log CFU/cm^2 after 3rd and 5th use, respectively. Whereas, TiO$_2$ coatings at 0.0625 and 0.125 mg/cm^2 with 1 min plasma treatment did not show a statistically significant ($P>0.05$) difference in the log reduction even after repeated use for 5 times (Table 4). Almost similar trends were observed for samples that are plasma treated for 5 or 15 min. It is apparent in most cases that the longer plasma treatment times (5 or 15 min) showed increased reductions initially up to 2 times washing. However, after 2 times repeated washing the coatings either showed a decreased or no change in log reductions compared to their first use. This can be partly attributed to the increased surface energy, hydrophilicity, and the exposure of reactive sites of NPs on the surface of coating immediately after the plasma treatment and eventual loss of weakly adhered NPs on the cutting board after repeated washing. Further, samples at higher NP loadings can still maintain a layer of TiO$_2$ after repeated washing and exhibit stable antimicrobial property. In a study by Ratova and Mills (2015) found that pre-treatment of TiO$_2$ loaded LDPE film with UVC light for 30 min prior to photocatalytic disinfection increased the presence of surface actives sites on the coating and improved the log reductions. However, too much exposure (≥ 60 min) to UVC light resulted in significant surface damage and loss of TiO$_2$ NPs. Similar, phenomenon can be attributed to the plasma treated TiO$_2$ coatings in the current study. Hence, it is critical to identify optimum conditions for the coating and subsequent plasma treatment so as to achieve highest bactericidal efficacy as well as physical stability for an extended period of usage. The results of the current

study indicate that the TiO_2 coatings with 0.0625 mg/cm^2 loading showed the highest bactericidal property. Plasma treatment for 1 to 15 min further improved its bactericidal efficacy. However, plasma treatment for 5 min was found to be the optimum to retain highest bactericidal property and coating physical stability on repeated use.

4. Conclusions

TiO_2 coated antimicrobial cutting board was successfully developed in this study. A method was proposed to create a stable TiO_2 coating on a pre-formed plastic HDPE cutting board. The concentration of TiO_2 (i.e. loading) in the coating has a significant effect on the distribution and surface coverage. Increasing the TiO_2 loading from 0.0125 to 0.125 mg/cm^2 has significantly increased the log reduction. However, TiO_2 loading of 0.0625 mg/cm^2 was found to be the optimum to achieve a reduction of up to 1.85 log CFU/cm^2 at 0.5±0.05 mW/cm^2 UVA intensity in 3h. Oxygen plasma treatment of the TiO_2 coating for 1 to 15 min has further improved the coating bactericidal efficacy. However, plasma treatment for a minimum of 5 min was found to be optimum to achieve the log reduction of up to 2.67 CFU/cm^2. The developed TiO_2 coatings on the cutting board have shown high durability and consistent photocatalytic bactericidal property even after repeated use for up to 5 times. This study showcased the feasibility to develop a durable antimicrobial cutting board with photocatalyst TiO_2 NPs to help mitigate the risk of potential microbial cross-contamination. Moreover, further studies need to be conducted to better characterize the effect of plasma treatment on the coating as well as to optimize coating method.

Acknowledgements

References

Barker, J., Naeeni, M., & Bloomfield, S. F. (2003). The effects of cleaning and disinfection in reducing *Salmonella* contamination in a laboratory model kitchen. *Journal of Applied Microbiology*, 95(6), 1351-1360.

Berrang, M.E., Frank, J.F., & Meinersmann, R.J. (2010). *Listeria monocytogenes* biofilm formation on silver ion impregnated cutting boards. *Food Protection Trends*, Vol. 30, No.3, 168-171.

Carpentier, B. (1997). Sanitary quality of meat chopping board surfaces: a bibliographical study. *Food Microbiology*, 14, 31-37.

Cogan, T. A., Slader, J., Bloomfield, S. F., & Humphrey, T. J. (2002). Achieving hygiene in the domestic kitchen: the effectiveness of commonly used cleaning procedures. *Journal of Applied Microbiology*, 92(5), 885-892.

FDA (2014). Code of Federal Regulations 21CFR73.575 for listing of color additives exempt from certification. Title 21, Volume 1. Revised as of April 1, 2014.

FDA (2009). Food and Drug Administration, Report of the FDA Retail Food Program Database of Foodborne Illness Risk Factors, 2009 (accessed 7.22.16). http://www.fda.gov/downloads/Food/FoodSafety/RetailFoodProtection/FoodborneIllness andRiskFactorReduction/RetailFoodRiskFactorStudies/UCM224682.pdf

Foster, H. A., Ditta, I. B., Varghese, S., & Steele, A. (2011). Photocatalytic disinfection using titanium dioxide: spectrum and mechanism of antimicrobial activity. *Applied Microbiology and Biotechnology*, 90 (6), 1847-1868.

Hasmaliza, M., Foo, H.S., & Mohd, K. (2016). Anatase as antibacterial material in ceramic tiles. *Procedia Chemistry*, 19, 828-834.

Jung, C.-K., Bae, I.-S., Song, Y.-H., & Boo, J. H. (2005). Plasma surface modification of TiO_2 photocatalysts for improvement of catalytic efficiency. *Surface & Coatings Technology*, 200, 1320-1324.

Kim, Y., Yoo, B. J., Vittal, R., Lee, Y., Park, N.-G., & Kim, K.-J. (2008). Low-temperature oxygen plasma treatment of TiO_2 film for enhanced performance of dye-sensitized solar cells. *Journal of Power Sources*, 175, 914-919.

Kusumaningrum, H. D., van Asselt, E. D., Beumer, R. R., & Zwietering, M. H. (2004). A quantitative analysis of cross-contamination of *Salmonella* and *Campylobacter* species via domestic kitchen surfaces. *Journal of Food Protection*, 67, 1892-1903.

Lan, Y., Lu, Y., & Ren, Z. (2013). Mini review on photocatalysis of titanium dioxide nanoparticles and their solar applications. *Nano Energy, 2*(5), 1031-1045.

Marugan, J., van Grieken, R., Sordo, C., & Cruz, C. (2008). Kinetics of the photocatalytic disinfection of *Escherichia coli* suspensions. *Applied Catalysis B-Environmental, 82*(1-2), 27-36.

Monnin, A., Lee, J., & Pascall, M.A. (2012). Efficacy of neutral electrolyzed water for sanitization of cutting boards used in the preparation of foods. *Journal of Food Engineering*, 110, 541-546.

Moretro, T., Hoiby-Pettersen, G.S., Habimana, O., Heir, E., & Langsrud, S. (2011). Assesment of the antibacterial activity of a triclosan-containing cutting board. *International Journal of Food Microbiology*, 146, 157-162.

Moretro, T., Hoiby-Pettersen, G.S., Halvorsen, C.K., & Langsrud, S. (2012). Antibacterial activity of cutting boards containing silver. *Food Control*, 28, 118-121.

Muranyi, P., Schraml, C., & Wunderlich, J. (2010). Antimicrobial efficiency of titanium dioxide-coated surfaces. *Journal of Applied Microbiology, 108*(6), 1966-1973.

Sobczyk-Guzenda, A., Pietrzyk, B., Jakubowski, W., Szymanowski, H., Szymanski, W., & others (2013). Mechanical, photocatalytic and microbiological properties of titanium dioxide thin films synthesized with the sol-gel and low temperature plasma deposition techniques. *Materials Research Bulletin*, 48, 4022-4031.

Thormar, H., & Hilmarsson, H. (2012). Glycerol monocaprate (monocaprin) reduces contamination by *Escherichia coli* and *Salmonella eneteritidis* on hard surfaces. *Food Control*, 25, 505-510.

Todd, E. C. D., Greig, J. D., Bartleson, C. A., & Michaels, B. S. (2009). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 6. Transmission and survival of pathogens in the food processing and preparation environment. *Journal of Food Protection*, 72, 202-219.

Ratova, M., & Mills, A. (2015). Antibacterial titania-based photocatalytic extruded plastic films. *Journal of Photochemistry and Photobiology A: Chemistry*, 299, 159-165.

Wei, X., Yang, Z., Tay, S.L., & Gao, W. (2014). Photocatalytic TiO_2 nanoparticles enhanced polymer antimicrobial coating. *Applied Surface Science*, 290, 274-279.

Yemmireddy, V.K., & Hung, Y.-C. (2015a). Effect of different binding agents on physical stability and bactericidal activity of photocatalytic TiO_2 nanocoatings on food contact surfaces. *Food Control*, 57, 82-88.

Yemmireddy, V.K., & Hung, Y.-C. (2015b). Selection of photocatalytic bactericidal titanium dioxide (TiO_2) nanoparticles for food safety applications. *LWT- Food Science and Technology,* 61, 1-6.
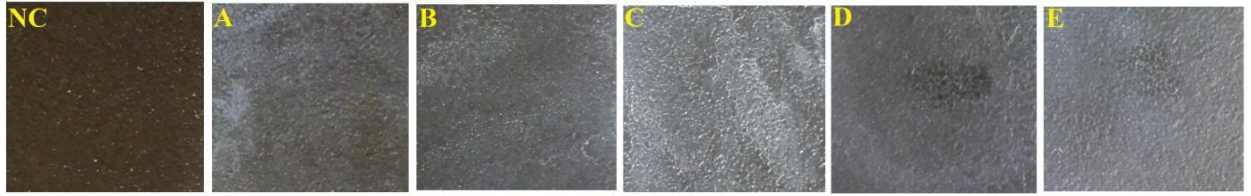
A-1

Cross-sectional view
(Thickness 461±15.6 μm)

A-2

25μm

Top-view
(TiO₂ at 0.0125 mg/cm²)

A-3
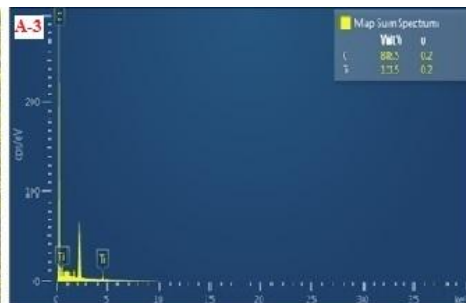
EDS spectra: Ti 11.5 %, C 88.5 %

B-1

Cross-sectional view
(Thickness 505±10 μm)

B-2

50μm
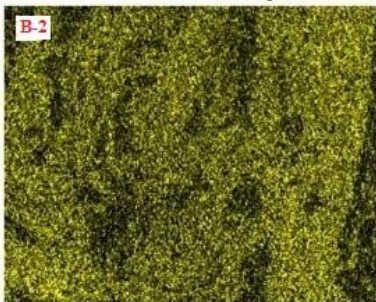
Top-view
(TiO₂ at 0.0625 mg/cm²)

B-3
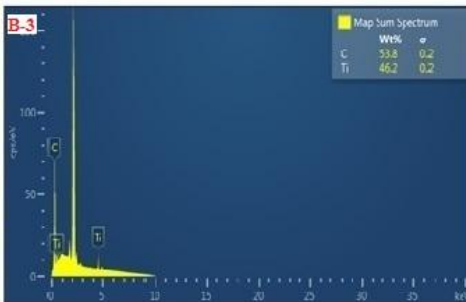
EDS spectra: Ti 46.2 %, C 53.8 %
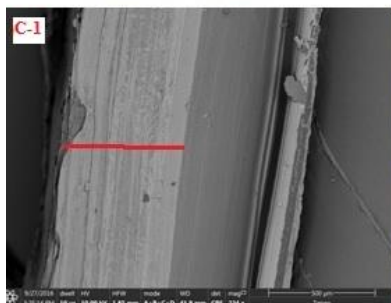
C-1

Cross-sectional view
(Thickness 551±32 μm)

C-2

50μm
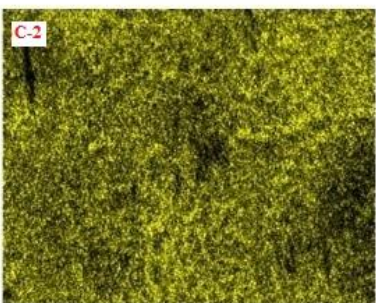
Top-view
(TiO₂ at 0.125 mg/cm²)

C-3
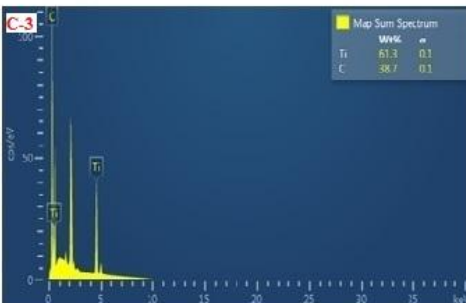
EDS spectra: Ti 61.3 %, C 38.7 %
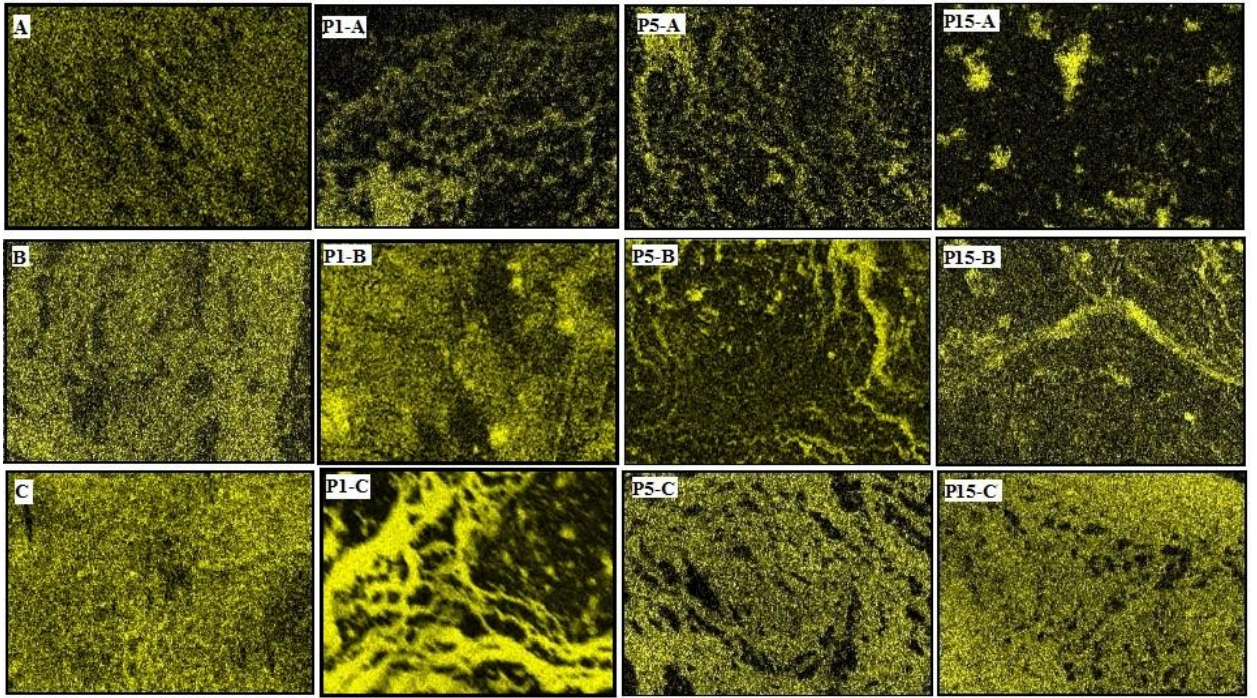
Table 1. Elemental Ti percent in different TiO_2 coatings on cutting board as evinced by EDS spectral analysis

Plasma treatment time[1] (min)	Percent elemental Ti in different coating samples		
	Sample-A[2]	Sample-B[2]	Sample-C[2]
0	10.8 ± 1.86^a	40.2 ± 6^a	50.4 ± 10.95^a
1	12.7 ± 1.25^a	37.9 ± 6.85^a	24.9 ± 19.75^a
5	7.9 ± 2.6^a	20.3 ± 12.1^a	36.6 ± 13.6^a
15	5.5 ± 1.95^a	22.5 ± 3.05^a	32.5 ± 12.25^a

[1]TiO_2 coatings plasma etched for different times at high power setting using a Harrick® plasma system.
[2]Samples A, B, and C are TiO_2 coatings at 0.0125, 0.0625, and 0.125 mg/cm^2 NP loadings.
Mean values with same superscript in the same column are not significantly different ($P > 0.05$)

Table 2. Effect of TiO_2 loading and the number of coats on the bactericidal activity

TiO_2 loading[1] (mg/cm^2)	Log reduction[2] (CFU/cm^2) at UV-A intensity of 0.5±0.05 mW/cm^2 for 3h	
	Single coating[3]	Multiple coatings[4]
0	$^D0.37^b$	-
0.0125	$^{DC}0.67^b$	-
0.0625	$^{BC}1.26^a$	$^A1.87^a$ (5 coatings of 0.0125 mg/cm^2)
0.125	$^{BC}1.18^a$	$^{BA}1.38^a$ (2 coatings of 0.0625 mg/cm^2)

[1]TiO_2 coated cutting board at different NP loadings (where 0 mg/cm^2 loading means cutting board with no TiO_2 coating which is just treated under UVA light)
[2]Mean values with same lower case superscript in the same column and same upper case superscript in the table are not significantly different ($P > 0.05$)
[3] Single coating of TiO_2; [4] Multiple coatings of TiO_2

Table 3. Effect of plasma treatment time on the bactericidal activity

TiO$_2$ loading[1] (mg/cm^2)	Log reduction[2] (CFU/cm^2) at 0.5 ± 0.05 mW/cm^2 for 3h			
	Plasma treatment time[3] (min)			
	0	1	5	15
0	$^{A}0.37^{b}$	$^{A}0.37^{c}$	$^{A}0.37^{d}$	$^{A}0.37^{c}$
0.0125	$^{B}0.67^{b}$	$^{BA}1.21^{b}$	$^{A}1.73^{c}$	$^{BA}0.91^{bc}$
0.0625	$^{C}1.26^{a}$	$^{B}1.97^{a}$	$^{A}2.67^{a}$	$^{B}2.01^{a}$
0.125	$^{B}1.18^{a}$	$^{B}1.43^{ba}$	$^{A}2.22^{b}$	$^{B}1.42^{ba}$

[1]TiO$_2$ coated cutting board at different NP loadings in mg/cm^2
[2]Mean values with same lower case superscript with in the same column and with same upper case superscript in the same row are not significantly different ($P>0.05$)
[3]TiO$_2$ coatings plasma etched for different periods at high power setting using a Harrick® plasma system

Table 4. Effect of repeated use on the bactericidal efficacy of plasma treated and untreated TiO$_2$ coatings on the cutting board

TiO$_2$ loading[1] (mg/cm^2)	Plasma treatment time[2] (min)	Log reduction[3] (CFU/cm^2) at 0.5 ± 0.05 mW/cm^2 for 3h			
		Number of repeated uses[4]			
		1	2	3	5
0.0125	0	0.67^{b}	1.04^{a}	0.61^{b}	0.51^{b}
	1	1.21^{a}	1.39^{a}	0.63^{b}	0.64^{b}
	5	1.73^{a}	2.44^{a}	0.85^{b}	0.78^{b}
	15	0.91^{b}	2.00^{a}	1.19^{ba}	1.01^{b}
0.0625	0	1.26^{a}	1.52^{a}	1.50^{a}	1.35^{a}
	1	1.97^{a}	1.61^{a}	2.34^{a}	2.44^{a}
	5	2.67^{ba}	3.13^{a}	2.28^{b}	2.15^{b}
	15	2.01^{c}	2.73^{a}	2.37^{b}	2.38^{b}
0.125	0	1.18^{a}	1.40^{a}	2.12^{a}	1.31^{a}
	1	1.43^{ba}	1.22^{ba}	1.11^{b}	1.80^{a}
	5	2.22^{b}	3.19^{a}	1.50^{b}	1.94^{b}
	15	1.42^{a}	1.87^{a}	1.56^{a}	1.95^{a}

[1]TiO$_2$ coated cutting board at different NP loadings in mg/cm^2
[2]TiO$_2$ coatings plasma etched for different periods at high power setting using a Harrick® plasma system
[3]Mean values with same lower case superscript with in the same row are not significantly different ($P>0.05$)
[4]TiO$_2$ coated samples that are used repeatedly for 1, 2, 3, and 5 times

Fig 1. Images of TiO$_2$ coating on HDPE cutting board at different NP loadings in mg/cm^2: (NC) control with no TiO$_2$ coating, (A) 0.0125 (B) 0.0625, (C) 0.125, (D) 5 coatings of 0.0125 mg/cm^2 each to achieve a total loading of 0.0625 mg/cm^2, and (E) 2 coatings of 0.0625 mg/cm^2 each to achieve a total loading of 0.125 mg/cm^2

Fig 2. SEM and EDS analysis of the coatings at 0.0125 mg/cm^2 (A), 0.625 (B), and 0.125 (C) mg/cm^2 TiO$_2$ NP loadings. A-1, B-1, and C-1 are cross-sectional SEM images representing mean thickness of the coatings; A-2, B-2, and C-2 are surface topography of the coatings by SEM top-view, and; A-3, B-3, and C-3 are EDS spectra and elemental analysis of each coating

Fig 3. SEM images of untreated TiO$_2$ coatings at 0.0125 (A), 0.625 (B), and 0.125 (C) mg/cm^2 NP loadings (first column) and plasma treated TiO$_2$ coatings of A, B, and C for 1 (P1-A, P1-B, and P1-C), 5 (P5-A, P5-B, and P5-C), and 15 (P15-A, P15-B, and P15-C) min, respectively. (Scale: 50 μm except for Fig 3A which is scaled at 25 μm)