Core/Shell Quantum-Dot-Photosensitized Nano-TiO_2 Films: Fabrication and Application to the Damage of Cells and DNA

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Through the use of monodisperse core/shell quantum dots (QDs) as photosensitizers for the first time, a novel strategy for the fabrication of QD-photosensitized nano-TiO_2 films was demonstrated. Core/shell QDs were self-assembled on nano-TiO_2 films through carboxyls as anchoring groups to metal oxides. Atomic force microscopy and some other experiments showed the fabrication strategy is successful. Reactive oxygen species detection experiments indicated that such films have photosensitization ability. The results of bactericidal and DNA damage experiments demonstrate that such films have excellent photoactivity.

Introduction

Nanocrystalline titanium dioxide (TiO_2) has been extensively used over the last three decades in fields of photocatalysis.[1−4] However, its photoactivity exists only when illuminated by UV light with wavelengths below 385 nm, which greatly limits the application of TiO_2. To extend its application and make the most of inexhaustible solar energy, dye-sensitized TiO_2 films have appeared and been widely used in fields of photocatalysis and photoelectrochemical solar cells.[5−11] Current approaches to enhance the photosensitization ability are to tune the absorption spectrum and redox properties of different dyes. This generally involves rather complicated synthetic routes.

Quantum dots (QDs) are inorganic semiconductor nanocrystals with unique optical properties. Since Weller's group[12] reported the photosensitization of highly porous TiO_2 electrodes by quantum-sized CdS, QDs have received significant attention as photosensitizers.[13−15] QDs have some advantages compared with organic dyes. First, the band gap of QDs can be easily tuned by changing the particle size instead of the chemical composition. Second, QDs have a continuous excitation spectrum and higher extinction coefficient. Third, QDs have better chemical stability.

In previous studies, QD-photosensitized TiO_2 films were fabricated based on the direct growth of semiconductor particles on TiO_2 films[13,14] or the arrested precipitation process.[15] QDs were anchored onto TiO_2 films simultaneously when they were synthesized. Because of difficult control of interface processes, this fabrication strategy resulted in nonoptimal size distributions and less controllable surface properties. Furthermore, only bare QDs have been used as photosensitizers up to now. Core/shell QDs have better photostability compared with bare QDs, and they also have electronic accessibility.[16] In the present study, core/shell QDs were used as photosensitizers for the first time, and a novel fabrication strategy was proposed. The synthesis of core/shell QDs was decoupled from the fabrication of QD-photosensitized TiO_2 films. And the fabrication procedure of core/shell QD-photosensitized TiO_2 films is easy to manipulate. In addition, previous attention of such QD-photosensitized TiO_2 films was only the field of photoelectrochemical solar cells. In this work, the photoactivity of such films was characterized by the damage of cells and DNA.

Experimental Section

Cultures. *Escherichia coli* AB-9112 was grown in Luria-Bertani (LB) medium, cultured at 37 °C with shaking, and harvested in the late exponential phase of growth. The harvested bacteria were centrifuged at 7000 rpm for 3 min, and the wet pellets were resuspended in ultrapure water and recentrifuged at 7000 rpm for 3 min to remove the growth medium. And the final pellets were resuspended in ultrapure water for analysis. Yeast cells (*Saccharomyces cerevisiae* AY) were grown in 5 mL of YPD (1% yeast extract, 2% tryptone, 2% glucose) with shaking at 30 °C overnight, and a 2% dilution of the cells was grown in 50 mL of YPD with shaking at 30 °C for 5h, harvested by centrifugation at 7000 rpm for 3 min, and washed three times with sterile water to remove the growth medium. And the final pellets were resuspended in ultrapure water for analysis.

Preparation of TiO_2 Films and QDs and Fabrication of QD-Photosensitized Nano-TiO_2 Films. The preparation of TiO_2 films was as described in the literature.[17] Core/shell CdSe/ZnS quantum dots in hexane were prepared according to the method developed in our laboratory.[18] Nearly monodisperse CdSe quantum dots were first synthesized according to the scheme reported by Peng,[19] and then core/shell CdSe/ZnS quantum dots were produced by adding hexamethyldisilathiane ($(TMS)_2S$) and $Zn(Ac)_2$ precursors dropwise into a freshly prepared CdSe trioctylphosphine oxide (TOPO)/hexadecylamine (HAD) solution at 200 °C. The resultant CdSe/ZnS products were dissolved in hexane, precipitated, washed several times to remove excess TOPO on the surface of QDs with ethanol, and finally dried. The quantum dots were ultrasonically resuspended in dimethylformamide (DMF). Excess 3-mercaptopropionic acid was added, and the mixture was shaken

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violently for 30 min at room temperature, followed by centrifugation to discard the precipitate. The 3-mercaptopropionic-acid-modified QDs in the supernatant were precipitated again with 4 M NaOH and excess tetrahydrofuran, removing the solution, and the resulting pellet of 3-mercaptopropionic-acid-modified QDs was dispersed in water.[20] The fabrication of QD-photosensitized nano-TiO_2 films was as follows: 3-Mercaptopropionic-acid-modified QDs were dissolved in ultrapure water. A nano-TiO_2 film was then immersed into this aqueous QD solution for self-assembly for 10 h, rinsed with ultrapure water, and dried by nitrogen.

Photoactivity Experiments. Two core/shell QD-photosensitized nano-TiO_2 films were immersed in 1 mL of bacterial suspension (about 10^8 cells/mL) or pUC18 DNA solution (3 μg/mL), respectively. The photoactivity reaction was carried out by overhead illumination of the bacterial suspension and DNA solution with a 125 W high-pressure mercury lamp (ShanghaiYaMingElectric Bulb Holding Co. Ltd.). The light with wavelengths below 420 nm was filtered. The light intensity reaching the surface of the bacterial suspension was 0.7 mW/cm^2. At different time intervals, 50 μL of the bacterial suspension and DNA solution were transferred and used immediately for assays. The control experiments using nano-TiO_2 films and QDs were also done.

Atomic Force Microscopy Imaging. Samples were analyzed using a Picoscan atomic force microscope (Molecular Imaging, Tempe, AZ) in MAC mode with commercial MAClever II tips (Molecular Imaging), with a spring constant of 0.95 N/m. At every time interval, 20 μL of the bacterial suspension was dropped on a 2 $\times$ 2 cm^2 poly-L-lysine-coated glass slide, incubated for 10 min, rinsed with ultrapure water, and dried with nitrogen for atomic force microscopy (AFM) imaging; 10 μL of DNA is deposited onto freshly cleaved mica and incubated for 5 min, rinsed with ultrapure water, and dried with nitrogen for AFM imaging.

Reactive Oxygen Species Detection Experiments. Core/shell QD-photosensitized nano-TiO_2 films were immersed in 1 mL of yeast suspension (about 10^7 cells/mL). The photoactivity reaction was carried out as in the above photoactivity reaction experiments. After 30 min of illumination, 150 μL of the yeast suspension was transferred and resuspended in dichlorofluorescin diacetate (DCFH-DA, 10 μmol/L, final concentration), incubated at 37 °C for 20 min, and then centrifuged at 7000 rpm for 3 min, and the wet pellets were resuspended in PBS and recentrifuged at 7000 rpm for 3 min. The final pellets were resuspended in PBS for fluorescence imaging. The control experiments using nano-TiO_2 films and QDs were also done.

Cell Viability Assay. Loss of viability was examined according to the viable count procedure. Forty microliters of serially diluted cell suspensions was respectively spread onto LB agar plates. All plates were incubated at 37 °C for 24 h, and the numbers of colonies on the plates were counted.

Results and Discussion

Fabrication of QD-Photosensitized Nano-TiO_2 Films. As we know, QD-photosensitized TiO_2 films were mostly fabricated based on the direct growth of semiconductor particles on TiO_2 films in previous studies. However, the self-assembly method, which is very simple and easy to manipulate, has been widely used in anchoring dyes to the surface of nanocrystalline semiconductors for fabrication of dye-sensitized semiconductor nanoparticles.[9] In the present study, nearly monodisperse CdSe/ZnS quantum dots were modified with 3-mercaptopropionic acid and dissolved in ultrapure water. A nano-TiO_2 film was then immersed into this QD aqueous solution for self-assembly through the carboxyl groups; thus a self-assembled monolayer (SAM) was formed on the TiO_2 film, which is very much similar to the formation of alkanethiolate SAMs on gold.

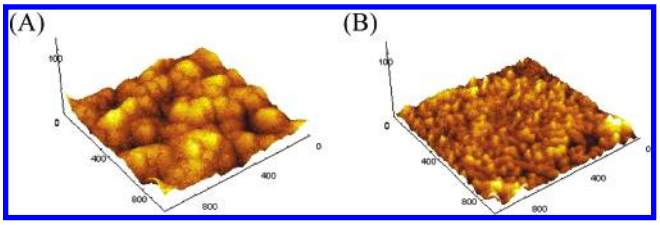

Figure 1. AFM three-dimensional images in topographic mode of a nano-TiO_2 film (A) and QD-photosensitized nano-TiO_2 film (B). Image sizes: 1.0 $\times$ 1.0 μm^2.

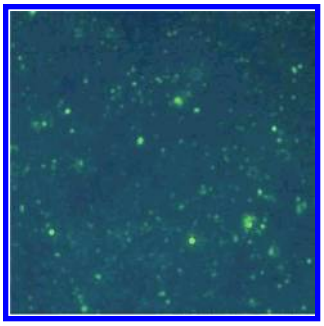

Figure 2. Fluorescence image of a QD-photosensitized nano-TiO_2 film. Image size: 22 $\times$ 22 μm^2.

First, the successful fabrication of QD-photosensitized nano-TiO_2 films was confirmed by contact angle experiments. The contact angles of nano-TiO_2 films and QD-photosensitized nano-TiO_2 films were 84° and 52°, respectively. The hydrophilicity of the films changed largely, which suggests that the QDs modified with 3-mercaptopropionic acid were self-assembled on nano-TiO_2 films.

AFM experiments showed that QDs on such core/shell QD-photosensitized TiO_2 films were of excellent monodispersity. Figure 1A displays an AFM image of a bare nano-TiO_2 film. The average size of TiO_2 in the film was ca. 50 nm. After self-assembly with core/shell QDs, monodisperse QDs of ca. 5 nm were observed on the nano-TiO_2 film (Figure 1B). Because of the highly luminescent properties of QDs, fluorescence experiments were also performed to characterize the QD-photosensitized nano-TiO_2 film. Figure 2 shows the fluorescence image of the QD-photosensitized nano-TiO_2 film. Many small starlike fluorescent dots were observed, demonstrating that the QDs self-assembled on the film were well-dispersed and did not form agglomerates, consistent with the AFM experiments.

Photosensitization Ability Confirmed by Reactive Oxygen Species Detection Experiments. Above experiments confirmed the successful self-assembly of QDs on TiO_2 films. However, it is not clear whether such films have photosensitization ability. If such films have photosensitization ability, similar to dye-sensitized TiO_2 films,[8] when they are illuminated by visible light, then electrons in the ground state of the QDs will be excited to the excitation state and subsequently injected to the conduction band of TiO_2, and then the injected electrons will be scavenged by the surface-adsorbed O_2 to yield reactive oxygen species (ROS), which have strong oxidation abilities.

DCFH-DA has been widely used as a substrate for measuring cellular ROS.[21,22] DCFH-DA is hydrolyzed by esterases to dichlorofluorescin (DCFH), which is trapped within the cell. This nonfluorescent molecule is then oxidized to fluorescent dichlorofluorescin (DCF) by ROS. So, in the present study,

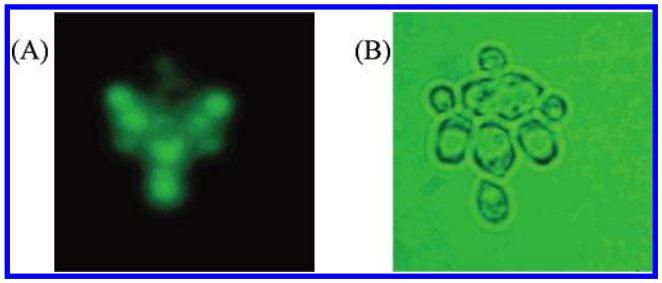

Figure 3. Fluorescence (A) and bright-field (B) images of yeast cells that have been illuminated 30 min in the presence of QD-photosensitized nano-TiO_2 film and incubated with DCFH-DA at 37 °C for 20 min. Image sizes: 26 × 26 μm^2.

DCFH-DA was used as an indicator for the photosensitization of such films.

Figure 3 shows the fluorescence and bright-field images of yeast having been mixed with DCFH-DA obtained from the same area. When yeast cells were illuminated for 30 min in the presence of QD-photosensitized nano-TiO_2 films and incubated with DCFH-DA for 20 min, fluorescence was found in the yeast cells (Figures 3A and 3B). When the yeast cells were not illuminated or illuminated for 30 min in the absence of such films, no fluorescence was observed. It could be concluded that when yeast cells were illuminated for 30 min in the presence of QD-photosensitized nano-TiO_2 films, ROS were generated. When such treated yeast cells were incubated with DCFH-DA, DCFH-DA was hydrolyzed by esterases and then oxidized to fluorescent DCF by the generated ROS. These results indicated such films have photosensitization abilities.

Application to the Damage of Cells and DNA. Above reactive oxygen species detection experiments proved the generation of ROS, which have strong oxidation ability, so the bactericidal and DNA damage applications of such films were tested here.

Bactericidal activity of TiO_2 has been studied in our laboratory[4] and elsewhere.[2,3] However, bactericidal activity of QD-photosensitized nano-TiO_2 films has not been investigated up to now. On the basis of our previous study of bactericidal activity of TiO_2, the cell damage induced by QD-photosensitized nano-TiO_2 films was observed using AFM. Figure 4 shows AFM images of native *E. coli* (Figure 4A) and *E. coli* illuminated for 90 min by visible light in the presence of QD-photosensitized nano-TiO_2 films (Figure 4C). It was found that the cells were damaged greatly after illumination for 90 min. The cell morphology changed greatly, and some groovelike rifts appeared in the cell body. The corresponding surface structure changed also (Figures 4B and 4D); the rodlike granules that have been proven to be patches of lipopolysaccharide[23] decomposed and disappeared. The control experiments using bare nano-TiO_2 films and QDs, respectively, showed nearly no change in cell morphology and cell surface structure (data not shown here). The viability of illuminated cells was then determined by colony counting experiments. It was found that nearly 90% of the *E. coli* cells died after 90 min of illumination. These results imply that QD-photosensitized nano-TiO_2 films have excellent bactericidal activity. Similar to dye-sensitized TiO_2 films,[8] when such films were illuminated by visible light, $O_2^{\bullet -}$ and $^{\bullet}OH$ radical would be generated, which then decompose the cell walls and cell membranes and result in the death of cells.

The photoactivity of such films was also verified by DNA damage experiments. The films were immersed into a solution of pUC18 DNA and illuminated for different lengths of time. DNA conformation changes were detected by agarose gel electrophoresis (Figure 5). A new band appeared after 30 min of illumination, and the band of supercoiled pUC18 DNA disappeared after 120 min of illumination. Then the changes of DNA conformation were confirmed by AFM images of native pUC18 DNA and pUC18 DNA illuminated for 120 min (Figure 6). The results showed that the conformation of pUC18 DNA changed from supercoiled to circular, demonstrating that pUC18 DNA was damaged by QD-photosensitized nano-TiO_2 films.

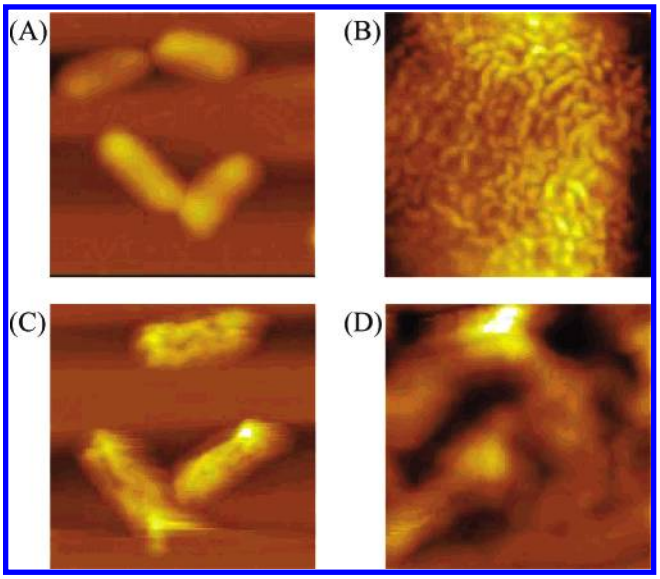

Figure 4. AFM images, in topographic mode (A−D) of native *E. coli* and *E. coli* illuminated for 90 min by visible light in the presence of QD-photosensitized nano-TiO_2 films. Images of intact bacteria (A and C) and the corresponding surface structures of individual bacterium (B and D). Image sizes: (A and C) 6 × 6 μm^2; (B and D) 1.2 × 1.2 μm^2.

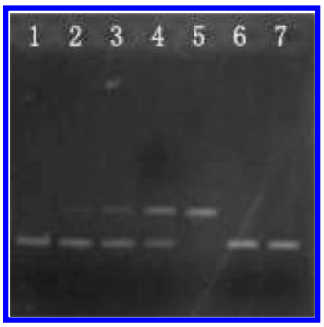

Figure 5. DNA electrophoresis of pUC18 DNA illuminated by visible light in the presence of QD-photosensitized nano-TiO_2 films, bare nano-TiO_2 film, or QDs at different time intervals. pUC18 DNA in the presence of QD-photosensitized nano-TiO_2 films standing for 60 min in dark (2); pUC18 DNA in the presence of QD-photosensitized nano-TiO_2 films illuminated at time intervals of 0 (1), 30 (3), 60 (4), and 120 min (5); pUC18 DNA illuminated for 60 min in the presence of a bare nano-TiO_2 film (6) or QDs (7).

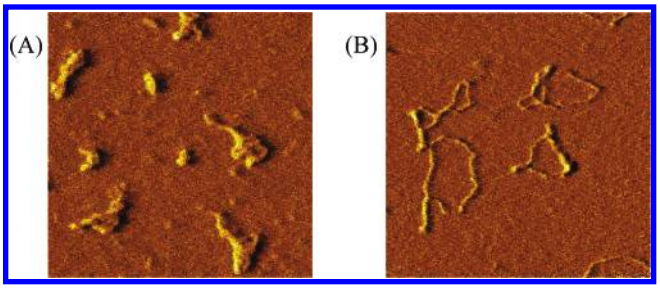

Figure 6. AFM images, in topographic mode (A and B), of native pUC18 DNA and pUC18 DNA illuminated for 120 min by visible light in the presence of QD-photosensitized nano-TiO_2 films. Image sizes: (A) 1.5 × 1.5 μm^2; (B) 800 × 800 nm^2.

Conclusion

A novel strategy for the fabrication of QD-photosensitized nano-TiO_2 films was demonstrated in the present study. The core/shell QDs were used as photosensitizers for the first time. The excellent photoactivity of such films was confirmed through cell and DNA damage. These films may also be useful for photoelectrochemical solar cells and nonlinear optical applications.

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