Bioconjugate Chemistry

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Targeted Disinfection of *E. coli* via Bioconjugation to Photoreactive TiO_2

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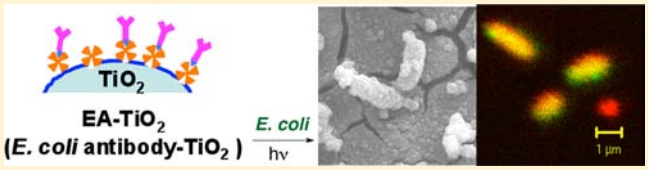
Supporting Information

ABSTRACT: The selective control of pathogenic bacteria is an ongoing challenge. A strategy is proposed that combines targeted binding of the bacterium, using antibodies, with their photoactivated oxidative destruction. Photoactive colloidal TiO_2 was first derivatized with *E. coli* antibodies (EA-TiO_2). When mixtures of the organisms *E. coli* and *Pseudomonas putida* (*P. putida*) were exposed to modified EA-TiO_2, the particles preferentially selected *E. coli* for surface binding. Two consequences arose from surface bioconjugation: bacteria were found to flocculate upon mixing at appropriate ratios of EA-TiO_2/*E. coli*, and EA-TiO_2-bound *E. coli* underwent cell death after exposure to UV light. In the former case, flocculation of the bacteria was optimal at ~50 EA-TiO_2 particles per *E. coli*. Selective flocculation provides an alternative strategy for pathogen removal. With respect to UV disinfection, as few as 26 EA-TiO_2 particles per *E. coli* gave a 10 000-fold decrease in viable bacteria. Thus, it is possible to selectively target and kill one type of bacteria in a mixture of pathogens. The results give support to the proposal that photocatalytic TiO_2 most effectively delivers an oxidizing agent when the titania is bound to the bacterial surface.



INTRODUCTION

In 1972, Fujishima and Honda[1] reported that anatase, a form of TiO_2 (titania), decomposed water in the presence of UV light, which initiated an enormous amount of work on TiO_2 photocatalysis.[2−4] Under near UV irradiation (<380 nm), TiO_2 is electronically excited to produce electrons and holes on the titania surfaces. These species interact with air to give singlet oxygen[5−7] and with water to give hydroxyl radicals and other reactive species that can decompose most organic materials by oxidation.[2,4] Matsunaga and co-workers were the first to report that the products of TiO_2 photocatalysis could be used to kill bacteria.[8] Subsequent studies have reported the effects of disinfection as a function of bacteria type,[9,10] TiO_2 type,[11] pH,[12] specific ions,[9] media, and other organics. A number of reviews are available.[13,14]

The mechanisms by which the reactive oxygen species kill bacteria have also received much attention. Some propose that the cell walls[12−15] are disrupted, whereas others claim that DNA/RNA damage is important.[16−18] Another proposal suggests that iron released by ruptured cells undergoes a photo Fenton reaction to generate further reactive species.[19]

There seems to be a general consensus that the colloidal photocatalytic TiO_2 particles must be proximate to or, preferably, directly deposited on the bacteria for effective disinfection. It is known that very reactive radicals have a short lifetime, and thus a short diffusion length. Indirect evidence supporting this proposal is that P25, a commercial anatase/rutile mixture commonly used in photocatalysis studies, is most effective at pHs where it is positively charged and binds to negatively charged *E. coli*.[8] Similarly, the presence of phosphate,[12] which enhances both TiO_2 negative charge and colloidal stability (and thus repels the bacteria), inhibits photodisinfection. Gogniat and co-workers described clever experiments with flow cytometry that give support to the proposal that bacteria were killed by TiO_2 bound to the bacteria surfaces.[12] However, Kikuchi and co-workers showed that bacteria were killed even though the bacteria were separated from the TiO_2 by a 50 μm membrane.[20] On the basis of the influences of enzymatic peroxide scavengers and of free radical scavengers, they made compelling arguments that the reactive hydroxyl radicals formed much more stable hydrogen peroxide that then diffused across the membrane.

Photocatalytic TiO_2 has also been shown to kill mammalian cells and thus has been evaluated for killing cancer cells.[21−23] Usually, cancer therapies involve targeting the killing agent to specific cells. We have found only one reference to the use of

Received: November 1, 2012
Revised: January 28, 2013
Published: February 20, 2013

photocatalytic TiO_2 with targeting—Xu et al. describe titania with physically adsorbed antibodies to promote interactions with specific cells.[24,25]

We have previously developed methods to covalently couple biotin to photocatalytic TiO_2.[26] Using streptavidin as a coupling protein, it was possible to show that the biotinylated TiO_2 displayed specific deposition onto cellulose coated with biotinylated cellulose binding domain.[27] In this paper, we report what we believe is the first example of photocatalytic TiO_2 with chemically immobilized antibodies to *E. coli*. Our work demonstrates two important features. First, it is possible to selectively target and kill one type of bacteria in a mixture of model pathogens. Second, the results give further support to the proposal that photocatalytic TiO_2 is most efficient when the titania is bound to the bacterial surface.

EXPERIMENTAL SECTION

Materials. TiO_2 particles (anatase, 3.9×10^3 kg/m^3, 99%) was purchased from Aldrich and used as received. Biotinylated TiO_2 nanoparticles (Biotin-TiO_2) were prepared by treating TiO_2 nanoparticles with 3-aminopropyltriethoxysilane in anhydrous DMSO, followed by reaction with *N*-hydroxysuccinimidobiotin as previously described (Figure 1).[23] Streptavidin from *Streptomyces avidinii* (Fluka), atto-610-streptavidin (Fluka), *N*-hydroxysuccinimidobiotin (Sigma), and biotinylated and *E. coli* antibody (ab20640, Abcam) were used as received.

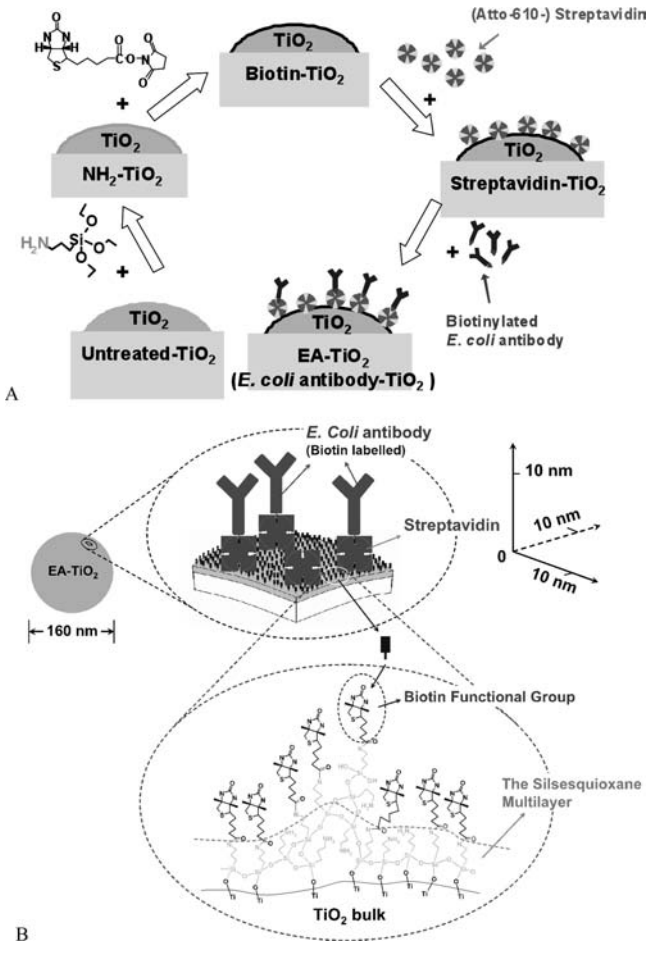


Figure 1. (A) Preparation of streptavidin-TiO_2 and EA-TiO_2. (B) Structural details of the EA-TiO_2 surface.

E. coli/coliform count plates (Petrifilm) were purchased from 3M Company and used according to the manufacturer's instructions. Phosphate buffered saline (PBS) buffer (137 mM NaCl, 2.7 mM KCl, 4.2 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.0) was autoclaved before use. Milli-Q water was used for preparing all the aqueous solutions.

Instrumental Methods. The size and size distribution of TiO_2 particles were obtained on a Mastersizer 2000 (Malvern Instruments). EA-TiO_2 (6 mg L^{-1}), untreated-TiO_2 (3.6 mg L^{-1}), or streptavidin-TiO_2 (6 mg L^{-1}) were dispersed in filtered (0.22 μm Millipore) PBS buffer (140 mM, pH 7) using ultrasonication for 2 min before measurements. Specific surface areas of TiO_2 samples were determined by nitrogen adsorption using a Quantachrome Nova 2200. Optical microscopy images were obtained on a Zeiss EL-Einsatz microscope equipped with a QICAM QImaging camera.

Scanning electron microscopy images were obtained on a JEOL JSM-7000F scanning electron microscope. All specimens were washed then diluted with Milli-Q water. After drying on aluminum stubs, samples were sputter-coated with gold.

Optical density (absorbance) was measured with a Beckman Coulter UV–vis DU800 spectrophotometer, software version 2.0. The extinction coefficient for streptavidin was determined to be 3.4 L g^{-1} cm^{-1} from the calibration curve. Confocal laser scanning microscopy (CSLM) images of GFP *E. coli*, EA-TiO_2, and Streptavidin-TiO_2 (both labeled with atto-610-streptavidin) in PBS buffer were obtained on Zeiss LSM 510 confocal microscope.

Electrophoretic mobilities of bacteria and all TiO_2 specimens were measured on a ZetaPlus zeta potential analyzer (Brookhaven Instruments Corp.) operating in phase analysis light scattering mode. Measurements were performed in PBS buffer (140 mM, pH 7). The mean and standard deviation were calculated from 10 runs (15 cycles per run).

Cell Culture. Luria–Bertani media were prepared from 10 g of tryptone, 5 g of yeast extract (EMD), and 10 g of NaCl (Aldrich) per liter. *E. coli* K-12 strain DH5α (Invitrogen, Burlington, Canada) was transformed with plasmid pCA24N, which carries the gene coding for a green fluorescent protein variant (GFPuv4) with enhanced fluorescent intensity.[28] The transformed *E. coli* was inoculated into freshly sterilized Luria–Bertani media supplemented with 0.1% chloramphenicol (Fluka) solution (34 μg/mL in ethanol) from a single colony, grown up aerobically (37 °C, 180 rpm) to a to final OD_{600} of 1.0. To obtain green fluorescent protein expression, the cells were grown to an OD_{600} of 0.6–0.8 and protein expression was induced at that point with addition of isopropyl β-D-1-thiogalactopyranoside (Fermentas, Burlington, ON, Canada) to a final concentration of 0.5 mM.

P. putida LV 2–4 (culture: CRIFS C852) was kindly provided by the Canadian Research Institute for Food Safety. *P. putida* was cultured in the same conditions as *E. coli*, except that the temperature was set at 30 °C.

After culturing, the two types of bacteria were collected separately by centrifugation at 8500 rpm for 10 min. The cell pellet was dispersed in PBS buffer and centrifuged again. This wash process was repeated 3 times, for complete removal of the culture broth. Finally, the resulting pellets were separately suspended and diluted to a final cell concentration of 3.8×10^8 colony-forming units per milliliter (CFU/mL) in sterile PBS buffer. These solutions were stored at 4 °C for further use in the photodisinfection experiments.

dx.doi.org/10.1021/bc300581t | *Bioconjugate Chem.* 2013, 24, 448–455

Bacterial Concentrations. Bacteria concentrations were measured before and after photodisinfection. An aliquot (0.1 mL) of bacterial suspension was inoculated into *E. coli* Luria–Bertani-agar medium plates and another aliquot (1 mL) into the *E. coli*/coliform count plates (Petrifilm, 3M) at 37 °C. After 48 h, *E. coli* colonies on *E. coli*/coliform count plates were counted. Note that *E. coli* was specifically identified on the Petrifilm plates by the presence of gas bubbles attached to the colonies, whereas the total bacteria concentration came from the Luria–Bertani-agar plates.

Optical density measurements at 600 nm, OD_{600}, were used as approximate measures of bacteria concentration to facilitate sample preparation. For CFU/mL of $\sim 3.8 \times 10^8$, the corresponding OD_{600} values for *E. coli* and *P. putida* were ~1.0 and ~0.1, respectively.[29]

Streptavidin-Coated TiO_2 (Streptavidin-TiO_2). Titania particle surfaces were treated by a series of reactions to ultimately give immobilized *E. coli* antibodies. The sequence of steps is illustrated schematically in Figure 1, which also serves as a summary of the particle nomenclature used herein. Biotinylated TiO_2 nanoparticles (Biotin-TiO_2) were prepared by treating TiO_2 nanoparticles with 3-aminopropyltriethoxysilane in anhydrous DMSO, followed by reaction with *N*-hydroxysuccinimidobiotin.[23]

The preparation of streptavidin-TiO_2 particles was handled in a laminar flow hood. Biotin-TiO_2 particles (1.2 mg) were sterilized and then dispersed in 3 mL of PBS buffer (pH 7) through ultrasonication (Bransonic Ultrasonic cleaner model 3510R-DTH, Branson Ultrasonic Corporation USA) for 2 min. Streptavidin solution (2 mL, 0.5 g/L in PBS) and the biotinylated TiO_2 suspension (3 mL) were then mixed and agitated at room temperature for 1 h in the dark. The product streptavidin-TiO_2 was isolated and purified as follows: (i) the particles were dispersed in 3 mL PBS buffer by ultrasonication for 2 min; (ii) particles were precipitated by centrifugation at 21 100 *g* for 10 min; and (iii) the supernatant liquid was decanted and the streptavidin content in the supernatant was measured by UV–vis at 282 nm. The washing process was repeated until the absorbance value reached the baseline (±0.0005), the absorbance of the blank PBS buffer, indicating that the unbound streptavidin molecules had all been washed off the biotinylated TiO_2 colloidal particles. The density of the bound streptavidin was calculated as a mass balance using the absorbance values of the decanted unadsorbed species. For some preparations, atto-610-streptavidin was used to give fluorescent particles.

***E. coli* Antibody Coated TiO_2 (EA-TiO_2).** Streptavidin-TiO_2 colloidal particles (1.2 mg) were mixed with biotinylated *E. coli* antibody (60 μg) in 1 mL PBS buffer at pH 7 for 1 h in the dark to prepare *E. coli* antibody-conjugated TiO_2 colloidal particles (EA-TiO_2) (Figure 1). First, streptavidin-TiO_2 colloidal particles (1.2 mg) were dispersed in 1 mL of PBS buffer through ultrasonication for 2 min. Then, following each addition (3 μL, 4 g L^{-1}) of biotinylated *E. coli* antibody solution to the streptavidin-TiO_2 colloidal particles suspension, the system was agitated for 20 min in the dark, and then centrifuged at 21 100 *g* for 10 min. The presence of antibody in the supernatant was probed by OD_{280} measurements. The process of addition-agitation-centrifugation-testing was repeated until the OD_{280} was higher than 0.004, indicating the addition of biotinylated *E. coli* antibody was slightly overdosed (3 μg biotinylated *E. coli* antibody in the supernatant liquid of the solution). The antibody content of the product was 60 ± 14 μg of biotinylated *E. coli* antibody per 1.2 mg of streptavidin-coated TiO_2 particles.

TiO_2 Deposition onto Bacteria. To demonstrate the specific, antibody-mediated deposition of EA-TiO_2 onto *E. coli*, a series of experiments was conducted in which particles were mixed with bacteria and the product was examined by optical and scanning electron microscopy. All suspensions, including *E. coli* at $OD_{600} \approx 1$, *P. putida* at $OD_{600} \approx 0.1$, and all TiO_2 specimens (at a concentration of 0.4 g/L) were prepared in PBS buffer (140 mM, pH 7.0) for the binding and control experiments.

In a typical experiment, 1 mL of *E. coli* suspension and 1 mL of EA-TiO_2 suspension were mixed on a shaking table (Barnstead/Lab-line USA model no. 4633) at a speed of 120 rpm for 1 h in the dark. The mixed suspension was then observed under a confocal microscope and scanning electron microscopy, respectively. After leaving the mixture untouched for 2 h, the supernatant liquid was observed under an optical microscope, while the sediment was also observed by both optical and scanning electron microscopy. A variety of different ratios of EA-TiO_2 particles/bacterium were examined.

Photodisinfection Experiments. The media, PBS buffer, plates, vials, micropipets, and loops were sterilized and the samples were handled in a laminar flow hood. In a typical experiment, *P. putida* (0.1 mL, 3.8×10^8 CFU/mL), *E. coli* (0.1 mL, 3.8×10^8 CFU/mL), with or without TiO_2 (0.1 mL, 0–0.8 mg/mL), were mixed in a vial, and a total of 0.3 mL of the mixture was added to one well in a BD Falcon 48-well culture plate (catalog no. 353078) that contained a 0.5 cm magnetic stirring bar. The plates, fitted with lids, were placed under a Black-Ray XX-15BLB UV Bench Lamp (365 nm). The samples were illuminated from the top with an incident intensity of 5.8 W m^{-2} (Traceable Ultra Violet Light Meter) after passing through the cell lid. These mixtures were exposed to UV irradiation for 0, 1, or 2 h, without or with agitation (30 rpm). After UV exposure, samples were diluted with sterile PBS buffer with appropriate dilution multiples, varying from 10^4 to 10^7, depending on the extent of killing. An aliquot (0.1 mL) of the solution was inoculated into *E. coli* LB-agar medium plates and another aliquot (1 mL) into the *E. coli*/coliform count plates (Petrifilm, 3M). After 48 h at 37 °C, colonies were counted.

The initial bacteria concentrations were estimated by optical density measurements. For each batch of experiments, the bacteria stock suspensions were plated so that more exact values could be assigned. Titania concentrations in our photodisinfection experiments were expressed as the number of primary TiO_2 particles per bacterium. The mass concentration of titania colloids was converted to the number concentration of primary TiO_2 particles assuming a suspension of spheres with an average particle diameter of 160 nm and a TiO_2 density of 3900 kg/m^3. The corresponding bacteria concentration was based on the CFU of the bacteria stock suspension. For example, mixing 0.1 mL TiO_2 suspension (0.8 g/L) with 0.1 mL bacteria suspension (3.8×10^8 CFU/mL) corresponds to 252 primary TiO_2 particles per bacterium.

UV Irradiation–Different Reaction Times. Equal concentrations of *E. coli* and *P. putida* were mixed with EA-TiO_2 at a ratio of 26 titania particles per *E. coli*. The mixtures were exposed to UV light (365 nm, 5.8 W m^{-2}) without agitation for 0, 1, or 2 h, respectively. After UV irradiation, the resulting mixtures were diluted with PBS buffer, and then cultured on LB-agar plates and *E. coli*/coliform count plates (Petrifilm, 3M). The colonies of *E. coli* were identified on the *E. coli*/coliform

dx.doi.org/10.1021/bc300581t | *Bioconjugate Chem.* 2013, 24, 448–455

Table 1. Summary of Properties of Derivatized TiO_2

sample	functional group	content functional group (mg)/TiO_2 sample (mg)	specific surface area (m^2 g^{-1})[a]	area per functional group (nm^{-2})	nature of surface modification
TiO_2	Ti-OH	-	16.0	-	-
TiO_2-NH_2[23]	aminopropylsilane	-	14.8	2.6	44 nm thick
Biotin-TiO_2[23]	biotin	-	14.4	2.0	0.77 biotin per amino group
Streptavidin-TiO_2	streptavidin	0.029	13.5	0.02	50% surface coverage[a]
EA-TiO_2	anti-*E. coli* antibody	0.05 + 0.012	-	0.015	0.75 per steptavidin

[a]Based on the area per streptavidin of 4.5 nm × 5.5 nm.[30,31]

count plates, and the total colonies of *E. coli* and *P. putida* were counted on LB-agar plates by conventional plate counting.

RESULTS

Titania Derivatization. A series of steps were employed to convert the titania mineral surface into an *E. coli* antibody-coated particle EA-TiO_2 (Figure 1). The properties of the original anatase suspension and a detailed description of the first two steps that involve amination of titania surface with aminopropyltriethoxysilane and then biotinylation using biotin-NHS ester to give biotinylated TiO_2 (Biotin-TiO_2) have been previously described.[23] Biotin-TiO_2 suspensions had an average primary (unaggregated) particle size of ~160 nm, and a specific surface area of 14.4 m^2 g^{-1}. The density of amine groups was 2.6 nm^{-2} and of biotin groups was 2.0 nm^{-2}. The widely used affinity of streptavidin for biotin was used here to modify the mineral surface to give streptavidin-TiO_2 simply by adding streptavidin into a suspension of biotin-TiO_2, and then washing away unbound protein. The density of streptavidin groups was estimated to be 0.02 nm^{-2} based on measurements of the concentration of unbound streptavidin in the supernatant.

Antibody-coated titania, EA-TiO_2, was prepared by slowly titrating biotinylated *E. coli* antibody into a suspension of streptavidin-TiO_2 until excess antibody was detected by UV in the supernatant. Thus, 60 ± 14 μg of antibody was consumed by 1.2 mg of streptavidin-TiO_2 which, in turn, gave an antibody density of ~0.015 nm^{-2}. Table 1 summarizes the properties of the starting titania and the changes that resulted from each of the three subsequent treatment steps.

The average primary particle size for titania and the surface-modified derivatives was ~160 nm (for micrographs, see Supporting Information). However, even though the particles are charged, they formed loose aggregates whose size could be characterized by Fraunhofer diffraction. The evolution of the size distribution of the aggregates of TiO_2 particles through the various treatments is summarized in Figure 2. The initial titania suspension had a monomodal particle size distribution centered around an average size of 1.6 μm and a dispersity (D90-D10/D50) of 1.8. However, each subsequent processing step displayed a small second peak around 20 μm, suggesting some aggregation had occurred. Note that the aggregates have a very open structure so that specific surface areas (Table 1) better reflect the primary particle size and not the aggregate size.

The electrophoretic mobility of colloidal particles is very sensitive to the nature of functional groups on the particle surfaces.[32,33]

Table 2 summarizes the mobilities corresponding to each of the transformations of titania (Figure 1). Also shown are mobilities of the two bacteria types used in this work. All titania suspensions and both bacteria types were negatively charged at pH 7 in PBS buffer. Thus, electrostatic interactions between the different constituents of the dispersions are repulsive and tend to prevent aggregation.

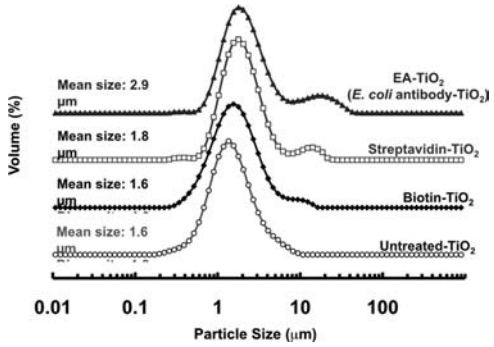


Figure 2. Size and size distribution of aggregates of untreated-TiO_2, biotin-TiO_2, streptavidin-TiO_2, and EA-TiO_2 colloidal particles in PBS buffer at pH 7.0.

Table 2. Electrophoretic Mobility Values for Titania Samples and Bacteria[a]

	electrophoretic mobility ($\times 10^{-8}$ m^2 V^{-1} s^{-1})	std. error	conductance (μS)
P. putida LV 2−4	−1.49	0.12	26100
E. coli K12 DH5α	−1.37	0.09	21700
untreated TiO_2	−1.82	0.12	26800
streptavidin-TiO_2	−1.43	0.22	26800
EA-TiO_2	−1.76	0.07	26800

[a]All measurements were performed in PBS buffer (140 mM, pH 7).

Interaction of EA-TiO_2 with *E. coli*. Fluorescence labeling was used to confirm that the antibody-coated titania bound to bacteria. Dual labels were utilized: fluorescently labeled (atto-610) streptavidin served as an indicator for the location of the titania particles, and *E. coli* that expressed green fluorescent protein (GFP) was used to follow binding. Figure 3A,B shows a fluorescent micrograph of a mixture of labeled EA-TiO_2 and *E. coli* GFP-bacteria (green) coated with EA-TiO_2 (red) appear yellow, confirming that primary titania particles (not the loose aggregates) were bound to the *E. coli* surface.

The colloidal stability of the bacteria was affected by the presence of the titania particles. With a large excess of titania particles (>200 EA-TiO_2 particles/bacterium), the entire surface of the bacteria became covered by a layer of TiO_2 primary particles (Figure 3C); the modified bacteria remained in suspension. However, at lower ratios of TiO_2 particles/bacterium, the bacteria assembled into >10−20 μm flocs that settled over time (Figure 3D). The size and extent of aggregate formation depended upon the ratio of titania particles to the number concentration of bacteria. Attempts to directly

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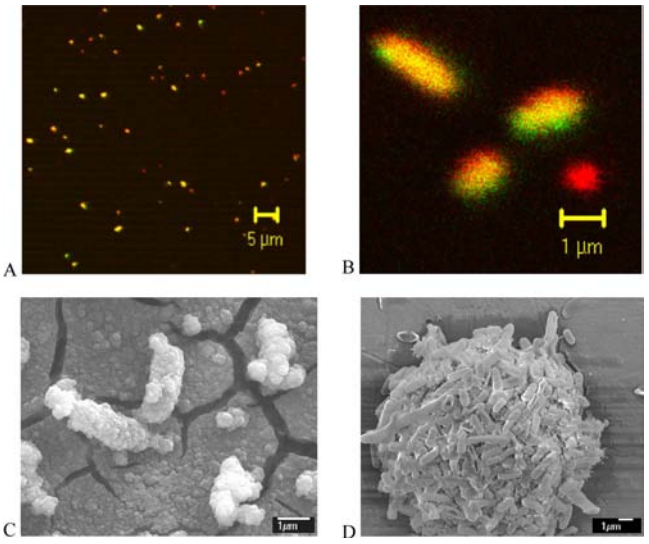

Figure 3. CLSM of GFP-*E. coli* and EA-TiO_2 (labeled with atto-610): (A) lower and (B) higher magnification. Yellow indicates that the red TiO_2 coated the green bacteria. Examples of *E. coli* (C) flocculated with EA-TiO_2 and (D) coated with (b) EA-TiO_2.

correlate floc size with the EA-TiO_2 particles/bacterium ratio were made using turbidity measurements of the dispersion and microscopy characterization of the precipitate. Maximum floc sizes were observed at around 50 EA-TiO_2 particles per *E. coli* bacterium with smaller flocs (but not of a consistent size) being observed with either fewer or more particles/bacterium. Note that these interactions only occurred with EA-TiO_2, as shown by a variety of control experiments (Supporting Information).[26] The ability to use such flocculation processes as a mechanism to separate unwanted pathogens simply by sedimentation could be of practical utility.

Bacterial Response to UV Irradiation. Three different control experiments demonstrated that photodisinfection could occur in the presence of TiO_2 and UV light, as is well-known (Supporting Information). The untreated TiO_2 or the intermediate streptavidin-TiO_2, however, caused only a minor decrease in the concentrations of viable *E. coli* (Supporting Information); *P. putida* was virtually unaffected by irradiation. There was no bactericidal effect on either *E. coli* or *P. putida* in the absence of TiO_2, or in the presence of the various control TiO_2 samples (Supporting Information).

Light was required for the production of reactive oxygen species, and disinfection was only efficient when the photocatalytic titania was bionconjugated to the bacteria (for controls, see Supporting Information). After irradiation, the concentrations of viable *E. coli* were shown to dramatically diminish (Figure 4) as functions of both the concentration and the type of titania particles. For example, one hour irradiation of *E. coli* dispersion in the presence of EA-TiO_2 induced nearly a 100-fold decrease in live bacteria when the ratio of TiO_2 particles to bacteria was greater than 10 (the mass concentration of TiO_2, the *x*-axis in Figure 4, is expressed as the number of primary TiO_2 particles per bacterium—see Experimental Section for details).

In most experiments involving TiO_2 photocatalysis, irradiation time is a critical variable—this work is no exception (Figure 5).[14] After two hours of irradiation, the *E. coli* antibody coated TiO_2 (EA-TiO_2) reduced the viable *E. coli* concentration

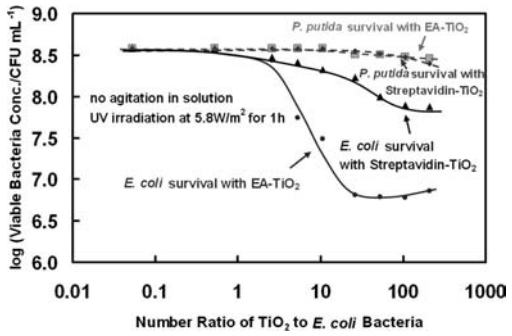

Figure 4. Influence of TiO_2 concentration on the viability of *E. coli* or *P. putida*. Only the antibody coated EA-TiO_2 specifically adsorbed onto *E. coli* and induced significant killing upon exposure to UV for 1 h.

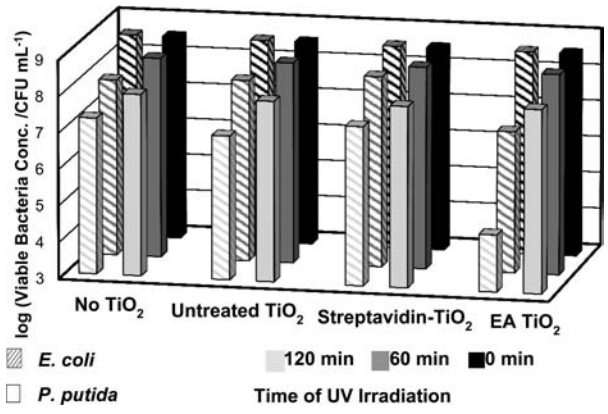

Figure 5. Selective photodisinfection of *E. coli* with EA-TiO_2 bearing *E. coli* antibodies. The TiO_2 concentration corresponded to 26 TiO_2 particles per *E. coli* and the samples were not agitated during irradiation.

by 10 000 (log 4), while after one hour the value was only about 30 (log 1.5).

Influence of Agitation on Selectivity of Photodisinfection. Agitation of the suspension had an interesting effect on photodisinfection. It was initially assumed that stirring would improve photodisinfection because the TiO_2 particles would remain in suspension and shear forces would help prevent the formation of large aggregates that could interfere with UV penetration into the dispersion. In fact, the results showed the opposite: gentle agitation lowered photodisinfection efficacy with EA-TiO_2. Figure 6 shows bacteria viability as a function of stirring rate during UV irradiation. With stirring, EA-TiO_2 performance decreased, whereas the streptavidin-TiO_2 efficiency as a photooxidant improved with increased stirring speed: note that the stirring was mild. We estimate that the Reynolds number for the highest stirring rate was approximately 50, well within the laminar flow regime.[34]

DISCUSSION

Based on the measurements described above, the titania particle surface is interpreted to have the following characteristics. Approximately 50% of the TiO_2 surface was covered with streptavidin and there was slightly less than one antibody tethered per streptavidin, as illustrated in Figure 1. There was a ~4-nm-thick layer of (cationic) aminosilane in the areas between bound streptavidin, in which 77% of the amino groups were derivatized with biotin. The electrophoretic mobility

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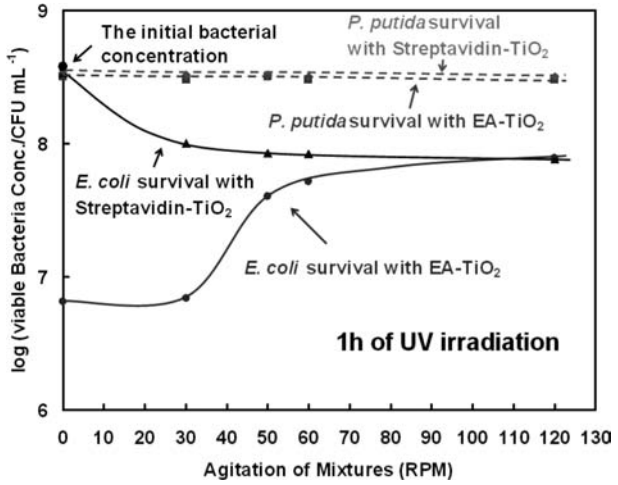

Figure 6. Influence of stirring on photodisinfection. Results correspond to 1 h irradiation time and 26 titania particles for every bacterium.

values of all the titania derivatives were negative (Table 2) suggesting that the protein dominates the electrokinetic properties of the final titania particles. The mixing of EA-TiO_2 with bacteria initiated three linked kinetic processes—aggregation, redispersion, and killing. The key limiting cases are illustrated in Figure 7.

Aggregation. In spite of the electrostatic repulsion between negatively charged EA-TiO_2 and *E. coli*, coaggregation was observed because the TiO_2 surface-bound antibodies formed adhesive contact with bacteria during Brownian encounters. If conducted in the dark, aggregation did not kill the bacteria. However, aggregation did negate the value of CFU as a measurement of bacterial load—ten living bacteria in a floc counts as only one CFU.[35] The aggregate size distribution (in the dark) depended mostly upon the ratio of titania particles to bacteria. The physical principles of heteroflocculation are well established for model systems.[36] For example, when there are many more titania particles then bacteria, the bacteria were completely coated with adsorbed titania such as shown in Figure 3C. By contrast, as the number concentration of bacteria approached that of the titania particles, it is expected that large flocs such as those shown in Figure 3D would form: the largest flocs were found to form at a ratio of ~50 EA-TiO_2/*E. coli* bacterium. As the number of EA-TiO_2 particles decreased or increased from about 50/bacterium, the bacteria/TiO_2 floc size decreased.

There was no evidence that EA-TiO_2 was able to conjugate to *P. putida*, suggesting that the aggregation process could be a simple way to selectively isolate specific bacteria by first aggregating and then physically separating the flocs by coarse filtration or centrifugation. In such a case, no benefit is taken of the photoproperties of the titania.

Redispersion. The irradiation of TiO_2 by UV light in the presence of water and/or oxygen leads to the formation of reactive oxygen species (ROS). In the presence of UV light with mixing, the titania/bacteria flocs redispersed. Previously, we have shown that amino-silane coating on treated titania is rapidly destroyed by exposure to UV light.[37] Therefore, in addition to killing the bacteria (next section), the ROS also destroy the tethers between the titania particles and the bacteria surfaces. With even gentle stirring, living bacteria can lose contact with the titania and thus survive (Figure 7).

Killing. Under quiescent conditions, the fragile titania-bacteria flocs remain intact sufficiently long for the ROS to kill the bacteria (Figure 7). The selective bacteria experiments (Figure 5) clearly indicate that those microbes in contact with the titania are most susceptible to light induced killing. Therefore, the strategy for maximum killing is to treat the bacteria in the dark to form the maximum number of titania contacts, followed by light exposure while avoiding excess hydrodynamic forces. Herein, we have demonstrated selective killing by using antibodies to give specific deposition of titania onto *E. coli*, leaving the *P. putida* unaffected. We recognize that, in most standard disinfection applications, one wishes to kill all bacteria. However, it is increasingly recognized that there are beneficial bacteria that, where possible, should be left alone. This approach may be appropriate for such applications requiring more sophisticated control of the bacterial population.

In summary, aggregation, redispersion, and photocatalytic bacteria killing are linked kinetic processes that are sensitive to the photophysical parameters (light flux, wavelength, photocatalytic efficiency, turbidity), hydrodynamic parameters, colloidal properties (zeta potential, van der Waals, steric effects), and the detailed structure of the modified titania surfaces (antibody density, silane layer thickness). These can be profitably exploited to selectively kill detrimental bacteria. Future advancements might include tethers that are less susceptible to photocatalytic decomposition.

■ CONCLUSIONS

Titania particles that selectively target *E. coli* in the presence of *P. putida* are readily prepared by a series of surface modifications culminating in the conjugation of an antibody selective for the bacteria. The antibody-coated TiO_2 (EA-TiO_2) lowered the concentration of viable *E. coli* by both photo-

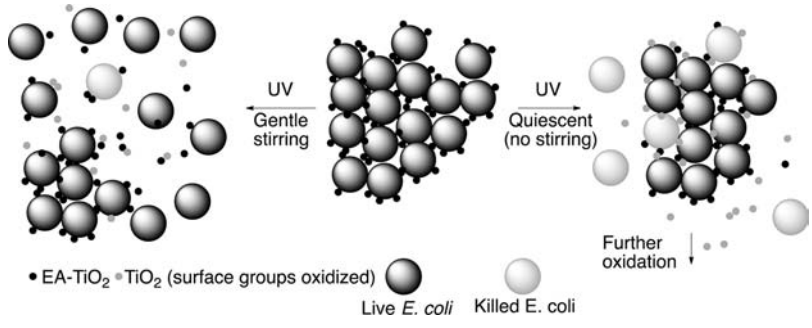

Figure 7. Model of oxidation processes with and without stirring showing that more ROS is delivered to the bacterium over time in the latter case.

dx.doi.org/10.1021/bc300581t | *Bioconjugate Chem.* 2013, 24, 448−455

catalysis and by flocculation. The lowest concentration of EA-TiO_2 giving maximum *E. coli* reduction corresponded to ~26 titania particles per bacterium. *E. coli* disinfection with EA-TiO_2 was decreased by stirring, suggesting that the disinfection benefits weak adhesion of EA-TiO_2 to *E. coli* even after the bioconjugating layer is oxidized.

■ ASSOCIATED CONTENT

Supporting Information

Tables showing control experiments that were performed with different reagents in the dark or in the presence of UV light (3 tables) and SEM/confocal images of control particles showing that EA-TiO_2 does not bind to *E. coli*. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Domtar Canada and the Sentinel Bioactive Paper NSERC Network for financial support of this research and Dr. Richard Gratton (Domtar) for helpful discussions. RP holds the Canada Research Chair in Interfacial Technologies.

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