A study on the antibacterial activity of one-dimensional ZnO nanowire arrays: effects of the orientation and plane surface

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Received (in Cambridge, UK) 7th June 2007, Accepted 7th August 2007
First published as an Advance Article on the web 17th August 2007
DOI: 10.1039/b708662h

In this study, ZnO nanowire arrays with different orientations were prepared. Confocal laser scanning microscopy (CLSM) and field-emission scanning electron microscope (FE-SEM) technique were employed for understanding the disparities in antibacterial activity between different orientations of ZnO nanoarrays. The effects of the different planes of ZnO nanowire were also discussed for the first time.

The synthesis and applications of nanosized zinc oxide (ZnO) especially one-dimensional (1-D) nanostructure is a highly desired objective.[1–3] It is a versatile material and has been used intensively because of its catalytic, electrical, optoelectronic and photochemical activities.[4–6] Furthermore, ZnO is also a well-known antibacterial agent.[7] Therefore, 1-D ZnO nanostructures have potential applications in antimicrobial coating or medical polymer technology. The properties of 1-D nanomaterials depend strongly on their dimensions and morphologies.[8,9] However, as the investigation of 1-D ZnO nanostructures has attracted increasing interest,[10] the interactions of 1-D ZnO nanostructures with biosystems are just beginning to be understood. There are still a variety of questions have to be addressed. Typically, for example, what is the relationship between the orientation of 1-D ZnO nanostructure and its corresponding antibacterial activity? Does antibacterial activity depend on surface membranes of different plane area? Besides those questions, how to directly and accurately evaluate the activity of nanostructure is also a big problem hindering this investigation.

The classical growth inhibition tests have several limitations, such as the use of environmentally unrealistic high cell density and antibacterial composites concentration; the inability to distinguish between live and dead cells; the inability to provide detailed information of treated cells and mechanisms of antibacterial activity.[11] Furthermore, these grown-based methods are time-consuming (more than 36 h) and severely underestimate the true value, especially when dealing with nano-sized agents. As the changes of the nano-sized agents directly impact cells before populations are affected, it is necessary to develop a rapid and more effective method for the quantitative antibacterial assessment of nano-sized material.

Theoretically, confocal laser scanning microscopy (CLSM) can solve this problem. The combination of CLSM and digital image analysis enables the high-resolution imaging of biological structures with negligible background interface.[12] Moreover, CLSM allows cells to be studied in their functional native (wet) environments, thus providing a direct and nondestructive assessment of bacterial activity with different nanostructure.[13] In this study, CLSM technique was employed for understanding the antibacterial activity of ZnO nanoarrays directly. Using this technique, the effect of in-use surfaces of different ZnO nanowire arrays on *Escherichia coli* was determined based on inhibition of esterase activity which was probed by fluorescein isothiocynate (FITC) and propidium iodide (PI).[14]

To investigate the relationship between antibacterial activity and orientation, single crystal 1-D ZnO nanowire arrays with different orientations were prepared according to Lévy-Clément[15] and Meng's reports.[16] The morphologies and chemical composition of the obtained ZnO nanowire arrays were characterized using a field-emission scanning electron microscope (FE-SEM) equipped with an energy dispersive X-ray detector (EDX). SEM images of three different ZnO nanoarrays are shown in Fig. 1. It can be seen that ZnO nanowires in three different arrays have a similar average diameter of around 150 nm. The obtained products were composed of zinc and oxygen with the atomic ratio of about 1 : 1 by EDX analysis (data not shown).

By measuring the changes of survival rates of cell suspension coated on different ZnO nanoarrays,‡ it was interesting to notice that the antibacterial activity was significantly different among ZnO nanoarrays. Table 1 shows that the membrane with comparatively less oriented ZnO nanoarrays (Fig. 1B) had higher antibacterial activity than the membrane with well-defined ZnO nanoarrays (Fig. 1A), and the membrane with randomly oriented ZnO nanoarrays (Fig. 1C) exhibited significantly superior antibacterial property. Detailed information was also obtained through CLSM observation. The integrity of *E. coli* after coating on different membranes for 90 min was observed. (a) ~ (d) in Fig. 2 are the representative morphologies of *E. coli* on four membranes. Partial disruption of cell membrane was found in Fig. 2c. In particular, the treated cells in Fig. 2d were obviously different and major damage was observed in the cell membranes. At the same time, no obvious disruption of cell membrane was observed in Fig. 2a and Fig. 2b. These results indicate that at a given size of ZnO nanowire, inhibition of bacterial growth depends on the orientation of 1-D ZnO nanostructure.

However, the mechanism of the antibacterial actions of 1-D ZnO nanoarrays is still under investigation. We speculate that the action of 1-D ZnO nanoarrays is broadly similar to that of ZnO nanoparticles. The antibacterial property of ZnO nanoparticles is considered to be due to the generation of hydrogen peroxide

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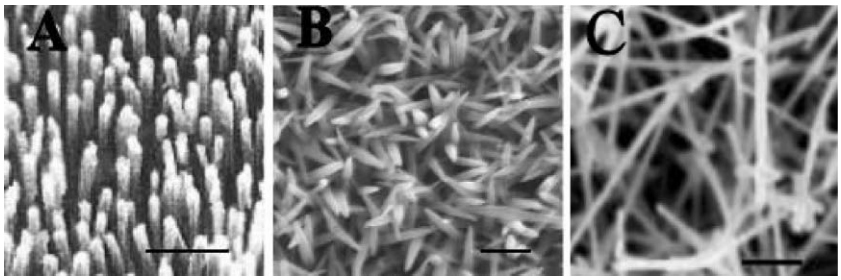

Fig. 1 SEM images of the structure of three ZnO nanowire arrays. Bar represents 2 μm.

Table 1 The survival of *E. coli* 90 min after coating on different membranes. The data are the mean of three independent experiments ± standard deviations

	SiO_2 (control)	ZnO (A)	ZnO (B)	ZnO (C)
E. coli	92.5 ± 1.7%	83.3 ± 8.5%	31. 8 ± 16.7%	17.4 ± 5.8%

(H_2O_2) which is effective for the inhibition of bacterial growth.[17] The H_2O_2 generated from ZnO could easily penetrate the cell wall of the bacteria.[18] Combined with the CLSM results, the concetration of H_2O_2 generated from 1-D ZnO nanoarrays seems to be connected with the orientation of the original samples.

For further investigation, the surface of ZnO nanoarrays after coating with *E. coli* was examined using SEM-EDX. Accumulation of the unknown layer was traced on the (0002) plane of ZnO nanowire (Fig. 3). In comparison, fewer layers were found on the (0001) plane. EDX indicates that the coatings consist of carbon and oxygen elements (The element of Au was from the coating layer used for SEM imaging). Therefore, it is logical to infer that the observed layer is the debris of cell membrane. This result is in agreement with the surface energy theory. Since the (0002) plane of ZnO with the highest surface energy is the most unstable plane, reasonably, the interaction between the (0002) plane of ZnO and bacterial membrane would also be more active. At present, however, we are unable to give a direct estimation of how the surface areas of (0002) plane and (0001) plane influence their killing activity. The disparities in antibacterial activities between the different planes were substantially increased on the surfaces of different orientation ZnO nanowire arrays. Consequently, we can provide a quantitative antibacterial assessment on the aggregation of different planes. As a result from former CLSM experiments, the membrane of randomly oriented ZnO nanoarrays, in comparison to other surfaces that contain less (0002) plane, showed obviously higher antibacterial activity.

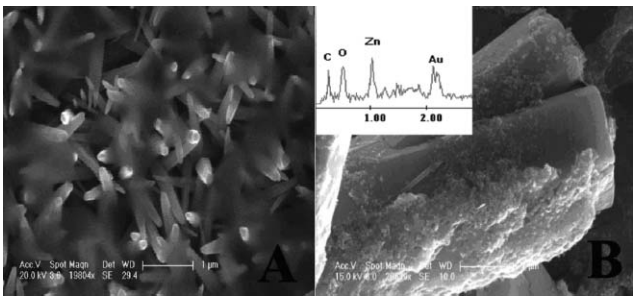

Fig. 3 SEM images of the surface of ZnO nanoarrays B after coating with *E. coli* film for 180 min (A); (B) is an enlarged image of sample (A) The inset in (B) is the EDX spectrum of (B).

In addition, the effects of the orientation of ZnO nanoarrays on *Staphylococcus aureus* has also been investigated. The changes in antibacterial action for *S. aureus* were similar to those for *E. coli.* However, the influence of orientation for *S. aureus* was less than for *E. coli*. The structure and chemical composition of the outer membrane of Gram-positive bacteria (*S. aureus*) is quite different from that of Gram-negative bacteria(*E. coli*). Therefore, the differences in antibacterial effects between *S. aureus* and *E. coli*

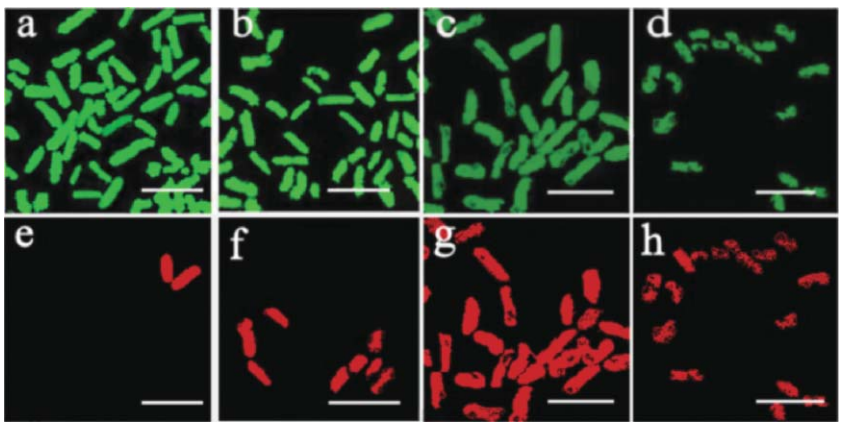

Fig. 2 CLSM images of *E. coli* on membranes of SiO_2 (a, e); ZnO nanowire arrays A (b, f); ZnO nanowire arrays B (c, g) and C (d, h) for 90 min. All cells were visible at an excitation wavelength of 488 nm due to staining with FITC (a, b, c and d), whereas only dead cells were visible at an excitation wavelength of 568 nm due to staining with propidium iodide (e, f, g and h). Bar represents 5 μm.

were assumed to be due to the different sensitivities of the cell membrane towards H_2O_2.

In conclusion, with the aid of CLSM technique, the relationship between the orientation and the antibacterial activity of ZnO nanoarrays was studied. The effects of the orientation and plane surface were also discussed. Moreover, a simple and nondestructive method for direct assessment of the antibacterial activity of nanostucture was developed here. This method could be easily scaled up for many applications in the nanomaterials community. Further studies on different nanostructures are planned.

This work was supported by the National Natural Science Foundation of China (No. 20475052) and the Project of Chinese Academy of Sciences (No. KJCX2. YW. H09).†

Notes and references

† *Experimental*: The preparation ZnO nanowires array A and C were carried out in a horizontal tube furnace by vapor transport process for 30 min. Zinc powder loaded in a quartz boat served as source material. ITO substrates with smooth face downward were laid above the source material at a vertical distance of 4 mm. Before being loaded, the ITO substrates were cleaned by organic solvent, etched by HF acid and washed with deionized water. The furnace was heated up to the desired temperature (430 °C for ZnO nanowires array A and 560 °C for ZnO nanowires array C, respectively) under a constant flow (100 sccm) of pure nitrogen gas. The temperature was detected by a thermal couple close the substrate. The oxygen is unintentionally introduced by the residual O_2 in the nitrogen gas. The pressure during the growth process was kept at 1 torr. After deposition, each substrate surface was covered with a white gray layer. Electrodeposition of arrays of ZnO nanowires array B was the same as C. Lévy-Clément's repoert.[15]

‡ *E. coli* cells (IFO 3301 Strain) were used in this work. The growth medium was Luria Bertani (LB) medium (bactotryptone: 10 g L^{-1}; bactoyeast extract: 5 g L^{-1}; NaCl: 10 g L^{-1}). *E. coli* from the frozen bacterial solution were grown at 37 °C for 18 h under aerobic conditions, and then exponentially growing cells were harvested by centrifugation at 1000 rpm for 5 min and washed 3 times with sterile PBS. The cells were then resuspended and diluted to 2×10^5 colony forming units (CFU) mL^{-1} with sterile PBS, which was determined by the plate count method. 20 μL of the diluted cell suspension was pipetted onto different membranes for CLSM study.

Cell viability was determined using fluorescent staining and confocal scanning laser microscopy (CSLM). According to kristi's report,[14] a fresh working solution was made daily by adding 50 μL of 5 mg fluorescein isothiocyanate (FITC) (Sigma) per milliliter of absolute ethanol and 40 μL of 1 mg of propidium iodide (Sigma) per milliliter of PBS (pH = 7.0, stored in the dark at 4 °C) to 1.9 mL of PBS. 10 μL such working solution was added to cover films of cells immobilized on the different membranes. The combination was then flushed gently with PBS to reduce background after incubation at 37 °C for 30 min.

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