Ultraviolet-Light-Induced Bactericidal Mechanism on ZnO Single Crystal

--Supplementary Material

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1) Experimental Section

The judgment of Zn plane and O plane of ZnO single crystal The ZnO wafers with size of 5×5×1mm and the resistance in MΩ•cm grade were purchased from HeFei KeJing Materials Technology Co., LTD, China. ZnO is a polar crystal belonging to hexagonal crystal system, and c-axis is the polar axis. The face of +c is terminated with Zn atoms, and –c is terminated with O atoms. A conventional way to define the O or Zn plane is via surface etching treatment. As to ZnO grown from hydrothermal method, the hexagonal pyramid-like etch pits could be observed in Zn plane, while the hillock-like etch pits could densely distribute on the O plane [1]. The purchased ZnO wafers were polished and rinsed by ethanol and acetone respectively for 20min, then etched by diluted HCl solution. Fig.S1 illustrates the microscopy images of ZnO crystal etched in the diluted HCl solution. The plane with hexagonal etch pits is defined as Zn plane, and the other plane is O plane.

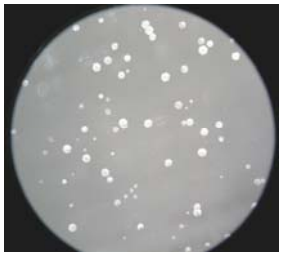
a)  b) 

Fig. S1 Microscope images of ZnO etched by dilute HCl a) Zn plane b) O plane

Preparation of bacterial cells The *E. coli* cells were incubated in LB medium (NaCl 10 gL^{-1}, tryptone 10 gL^{-1}, yeast extract 5 gL^{-1}, pH 7.2) at 37□ with shaking, and harvested in the exponential phase of growth. Then the harvested bacteria were centrifuged at 4000rpm for 2min, and the wet pellets were rinsed for two times by sterile distilled water. The final pellets were resuspended in sterile distilled water for usage.

Photo-catalytic experiment The sketch of experimental device is shown in Fig. S2. ZnO wafers were adhered in the interior side of a colorimetric ware with the Zn plane or O plane exposing to the UV source respectively. Then 600μl bacterial suspension prepared from above was dropped to the colorimetric ware. For the purpose of avoiding the *E. coli* being contaminated by dusts during experiments, the colorimetric ware is slightly covered by a cap. Under this condition the air including O_2 can enter the system freely. Then the colorimetric ware was irradiated under 365 nm UV light for 1h. The situation of i)no irradiation (with or without the present of ZnO) ii)irradiation without ZnO were used as control experiments.

Fig. S2 Sketch of photo-catalytic experimental facility

During the photo-catalytic process, the intensity of the light arriving at the other side of the crystal can be calculated according to the Lambert law ($I = I_0 \cdot e^{-\alpha L}$), where α and L represents absorptivity coefficient and the path length of the sample, respectively. ZnO is a kind of direct band gap semiconductor that features strong absorption in the UV region (α is about 10^5 ~10^6 cm^{-1}). The thickness of ZnO single crystal used in this work is 0.1 cm. Then the calculated transmitted intensity of the UV light arriving at the other side of ZnO single crystal is almost zero. Thus it can be assumed that only the side that illuminated by UV light directly is acted in the bactericidal experiments.

Bacteriological tests For illustrating the real-time growth status of *E. coli* after different irradiation treatments, bacteriological tests were conducted as follows: 250μl bacterial suspension was withdrew and added into 25ml LB medium, re-grew at 37℃ with shaking. The growth of the *E. coli* was monitored turbidometrically by determining the optical density at 600nm (OD_{600}) using a UV-Vis spectrophotometer (Perkin-ElmerLambda35). However, the numerical estimates of the extent of the death are provided by plate culture count instead of deducing from OD_{600}. After irradiation treatment,

about 200μl irradiated bacterial solution was taken out and diluted into the required multiple (10^6), and plated on LB agar. The colonies were counted after incubation at 33□ for 24h. The bacteriological test was performed in triplicate for statistical purpose.

AFM experiments The morphological changes were analyzed by AFM (Multimode NS3A-02NanoscopeIIIa) in tapping mode with RTESP7 tips. Tips are with a spring constant of about 20N/m and a resonance frequency of about 200-400 kHz. After irradiation treatment, a droplet of 5 μl bacterial suspension was placed on a freshly cleaved mica substrate and dried under N_2 gas for 3 min immediately. All images were obtained with a resolution of 256 by 256 pixes. Bacteria were scanned in both directions several times before capturing an image to help obviate tip artifacts, such as hysteresis.

TEM experiments HRTEM analyses were performed using a JEOL JEM2010 HRTEM at 200kV. After irradiation treatment, bacterial suspension was washed twice and fixed with 2% glutaraldehyde for 1.5h. Samples were then postfixed with 1% osmium tetroxide for 30 min and dehydrated in acetone series (35, 50, 70, 80, 95 and 100%) for 3 min each. After dehydration by 100% acetone, samples were then embedded in SPI-PON 812 resin. Ultra thin sections were cut using an ultramicrotome (Lecia EM UC6) and stained with uranyl acetate and lead citrate.

Analysis of K^+ concentration The concentration of K^+ in the bacterial suspension under different treatment conditions was detected by inductively coupled plasma (ICP-AES) (Jobin Yvon Ultima2).

2) The re-growth figure of *E. coli* after 365nm irradiation treatments

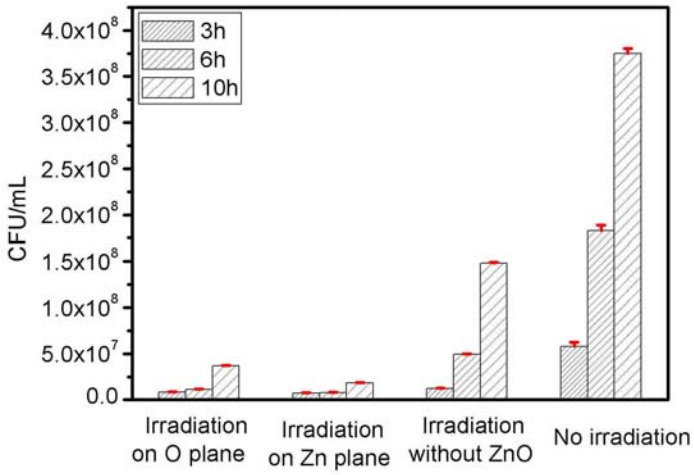


Fig. S3 The real-time growth status of *E. coli* cells in LB liquid medium after 365nm irradiation treatments for 1 hour.

3) Control experiments in the absence of UV light

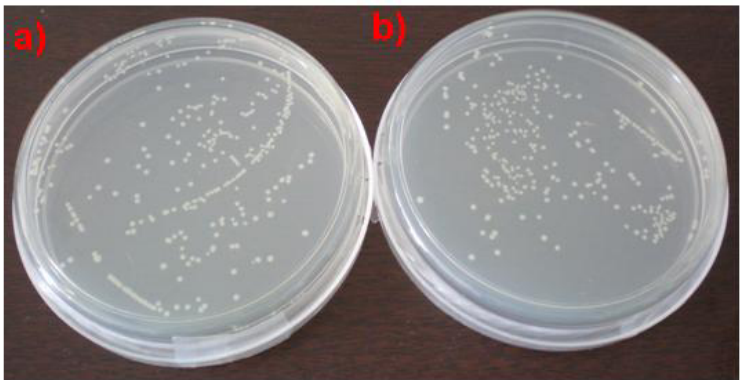


Fig. S4 The image of the bacterial re-growth condition in the absence of UV light. It reveals that, in the absence of UV light, there is no obvious bactericidal activity no matter ZnO single crystal was present or not. a) No irradiation without ZnO b) No irradiation with ZnO

4) Large scale AFM image of *E. coli* irradiation with 365nm UV on Zn plane for 1h

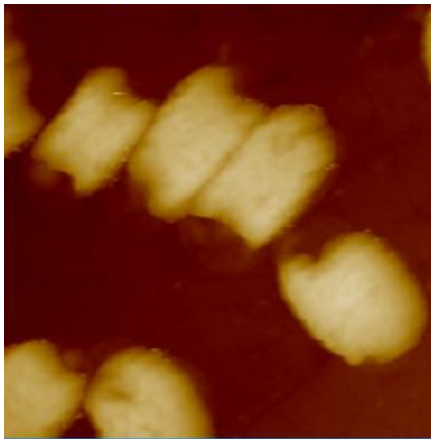


Fig. S5 Large scale AFM image shows that most of the cells were destructed from the longitude ends of bacterium. Scan size：4.5μm

5) The changes of the width-to-length ratio for *E. coli* under different treatment conditions

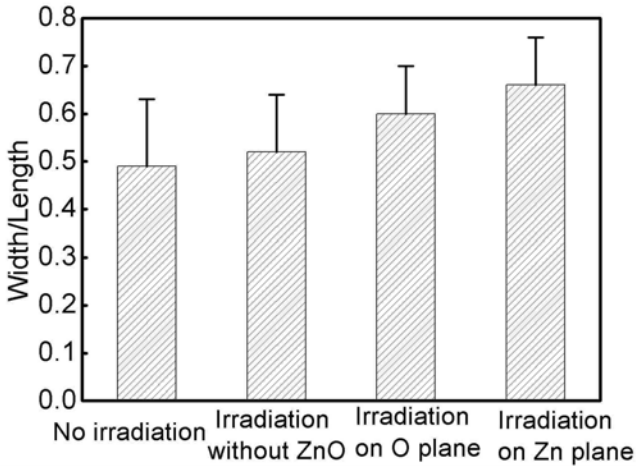


Fig. S6 Statistics of the width-to-length ratio for *E. coli* cells under different treatment conditions. Irradiation time: 1h.The standard deviation was used to statistical analysis for 15-18 *E. coli* bacteria in each situation for three times from AFM images.

Table S1. With the significance level set as 0.05, t-test statistical results for the comparison of difference degree on the width-to-length ratio of *E.coli* under different irradiation treatment.

The comparison of difference degree on the width-to-length ratio of *E.coli* under different irradiation treatment	p value	Significance of difference α=0.05
Irradiation on Zn plane and no irradiation	0.00748	Yes
Irradiation on O plane and no irradiation	0.09155	No
Irradiation without ZnO and no irradiation	0.79058	No
Irradiation on Zn plane and Irradiation on O plane	0.10438	No

It revealed in Table S1 that with the significance level set as 0.05, the width-to-length ratio of irradiation on the Zn plane and no irradiation presented evident statistical difference, while there was no significant statistical difference between the irradiation on the O plane and no irradiation. The p value for irradiation on Zn plane and irradiation on O plane was 0.10438. However, the difference of photocatalytic bactericidal activity between Zn plane and O plane can not be judged only from the P value of the AFM images. Since all the results including the plate culture count and the following K^+

concentrate changes, the re-growth figure (Fig.S3) indicated that the Zn plane has higher photocatalytic bactericidal activity than the O plane.

6) The surface damage situation of *E. coli* under UV lights with different wavelength

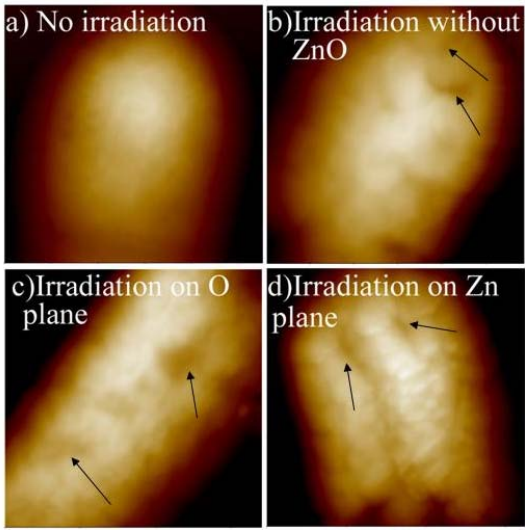


Fig.S7. AFM amplified image shows that few pits are distributed on the bacterial surface after UV light irradiation no matter ZnO single crystal are presented or not. Scan size：1.5μm

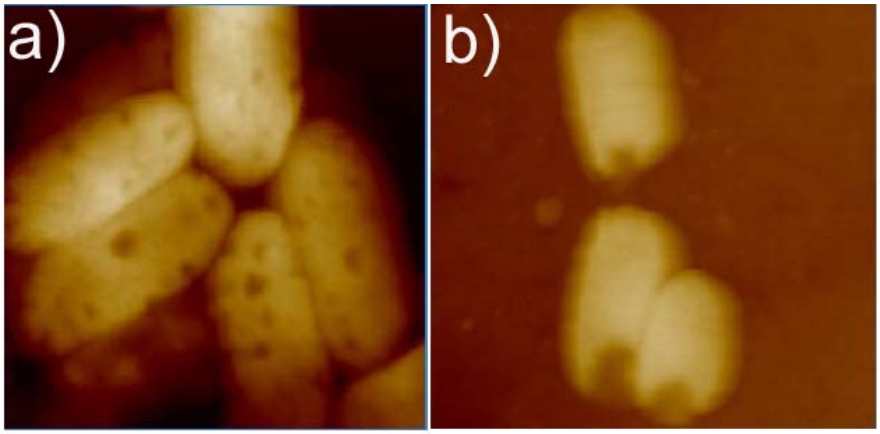


Fig. S8 AFM images of *E. coli* a) Irradiated by 254 nm UV light without the present of ZnO, b) Irradiated by 365nm UV light with the present of ZnO single crystal. Scan size: 4.50μm. Irradiation time: 1h

6) The sketch of *E. coli* cutting via different direction

The bacteria in TEM images are different from AFM images. While it is worthy to mention that the sample for TEM observation is the ultra thin sections part cut from cells. As illustrated in Fig.S9, the morphologies are much dependent on the cutting directions.

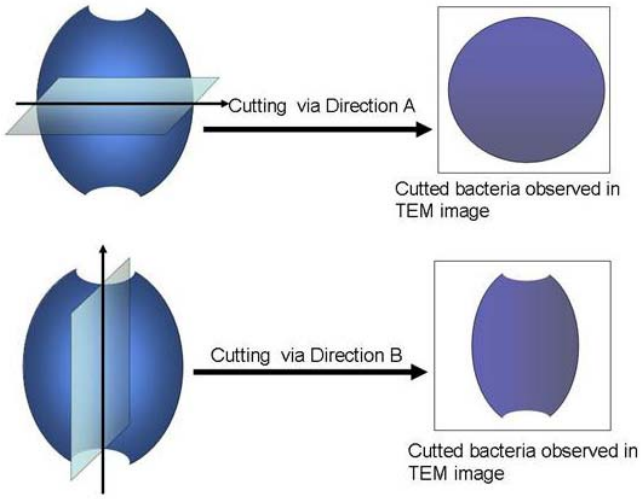


Fig.S9. The sketch of *E. coli* cutting via different direction before TEM analysis

7) The TEM micrographs of *E. coli* thin sections at large scale

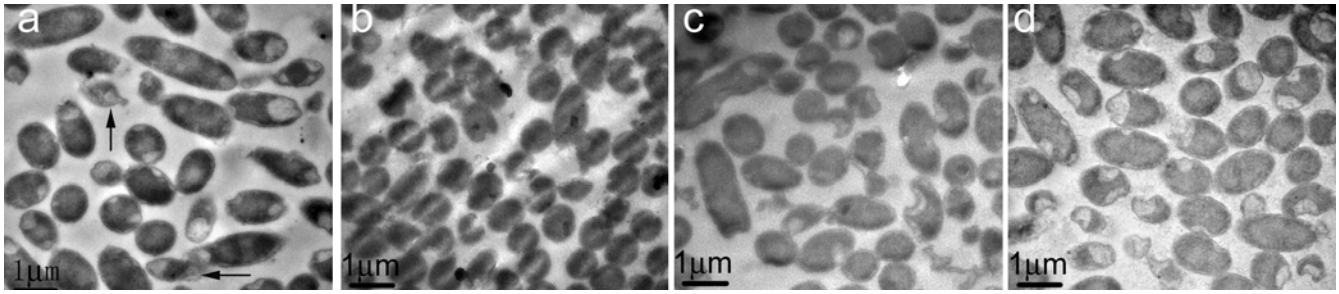


Fig. S10 TEM micrographs of *E. coli* thin section under different treatment conditions a) No irradiation , b) Irradiation without ZnO , c) Irradiation on O plane , d) Irradiation on Zn plane , Irradiation time: 1h

Reference:

[1] A.N.Mariano, R.E.Hanneman, *Journal of Applied Physics* 1963, **34**, 384.