Ultraviolet-light-induced bactericidal mechanism on ZnO single crystals†

Yonghao Wang,[a] Feng Huang,*[a] Danmei Pan,[b] Bin Li,[b] Dagui Chen,[a] Wenwen Lin,[a] Xueyuan Chen,[a] Renfu Li[a] and Zhang Lin*[b]

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By comparing the photocatalytic bactericidal effect on different crystal faces of bulk ZnO crystal, we found that an electron degradation mechanism dominates the photocatalytic processes of ZnO material.

Nanoscale photocatalysts such as TiO_2, ZnO and ZnS present a good degradation effect on organic pollutants[1,2] or an obvious destruction effect on bacteria.[3–5] Currently, many researchers are endeavoring to improve the degradation or bactericidal activity of nanoscale photocatalysts.[6–8] It should be noted that the so-called bactericidal mechanisms of nanomaterials are actually more complex than expected. For example, it was found that nanoparticles of certain sizes can easily penetrate into bacterial cells, thus also generating an antimicrobial effect.[9,10]

At the same time, very little work has been carried out related to the photocatalytic bactericidal process on bulk single crystals, since bulk single crystals have a relatively small surface area and thus low photocatalytic activities. However, when bulk crystals are used, the direct toxic effects of nanosized photocatalysts can be avoided.[10,11] Moreover, for a polar crystal such as ZnO or ZnS, the different crystal planes can selectively enrich different carriers.[12] Thus studies of UV-light-induced disinfection efficiencies on selected crystal planes can provide essential correlations between photocarriers and photocatalytic activities, and further help to deduce the dominating reaction mechanism during photocatalytic processes.

In this work, a bulk ZnO single crystal was chosen for achieving the above research goal. Under the irradiation of 365 nm UV light, the survival, morphologies and cell wall permeabilities of *Escherichia coli* on the Zn plane (0001) and the O plane ($000\bar{1}$) were explored. The comprehensive analyses of the surface and intracellular structures of rod-shaped *E. coli* cells disclose an unobserved disinfection character in which the cells are preferentially damaged *via* the ends of the rod. Furthermore, it is firstly revealed that the Zn plane has a much better photocatalytic bactericidal effect than the O plane. This discovery revealed clearly that, unlike generally thought, the electron-generated free radicals, rather than hole-generated ones, contribute predominantly to the photocatalytic bactericidal process induced by ZnO semiconductor materials.

The numerical estimates of the extent of the death are provided by plate culture count (CFU mL^{-1}). It was found that the propagation of *E. coli* cells was inhibited seriously when 365 nm UV light irradiation treatment was conducted on both the Zn and O planes of bulk single crystal ZnO (Fig. 1 and Fig. S3†). Statistical analyses by colony counting revealed that after different irradiation treatments, the amounts of surviving *E. coli* represented by colony-forming units (CFU mL^{-1}) were 3 ($\pm$1.5) $\times$ 10^7, 1.2 ($\pm$0.5) $\times$ 10^8, 6.6 ($\pm$3.2) $\times$ 10^8 and 2.4 ($\pm$1.3) $\times$ 10^8 for irradiation on the Zn plane, irradiation on the O plane, no irradiation and irradiation without ZnO respectively. As a result, the survival rate can be estimated as 4.5% and 18% for irradiation on the Zn plane and irradiation on the O plane, respectively. It shows that the Zn plane (0001) presents a much stronger photocatalytic bactericidal effect than the O plane ($000\bar{1}$).

The bactericidal effects on different crystal faces were further confirmed by AFM morphologic observation. As revealed in Fig. 2, under the irradiation of 365 nm UV light,

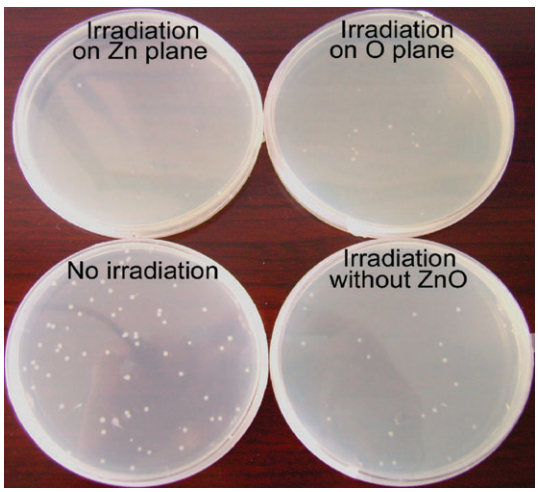

Fig. 1 Images of bacterial colonies showing re-growth situations of surviving *E. coli* on agar plates after different irradiation treatments. (UV wavelength: 365 nm; irradiation time: 1 h; incubation conditions: 33 °C for 24 h.) The judgement of the Zn plane and the O plane of the ZnO single crystal followed the conventional method (Fig. S1†).[13] "No irradiation" means "no irradiation with ZnO"; actually, the control experiment in the absence of UV light, presented in Fig. S4,† shows the bactericidal activity is the same no matter whether the ZnO single crystal is present or not.

[a] *Key Laboratory of Optoelectronic Materials Chemistry and Physics, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences Fuzhou, Fujian, 350002, China. E-mail: fhuang@fjirsm.ac.cn; Fax: +86 591 83705474; Tel: +86 591 83792630*
[b] *State Key Laboratory of Structural Chemistry, Fuzhou, Fujian, 350002, China. E-mail: zlin@fjirsm.ac.cn*

† Electronic supplementary information (ESI) available: Experimental section; real-time growth status of *E. coli* after 365 nm irradiation treatment; control experiment in the absence of UV light; surface damage situation of *E. coli* under UV light of different wavelengths; AFM image at a large scale; changes of the width–length ratio for *E. coli* under different treatment conditions; sketch of *E. coli* cutting *via* different directions in TEM experiments; TEM micrographs of *E. coli* thin sections at a large scale. See DOI: 10.1039/b912137d

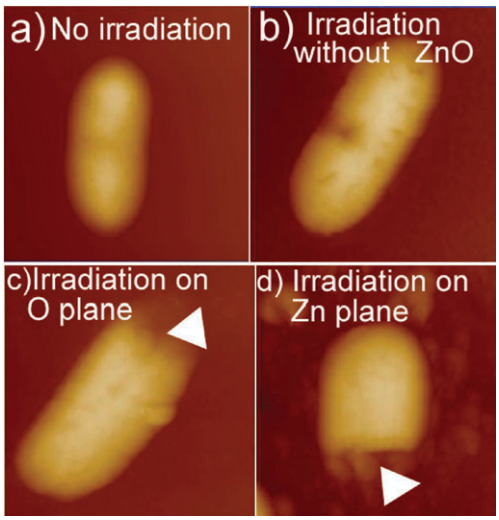

Fig. 2 AFM images of *E. coli* under different treatments (scan size: 4.0 μm; UV wavelength: 365 nm; irradiation time: 1.0 h). (a) Intact *E. coli* cells typically have a short-rod shape and smooth surface. (b) After irradiation with 365 nm UV light, the majority of the *E. coli* cells still keep the short-rod shape and smooth surface, while some have a few pits on the surface, indicating that the 365 nm UV source is slightly harmful to the *E. coli* cells (also see Fig. S7†). (c) On the O plane, bacteria are typically damaged from the end. (d) On the Zn plane, bacteria are not only damaged from the end, but the morphology also changes obviously, from rod- to round-shaped. When bacteria were destroyed by 254 nm UV light directly,[15] totally different surface characteristics from above were observed, see Fig. S8.† The phenomena that bacteria were destroyed from the ends might relate to the preferential distribution of negatively charged cardiolipin in the inner membrane of the two ends of *E. coli* cells,[16] which may be easily attacked by light-induced free radicals.

no matter whether on the Zn or on the O plane, the ends of most *E. coli* cells were damaged severely (denoted by triangles). The generality of the destruction of the bacteria from the ends is shown in the ESI (Fig. S5).† Since the transformation from rod-shaped into round-shaped is usually considered as the death characteristics of some rod-shaped bacteria,[14] the status of a cell can be analyzed *via* comparing the width-to-length ratio of the bacteria. The untreated (no irradiation) *E. coli* cells had a typical short-rod shape with a width-to-length ratio of 0.49 ± 0.14 (SD). Under irradiation on a ZnO single crystal, the width-to-length ratio of bacteria increased to 0.60 ± 0.10 (SD) on the O plane and 0.66 ± 0.10 (SD) on the Zn plane (Fig. S6†). According to a t-test analysis, with the significance level set as 0.05, the width-to-length ratios with irradiation on the Zn plane and no irradiation present evident statistical difference, while there is no significant statistical difference between the irradiation on the O plane and no irradiation. However, all the results including the plate culture count and the following K^+ concentration changes, and the re-growth figure (Fig. S3†) indicated that the Zn plane has higher photocatalytic bactericidal activity than the O plane.

The change of cell wall and the status of cell damage were studied using thin-section TEM micrographs and by cell permeability experiments. As shown in Fig. 3a and b, the cell walls were relatively intact when irradiated under 365 nm UV light without ZnO. However, in the presence of the

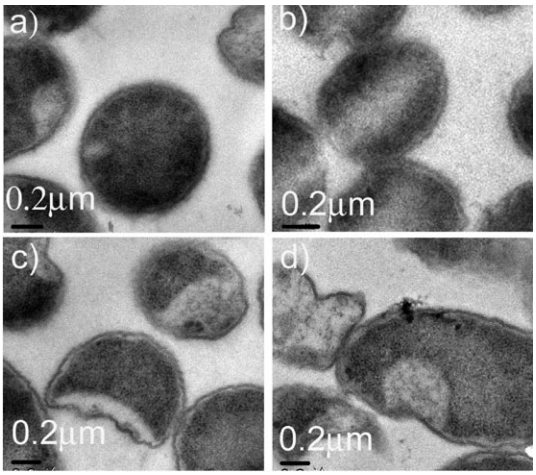

Fig. 3 TEM micrographs of *E. coli* thin section under: (a) no irradiation, (b) irradiation without ZnO, (c) irradiation on the O plane, (d) irradiation on the Zn plane (UV wavelength: 365 nm; irradiation time: 1.0 h). Different from AFM, the morphologies presented in the TEM images much depend on the thin section direction shown in Fig. S9.†

ZnO single crystal, the cell wall was destroyed seriously (Fig. 3c and d). Cell permeability can be determined by K^+ concentration in the supernatant (Fig. 4). Before the irradiation with UV light, the K^+ concentration was 1.51 mg L^{-1}. It increased to 3.19 mg L^{-1} and 2.55 mg L^{-1} after irradiation on the Zn and O planes, respectively. The percent increases are 111% and 69%, showing that the damage level to *E. coli* cell walls is more serious on irradiation on the Zn plane than on the O plane. As we stated above, the survival rate counting by plate culture count was about 4.5% and 18% for irradiation on the Zn and O planes, respectively. Data analysis revealed that, though the variation tendency for K^+ leakage and bacterial death are the same, there is no linear relation between these two experiments.

It is generally accepted that, in TiO_2 photocatalytic systems, photogenerated holes (h^+) play an important role during the

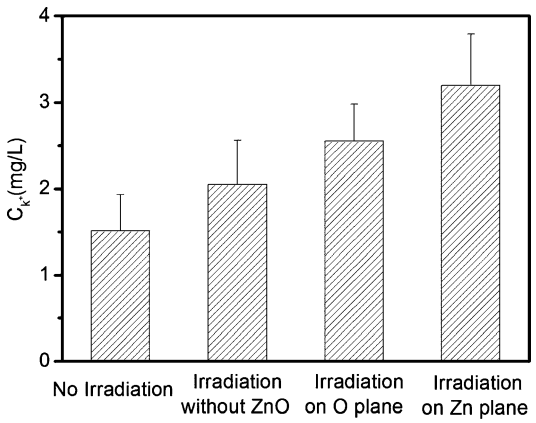

Fig. 4 K^+ concentration in the supernatant under different treatments (UV wavelength: 365 nm; irradiation time: 0.5 h). For the sample without any treatment, there was a background K^+ concentration, which was consistent with the existence of a few dead *E. coli* cells during the normal bacterial metabolic process (Fig. S10†).

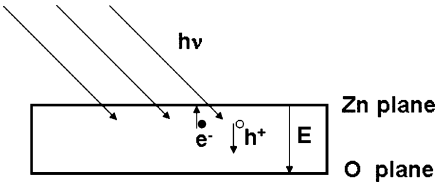

Fig. 5 Illustration of the drift tendency of UV-light-induced electrons and holes in a ZnO single crystal.

photocatalytic process.[17–19] The holes can oxidize OH^- directly, creating $^\bullet OH$ with strong bactericidal ability:

$$h^+ + OH^- \rightarrow {}^\bullet OH$$

$$h^+ + H_2O \rightarrow H^+ + {}^\bullet OH$$

$$2h^+ + 2H_2O \rightarrow 2H^+ + H_2O_2$$

$$^\bullet OH + \text{bacteria} \rightarrow \text{death of bacteria}$$

Theoretically, photogenerated electrons can also take part in the photocatalytic degradation process. However, this process needs the participation of oxygen. Firstly, the electrons should reduce the oxygen into a more active species O_2^-, and then the $^\bullet OH$ is generated:

$$e^- + O_2 \rightarrow O_2^-$$

$$O_2^- + H^+ \rightarrow HO_2^\bullet$$

$$2HO_2^\bullet \rightarrow O_2 + H_2O_2$$

$$e^- + H_2O_2 \rightarrow OH^- + {}^\bullet OH$$

$$^\bullet OH + \text{bacteria} \rightarrow \text{death of bacteria}$$

Generally, the electron degradation process is more complex. Thus, in much of the literature, the hole degradation process is assumed to be the major process for photocatalytic materials.

As mentioned above, owing to the positive and negative ionic charges on the zinc- and oxygen-terminated ±(0001) surfaces of ZnO, respectively, spontaneous polarization is induced across the ZnO single crystal from the Zn-terminated plane to the O-terminated plane. The light-induced electrons are inclined to accumulate on the Zn plane, while holes will move in the opposite way (Fig. 5). The observation that the Zn plane presented a better photocatalytic bactericidal effect than the O plane clearly revealed an example of an electron dominated photocatalytic bactericidal process. Similarly, for the decomposition of organic compounds, such as eosin B, the Zn plane also presents prior photocatalytic degradation compared with the O plane under the same irradiation conditions. Further studies into the photocatalytic degradation are in progress.

The discovery of the predominant electron degradation mechanism in a ZnO material, at least indicates that the photocatalytic mechanism in different materials might be different; the photocatalytic mechanisms of different semiconductors should be investigated independently.

Moreover, even for TiO_2 materials, whether the hole or the electron is dominant during photocatalytic process is still under-identified. Some recent theoretical studies claimed that the oxidation of surface OH^- and H_2O by photogenerated holes (h^+) in TiO_2 is less possible based on thermodynamics.[20–23] Instead, it is considered that the $^\bullet OH$ radicals can be produced from the hydrogen peroxide (H_2O_2) form *via* the reduction of molecular oxygen by the electrons in the conduction band.[23] We believe the discovery of the predominant electron degradation mechanism in ZnO material might give experimental support to these recent theoretical speculations.[20–23]

In conclusion, by using a ZnO single crystal, clear results were obtained giving insight into the photocatalytic bactericidal mechanism of ZnO. Unambiguous evidence showed that the Zn plane has a better bactericidal effect than the O plane. It is disclosed that an electron degradation mechanism dominates the ZnO bactericidal photocatalytic process.

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