Antibacterial performance of nanoscaled visible-light responsive platinum-containing titania photocatalyst in vitro and in vivo

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Background: Traditional antibacterial photocatalysts are primarily induced by ultraviolet light to elicit antibacterial reactive oxygen species. New generation visible-light responsive photocatalysts were discovered, offering greater opportunity to use photocatalysts as disinfectants in our living environment. Recently, we found that visible-light responsive platinum-containing titania (TiO2–Pt) exerted high performance antibacterial property against soil-borne pathogens even in soil highly contaminated water. However, its physical and photocatalytic properties, and the application in vivo have not been well-characterized.

Methods: Transmission electron microscopy, energy dispersive spectroscopy, X-ray photoelectron spectroscopy, X-ray diffraction, ultraviolet–visible absorption spectrum and the removal rate of nitrogen oxides were therefore analyzed. The antibacterial performance under in vitro and in vivo conditions was evaluated.

Results: The apparent quantum efficiency for visible light illuminated TiO2–Pt is relatively higher than several other titania photocatalysts. The killing effect achieved approximately 2 log reductions of pathogenic bacteria in vitro. Illumination of injected TiO2–Pt successfully ameliorated the subcutaneous infection in mice.

Conclusions: This is the first demonstration of in vivo antibacterial use of TiO2–Pt nanoparticles. When compared to nanoparticles of some other visible-light responsive photocatalysts, TiO2–Pt nanoparticles induced less adverse effects such as exacerbated platelet clearance and hepatic cytotoxicity in vivo.

General significance: These findings suggest that the TiO2–Pt may have potential application on the development of an antibacterial material in both in vitro and in vivo settings.

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1. Introduction

Disinfectants and antibiotics are antimicrobial agents extensively used in various in vitro and in vivo settings. They are essential for infection control and aid in the prevention of nosocomial infections. Compared to antibiotics, which provide comparatively selective activity against microorganisms, disinfectants typically have a broader biocidal spectrum and are usually used with inanimate objects [1,2]. Traditional chemical-based disinfectants, such as alcohols, aldehydes, iodine, phenols, and chlorine, have been used for centuries in environmental cleaning. Although these disinfectants are highly efficient against pathogenic microbes, they also have problems. These disinfectants are usually volatile, and their byproducts can be toxic and carcinogenic to humans. As the widespread use of antibiotics and the emergence of more-resistant and -virulent strains of microorganisms became great clinical challenges [3–8], the establishment and development of novel antibacterial strategies is, therefore, significant in the control of human pathogens and prevention of infectious diseases. From this point of view, photocatalyst-based antibacterial agents are conceptually feasible alternative approaches.

Classical photocatalyst generates pairs of electrons and holes (electron vacancy in valence band) upon ultraviolet light (UV) illumination. The electrons and holes induced by the reactions have strong reducing and oxidizing activities, and subsequently react with atmospheric water and oxygen (H2O and O2) to yield reactive oxygen species (ROS), such as hydroxyl radicals (•OH), superoxide anions (O2•−), and hydrogen peroxide (H2O2) [9]. These ROS can oxidize organic substances and exert the antibacterial function [10]. Titanium dioxide (TiO2) is the most widely used UV-responsive photocatalyst [11], which has been demonstrated to exert antibacterial effect in various forms that includes nanoparticles, thin films and polymer-composites [12–23]. The UV-light, however, can damage eyes and skin [24]. These side effects limit the use of UV-responsive photocatalysts in our living environments. Recently,
photocatalysts using visible light as alternative energy source were developed [25,26], which exerted antibacterial property [22,27,28]. These visible light responsive photocatalysts may also increase the light-spectrum absorption range, because the UV range of solar irradiation reached to the Earth’s surfaces is estimated to be only 2–3% [29]. At the same time, the emergence of antibiotic-resistant bacteria in public environments leads to an urgent need of alternative disinfection approaches [3–7]. Visible-light responsive photocatalysts have potential advantages for use in a variety of settings to reduce the transmission of pathogens in the public environments. Impurity doping of different materials such as anion and cation on TiO2 is a generally used strategy to extend the absorption spectrum into the visible-light range [25–28,30]. Since silver contains antibacterial property [31], a composite with silver nanoparticles is another approach to increase both antibacterial performance and visible-light responsiveness of titania photocatalysts [14,19,32]. Iron can extend the absorption of the light to the visible region and serve as an electron or hole scavenger on the surface of TiO2 to result in better separation of free carriers [33]. As visible-light responsive photocatalysts are currently under a developmental stage, their antibacterial property [27,28,34], however, is unable to compare with the high antibacterial performance of traditional chemical disinfectants [1]. Thus, development of a high performance photocatalyst is needed.

The antibacterial property of platinum-loaded titania (TiO2–Pt) was firstly reported to be activated by UV-irradiation [35]. Recently, we found that platinum-containing titania (TiO2–Pt) photocatalyst contains superior antibacterial property against soil-borne pathogens even in soil-contaminated water [36]. However, the physical and photocatalytic properties under visible light illumination and the potential antibacterial application in vivo have not been well-characterized. As a result, to characterize the crystal morphology, components of Pt element, Pt valence states, crystal structure and light absorption spectrum, we employed transmission electron microscopy (TEM), energy dispersive spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD) and UV–visible absorption spectrum, respectively. The photocatalytic performance was characterized by measuring the removal rate of nitrogen oxides (NOx). In addition, the antibacterial property of the TiO2–Pt against nosocomial infected pathogens Staphylococcus aureus, and Acinetobacter baumannii, and exotoxin-producing Streptococcus pyogenes was further evaluated under visible light illumination. The data suggest that TiO2–Pt substrates have superior visible-light induced bacterialidial property against human pathogens (approximately 2 log reduction) as compared to several other commercially available and laboratory prepared photocatalysts (ST01, P-25, C200, BA-PW25, etc. [23,34,37–40]; achieved approximately 0–1 log reduction under visible light). In addition, the potential in vivo usage was further evaluated by nanoparticle treatments using a mouse model, in which the potential applications in vivo are discussed.

2. Materials and methods

2.1. Preparation and characterization of photocatalysts

Following previously described methods [36], platinum-containing nano-structured TiO2 particles (TiO2–Pt) were prepared by the photoreduction process using chloroplatinic acid (H2PtCl6) and commercial TiO2 nanoparticles (ST01; Ishihara, Singapore) as a platinum precursor and a pristine photocatalyst, respectively. The photocatalyst crystal phase was identified using X-ray diffractometry with Cu Kα radiation (λ = 0.154 nm, D/Max RC; Rigaku, Tokyo, Japan). Material compositions were determined by X-ray photoelectron spectroscopy (SSI-M probe XPS system; Perkin Elmer, Waltham, MA). A diffuse-reflectance scanning spectrophotometer (UV-2450; Shimadzu, Kyoto, Japan) was employed to obtain the UV–visible absorption spectra of the powders. The reflectance data were converted to the absorbance values, F(R), based on the Kubelka–Munk theory. Measuring average particle size and morphology was performed by transmission electron microscopy (TEM, Philips Tecnai F20 G2 FEI-TEM). The specific surface area of the powders was measured by nitrogen adsorption using the Brunauer, Emmet, and Teller (BET) equation (Micrometrics Model ASAP 2400). The amount of Pt that was released from the TiO2–Pt surfaces into the deionized (DI) water (0.1 g TiO2–Pt powder in 100 mL H2O, pH 7.4) was measured by inductively coupled plasma atomic emission spectrometry (ICP-AES, JY 2000-2, Horiba).

2.2. Photocatalytic activity for NO oxidation

The NOx degradation was carried out at room temperature using an air stream containing 1.0 ppm NO as feedstock. The reaction system and procedure were described in detail in our previous papers [39,41–43]. The fundamental photocatalytic activity of prepared Pt/TiO2 was evaluated by the de-NOx photocatalytic activity implemented in relation to ISO22197-1 [44].

2.3. Bacterial strains and culture

Bacteria S. pyogenes (strain M29588), S. aureus (strain SA02) (without antibiotic resistance), and pan-drug resistance A. baumannii (PDRAB; strain M36788), are clinical isolates from the Buddhist Tzu-Chi General Hospital, Hualien, Taiwan. S. pyogenes and S. aureus were grown in tryptic soy broth supplemented with 0.5% yeast extract (TSBY) broth or TSBY broth agar (MDBio, Inc. Taipei, Taiwan) [27]. A. baumannii were maintained and grown in Luria–Bertani broth (LB) or LB agar (MDBio, Inc. Taipei, Taiwan) [27].

2.4. Photocatalytic reaction and detection of viable bacteria

The elimination of bacteria by photocatalysis was determined using previously described methods [27,28]. Bacteria and TiO2–Pt loaded 24-well plates (1 × 105 CFU + saline-rinsed TiO2–Pt nanoparticles 50 μg/ml) were placed under an incandescent lamp (Classictone incandescent lamp. 60 W; Philips) for photocatalytic reaction. The illuminations were initially carried out with illumination density of 48 mW/cm2 for 75 min (337.5 J/cm2). In dose-dependence experiments, illuminations were performed with illumination density of 28, 34 and 48 mW/cm2 for 30 min (1 × 103, 3 × 103, 1 × 104 lx; and 50.4, 61.2 and 86.4 J/cm2, respectively). In the kinetic analysis, illuminations were conducted with illumination density of 48 mW/cm2 for 10, 15, 30, 45 and 75 min (28.8, 43.2, 86.4, 129.6 and 337.5 J/cm2). The reaction temperature was 15 °C. After illumination, the surviving bacteria were quantified using the standard plating method. Commercially available UV-responsive photocatalysts ST01 [39], UV100 (Sachtleben, Germany) [34], Degussa P-25 (Evonik Degussa, Essen, Germany) [40] and UV/Vis-responsive photocatalyst BA-PW25 (Ecodevice, Tokyo, Japan) [37] were used as comparisons.

2.5. Transmission electron microscopy (TEM), scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) analyses

The bacterial samples were negatively stained and then analyzed by TEM using a Hitachi H–7500 transmission electron microscope. The SEM and EDS were analyzed using a JEM-3010 scanning electron microscope (JEOL, Japan) equipped with energy dispersive X-ray spectrometer following previously described methods (SEM [34,45], EDS [45]).

2.6. Air-pouch infection mouse model

Animal care and the air pouch infection were performed as described [36,45,46]. Anesthetic C57Bl/6j mice were subcutaneously injected with 1 ml air to form an air pouch. Suspensions of the S. aureus (log phase,
3 × 10⁵ CFU, 0.2 ml phosphate buffered saline (PBS) and the photocatalysts (D.I. H₂O rinsed twice; 500 μg/mL, 0.3 mL PBS) were injected into the air pouch sequentially. Bacteria–photocatalyst mixtures in the air pouches were then illuminated by skin-penetrative visible light using a cold-light source (KL-1500 LCD, Carl Zeiss; visible light, 60 mW/cm², 30 min), by which the mouse skin will not be damaged by heat during illumination. The number of surviving bacteria (CFU) was determined using the standard plating method. The research methods applied regarding the experimental mice were approved by the Institutional Animal Care and Use Committee of Tzu Chi University (approval ID: 95012).

2.7. Statistical analysis

All results were calculated from data at least of three independent experiments. T-test was used to assess statistical significance of differences in results of the antimicrobial effects. A P value of less than 0.05 (P < 0.05) was considered significant. The statistical tests were carried out and output to graphs using the Microsoft Excel (Microsoft Taiwan, Taipei, Taiwan) and SigmaPlot (Systat Software, Point Richmond, CA) software.

2.8. Supplemental methods

More detailed descriptions referring to the materials and methods used in this study could be obtained from the supplemental materials online.

Fig. 1. Transmission electron microscopy (TEM) images and spectrum analyses of TiO₂–Pt. The representative images were captured in bright field (A-1) and dark field (A-2), respectively. Through energy dispersive spectroscopy (EDS) analysis (A-3), Pt was found in the arrows pointed spots (A-1, 2). Scale bar: 20 nm. Deconvolution of Pt 4f XPS spectra for TiO₂–Pt (B), and XRD pattern (C) and UV–visible absorption spectra (D) of TiO₂–Pt and ST01 were indicated.
respectively. The results indicate that the addition of Pt produced insignificant changes in the specific surface area of TiO₂ particle size due to low Pt content and low calcination temperature in this preparation procedure. Furthermore, TEM observation confirmed that the prepared TiO₂ powders had an almost spherical and nonporous form. Therefore, this relationship can be expressed by a simple equation, assuming a spherical and nonporous particle:

\[ \rho \frac{\delta_{\text{BET}}}{\rho} = 6000 \ \text{μm} \text{/(g cm}^{-3}\text{)} \]

where \( \delta_{\text{BET}} \) is the calculated particle size (nm), \( \rho \) the density of TiO₂ (3.84 g/cm³), and \( \rho \) is the specific surface area (m²/g) [51]. Thus, the \( \delta_{\text{BET}} \) of TiO₂-Pt sample is around 8.6 nm by the above equation. The X-ray diffraction (XRD) patterns of TiO₂ samples were also examined (Fig. 1C). The peaks indicated as (101), (004), (200), (105), and (211) in Fig. 1C, revealed the reflection characteristics of TiO₂ anatase phase appearing in all samples (Fig. 1C). The rutile phase does not appear due to the low-temperature calcination. The crystallite sizes of the prepared photocatalysts are determined from a half width of (101) peak (2θ = 25.4°) using the Scherrer formula [52]. Accordingly, the anatase grain size of TiO₂-Pt and ST01 were around 9–7 nm, which is consistent with the observations of TEM images (Fig. 1A) and the estimated result from specific surface area. No peaks corresponding to crystalline phase of Pt metal and PtO₂ (2θ = 39.8°, 46.2°, 67.5°) were found due to small crystallite size and low Pt content. The results indicate that the addition of Pt salt produced insignificant changes in the primary TiO₂ particle size.

To investigate the light absorption property, UV–visible diffuse reflectance spectra of TiO₂–Pt and ST01 samples were analyzed (Fig. 1D). This reflectance data was converted by the instrument software to absorbance values, F(R), based on the Kubelka–Munk theory. TiO₂–Pt exhibited a good visible-light absorption tail up to 700 nm, and a sharp edge that extended to ~432 nm corresponding to a band gap of ~2.87 eV was observed (Fig. 1D). Therefore, the platinum species on the sample may play a role as the sensitizer for visible-light absorption.

### 3.2. Photocatalytic degradation of NO over TiO₂–Pt under visible light illumination

Nitrogen oxides (NOx) are air pollutants [53]. Oxidation of NO was used to determine photocatalytic reactivity in various TiO₂ photocatalytic applications [50,54–56]. Before the antibacterial test, the reaction of photocatalytic oxidation of NO was used to evaluate the visible-light-responsive activity and estimate the apparent quantum efficiency of TiO₂ samples. The electron–hole pair (e⁻–h⁺) generated upon light excitation is trapped at the TiO₂ surfaces as spatially separated redox active sites. Some studies report the formation of reactive oxygen species, such as superoxide ion (O₂⁻), atomic oxygen (O) O²⁻, OH and H₂O radicals on the surface of TiO₂ irradiated with UV light [11]. The general mechanism of NOx oxidation by photocatalyst is as follows. Hydrogen ions and hydroxide ions are dissociated from water. The active oxygen species are produced on the TiO₂ surfaces. The nitric monoxide is oxidized to nitric acid or nitrous acid by active oxygen species. Based on the gas-phase chemistry of NOx [54], NO is converted to HNO₃ as a consecutive photooxidation via a NO₂ intermediate.

\[ \text{NO} + \text{HO}_2^- \rightarrow \text{NO}_2 + \text{OH} \]  
\[ \text{NO}_2 + \text{OH} \rightarrow \text{HNO}_3. \]  

Photocatalytic TiO₂–Pt mediated removal of NO and NOx was compared with the pristine photocatalyst ST01, two UV-responsive photocatalysts UV100, P-25, and two visible-light responsive photocatalysts BA–PW25 (commercially available) [37] and C200 (a carbon-containing TiO₂) [23] (Fig. 2A). The activity levels of these photocatalysts fell in the order of TiO₂–Pt > C200 > BA–PW25 ≫ ST01 – UV100 – P25 under visible light (blue LED). The pure TiO₂ samples (ST01, UV100, and P25) have little photoactivities due to their large bandgap. The generation of NO₂ and selectivity of NO₂ (SelNO₂ = [NO₂ generated] / [NO₂ converted]) of the photocatalysts have a great effect on the NO–removal activity, as shown in Fig. 2B. For example, the TiO₂–Pt has 3.6 times the NOx removal activity of BA–PW25 even if the TiO₂–Pt has only 1.7 times the NO removal activity of BA–PW25. It might be that the Pt particle has a good affinity to NO₂. This is valuable for photocatalytic removal of NO, because NO₂ is an undesired intermediate in the consecutive oxidation of NO; the threshold concentrations of NO₂ and NO are 3 and 25 ppm, respectively [56].

According to the reaction scheme of the photo-oxidation of NO, the photocatalyst would be deactivated after long-term operation due to the coverage of active sites by adsorbed NO₃⁻ ions at the catalyst surface. However, the adsorbed NO₃⁻ ions could be easily washed away with 100 mL of D.I. water, enabling reuse of the catalyst. The removal rates of NOx over 1st, 2nd, and 5th-reused TiO₂–Pt samples were indicated (Fig. 2A). The photocatalytic activity was only slightly decreased and becomes stable without significant reduction after two reaction–reuse cycles, in which over 96% of the photocatalytic activity remains functional (Fig. 2, 2nd, and 5th reused groups). The ICP-AES quantification further confirmed that the minor reduction of photocatalytic activity is due to the release of unstable Pt particles during reuses. The Pt content of the as-prepared/original TiO₂–Pt sample was 62 μmol/g TiO₂. While the Pt content in the 1st-, 2nd-, and 5th-rinsed water were 3.2, 0.2, and 0 (not detectable) μmol/g TiO₂–Pt, respectively. This suggests that most Pt particles on the TiO₂ surfaces are quite stable and thus TiO₂–Pt may be suitable for the development of additional antibacterial applications.
3.3. Bactericidal property of TiO2–Pt photocatalysts

After physical characterizations, the bactericidal property of TiO2–Pt against S. aureus was compared with other visible light-responsive photocatalysts (Fig. 3A, 48 mW/cm², 75 min). The ultraviolet (UV) light responsive photocatalysts ST01, P-25 and UV100 [34,39,40] were used as negative controls under visible light illumination (Fig. 3A). TiO2–Pt samples were found to exhibit significantly higher bactericidal activity as compared with other tested samples including UV responsive photocatalysts ST01, P-25, UV100, visible-light responsive photocatalysts BA-PW25 (commercially available [37,38]), and C200 (a carbon-containing TiO2 [23,34]) (Fig. 3A, ***, P < 0.001, vs. respective dark groups).

To obtain dose-dependent and kinetic data, we further analyzed the antibacterial activity against clinically isolated S. aureus at various intensities (28, 34 and 48 mW/cm², visible light) for 30 min (Fig. 3B) or fixed illumination density (48 mW/cm²) at various time intervals (0, 10, 15, 30, 45 and 75 min; Fig. 3C). Our results revealed that photocatalytic TiO2–Pt substrates significantly reduced viable S. aureus cells within 30 min when exposed to visible light with doses higher than 34 mW/cm² (61.2 J/cm²; Fig. 3B). In addition, when the illumination density 48 mW/cm² was used, an efficient kill can be observed within 15 min (Fig. 3C, 15 min, TiO2–Pt-light groups). Illumination of TiO2–Pt by visible light significantly reduced the viable population of the bacteria (Fig. 3B and C; *, P < 0.05, **, P < 0.01, ***, P < 0.001, vs. respective untreated-dark groups).

To investigate the performance of TiO2–Pt to eradicate other pathogenic bacteria, the antibacterial property against pathogenic S. pyogenes (toxin producing) and A. baumannii (nosocomial spreading) was compared with S. aureus (48 mW/cm², 15 min; 43.2 J/cm²). Data revealed that the viable population of all tested pathogens was significantly reduced greater than 50% after the photocatalysis in this condition (Fig. 3D, *, P < 0.05, **, P < 0.01 and ***, P < 0.001, TiO2–Pt, dark vs. light groups). In addition, S. pyogenes and A. baumannii are more sensitive to the photocatalysis than S. aureus (Fig. 3D, S. pyogenes and A. baumannii vs. S. aureus, TiO2–Pt-light groups; *, P < 0.05, **, P < 0.01, ***, P < 0.001, vs. respective TiO2–Pt-dark groups).

3.4. Photocatalysis reduced the survival rate of S. aureus after macrophage engulfment

Photocatalysis induces bacterial deformation [23], by which it reduced the resistance of phagocytic clearance, and contributed to the antibacterial effect of nitrogen-doped TiO2 [28]. Thus, it was analyzed whether or not such antibacterial effect exists in this system (Suppl. Fig. S2). S. aureus was subjected to photocatalysis under a condition without significantly suppressing the viable bacteria (48 mW/cm², 5 min; Fig. 3C, no effect of TiO2–Pt-light, 10 min groups). After a relatively short phagocytosis course (J774A.1 mouse macrophages [57]), we found that photocatalysis did not influence the phagocytic efficiency of photocatalyzed bacteria (Suppl. Fig. S2A, tabellable; S2B, no significant differences among 0 h groups). However, the surviving bacteria significantly reduced in photocatalyzed groups after a longer engulfment and digestion by phagosomal enzymes (Suppl. Fig. S2B, TiO2–Pt-light, 4 h groups vs. all other 4 h groups; ***, P < 0.01). These results suggest that TiO2–Pt-mediated photocatalysis likely induced temporal and recoverable bacterial damages, which could be recovered on the Luria–Bertani (LB) agar (Fig. 3C, no effect of TiO2–Pt-light, 10 min),...
but became extra-burdens for those phagocyted bacteria to defend themselves against antibacterial mechanism of macrophages (Suppl. Fig. S2B, TiO2–Pt light vs. dark, 4 h groups, **, P < 0.01). As damages of bacterial cells also contribute to the antibacterial outcome, killing efficiency may not fatefully reflect the overall antibacterial property of a photocatalyst. Accordingly, as compared with the traditional plating method, this phagocytosis assay is more realistic and sensitive to reveal relative attenuation levels of the bacteria.

3.5. Electron microscopic imaging of bacterial damages

To investigate the damages on photocatalyzed S. aureus cells, transmission electron microscopy (TEM) images of negative stained samples (Fig. 4A–F) and scanning electron microscopy (SEM) images were taken (Fig. 4G–H). When compared with the untreated (Fig. 4A–B) and non-photocatalyzed groups (Fig. 4E–G; in the dark), TiO2–Pt-mediated photocatalysis (Fig. 4C–D, H) significantly induced damages on the bacteria. Photocatalysis causes perturbations on S. aureus cells (Fig. 4D, arrows), which are not observed in those bacteria without illumination (Fig. 4E–F). These results are in agreement with the SEM analysis, in which the deformation sites on the bacterial surfaces are revealed (Fig. 4H, white arrows, as compared with controls in the dark 5G).

3.6. Photocatalytic therapy mouse model

Aforementioned phagocytosis experiments indicated that TiO2–Pt–mediated photocatalysis can attenuate bacteria in vitro (Suppl. Fig. S2). Accordingly, photocatalytic attenuation and elimination of pathogenic bacteria may be feasible in vivo. Before the in vivo experiments, we successfully analyzed the spectrum and demonstrated TiO2–Pt-mediated antibacterial effect using mouse-skin penetrative visible-light (Suppl. Fig. S3). To further investigate the bactericidal property of TiO2–Pt in vivo, an air-pouch infection mouse model was performed. Bacterial suspensions of S. aureus (3 × 10^5 CFU) were injected into the air pouches underneath the mouse skins and then illuminated the injection sites with skin-penetrative visible light using a cold-light source (KL-1500 LCD, 60 mW/cm^2) for 30 min (Fig. 5A, experiment outline; Suppl. Fig. S4, experimental setting). Twenty-four hours later, the surviving bacteria (CFU) were then analyzed using the plating method. When compared with the control groups, both C200 (a carbon-containing TiO2) [23] and TiO2–Pt groups contained a reduced bacterial population after illumination (Fig. 5B, light vs. dark and untreated groups; *, P < 0.05, **, P < 0.01, vs. respective dark groups; †, P < 0.05, vs. C200 groups). In agreement with the in vitro analyses (Fig. 3A, Suppl. Fig. S3C), the bactericidal performance of TiO2–Pt is significantly higher than C200 (Fig. 5, TiO2–Pt vs. C200, light groups). These results suggest that TiO2–Pt photocatalyst may have potentials to be developed as an antibacterial agent in vivo.

4. Discussion

Heavy traveling and urbanization enable infectious diseases to spread worldwide more rapidly. The emergence of virulent and antibiotic resistant pathogens leads to a requirement of alternative disinfection strategies in public environments [3–8]. Development of visible-light-responsive photocatalysts may be a feasible approach to against these pathogens. Classical photocatalysts are UV-responsive. To extend the light-absorption into visible-light range, TiO2 composting with transition ions and/or anions is a common strategy to create intra-band gap states close to the conduction or valence band edges that cause visible-light absorption at the sub-band gap energies [26,29]. These materials are also able to inhibit the charge recombination in some conditions, thereby increase the photocatalytic activity [11,29]. As a result, the TiO2–Pt photocatalyst was prepared in this study. Through NO-oxidation analysis, we confirmed that TiO2–Pt has a higher visible-light inducible photocatalytic performance as compared with UV-responsive (P25, UV100), and UV/visible-responsive (C200 and BA-PW25) TiO2-based photocatalysts. In general, it has been reported that the TiO2 photocatalytic reactions proceed mainly by the contributions of active oxygen species, such as hydroxyl radical, OH•, superoxide radical, O2•−, and hydrogen peroxide, H2O2 [58–60]. Although these active species formation mechanisms have been suggested as photocatalytic oxidation and reduction of water and oxygen [58–60], the detailed mechanism on the TiO2 surface is unclear so far, and a lot of efforts have been spent to elucidate the precise mechanism by many research groups [59,61,62]. It is hard to estimate the real quantum efficiency (Φ) of photocatalyst. Therefore, the apparent quantum efficiency
\[ \Phi_{\text{app}} = \frac{\text{(mole of NO + NO}_2\text{) degraded}}{\text{Einstein of incident photons}}. \]

The apparent quantum efficiencies for TiO\textsubscript{2}–Pt, C200, BA–PW25, P25, UV100, and ST01 were 3.03, 1.88, 1.33, 0.41, 0.36, and 0.38%, which are consistent with the NO\textsubscript{x}-removal activity. In agreement with the estimation on the apparent quantum efficiencies and the performance in NO-oxidation experiments (Fig. 2), TiO\textsubscript{2}–Pt showed a superior bacterial property after illuminated by visible light. Survival of human pathogenic bacteria after photocatalysis showed a reduced-resistance against phagocytic clearance [28]. This suggests that such cellular damages and deformations may be an unaffordable burden when engulfed by a phagocyte. By contrast, these damages may be repairable if these bacteria were cultured in an optimal condition (e.g. on a dish with LB agar). In agreement with these observations, TiO\textsubscript{2}–Pt photocatalyzed S. aureus cells showed a relatively lower resistance against phagocytosis in prolonged treatments (Suppl. Fig. S2B, TiO\textsubscript{2}–Pt-light 4 h groups), indicating that TiO\textsubscript{2}–Pt-mediated photocatalysis also induce such bacterial damages. Even though bacteria cannot be completely eliminated by photocatalysis, these results suggest that the remaining survivors are also greatly attenuated, and thus provide a promising approach for disinfection of pathogenic bacteria. In addition, these results suggest that TiO\textsubscript{2}–Pt-mediated attenuation of bacteria could be feasible in vivo.

Even though antibacterial effect of TiO\textsubscript{2} photocatalysts has been well-documented in thin film and polymer composites [12–21,27,28,32], the use of nanoparticles is a relatively feasible approach to be applied in vivo. In the animal experiments, TiO\textsubscript{2}–Pt nanoparticle-mediated bacterial killing was successfully demonstrated in mice. We found that TiO\textsubscript{2}–Pt more efficiently eliminated pathogenic bacteria than another visible light responsive photocatalyst C200 in an air pouch model. Despite these results, evidences have implied that exposure to TiO\textsubscript{2} can cause side effects such as procoagulant and inflammatory responses [65–67]. Activation of platelets and coagulant factors by TiO\textsubscript{2} surfaces can lead to procoagulant activation and thus limit dental applications that make contact with patient tissue [65–67]. Through measuring the blood counts and a marker of liver-function aspartate aminotransferase (AST), here we found that the TiO\textsubscript{2}–Pt treatments did not induce significant pathogenic side effects in mice (Suppl. Fig. S5). Unlike commercially available BA–PW25 and carbon-containing TiO\textsubscript{2} C200, TiO\textsubscript{2}–Pt injections did not elicit side effects such as thrombocytopenia and liver damage (Suppl. Fig. S5); these two phenotypes implicate the platelet activation, procoagulant and inflammatory responses [46,65,66,68,69]). This suggested that TiO\textsubscript{2}–Pt is more suitable for in vivo usages as compared with other materials tested in this study. Literatures have indicated that adverse effects such as cyto- and geno-toxic effects of nanomaterials are primarily caused by the induction of oxidative stress [70,71]. Since oxidative stress can induce inflammation and liver damages [72], the different behavior of TiO\textsubscript{2}–Pt versus BA–PW25 and C200 in vivo may be resulted by similar mechanism. A detailed molecular pathway that leads to different responses in vivo after various TiO\textsubscript{2} photocatalysts treatments is an interesting issue and worthy to be further investigated.

Although the antibacterial performance of visible-light activated TiO\textsubscript{2}–Pt may not match with conventional disinfectants such as alcohols, iodine and chlorine, these disinfectants are not suitable for in vivo applications. As the antibacterial mechanism is different from the antibiotics, photocatalysts have potentials to be developed as an alternative approach to eliminate drug-resistant bacteria. In addition, compared to carbon and silver containing TiO\textsubscript{2}, on which the carbon is gradually decomposed and the silver is continuously releasing into the rinsed water that leads to a reduction of visible-light stimulated photocatalytic performance (authors’ unpublished results), TiO\textsubscript{2}–Pt is highly stable after various reuses. Furthermore, under visible light stimulation, TiO\textsubscript{2}–Pt exerts superior quantum efficiency and better bactericidal photocatalytic performance on the elimination of pathogenic bacteria when compared to several commercially available and laboratory-prepared UV/visible-responsive photocatalysts (UV-responsive: ST01, P25, UV100, and visible-responsive: C200, BA–PW25). At the same time, TiO\textsubscript{2}–Pt-mediated photocatalysis is able to eliminate subcutaneously infected bacteria. In addition to the killing, the bacterial damages induced prior to the exposure to host defense system also facilitated the phagocytic clearance. Thus, these findings suggest that the visible-light responsive TiO\textsubscript{2}–Pt photocatalyst have potential applications on the development of a highly effective antibacterial material in both in vitro and in vivo settings.
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Appendix A. Supplementary data
Supplementary data to this article can be found online at http://
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