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Ultrafast physical bacterial inactivation and photocatalytic self-cleaning of ZnO nanoarrays for rapid and sustainable bactericidal applications

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HIGHLIGHTS

• The ZnO nanoarrays exhibit an ultra-fast physico-bactericidal activity within 1 min.
• The ultra-fast bactericidal mechanism is attributed to the greater stress enabled by the sharp tips and uneven topography.
• The ZnO nanoarray tips can be re-exposed under a mild UV light source, and the surface has sustainable bactericidal properties.

GRAPHICAL ABSTRACT

ABSTRACT

Various nanostructured surfaces have been developed recently to physically inactivate bacteria, for reducing the rapidly spreading threat of pathogenic bacteria. However, it generally takes several hours for these surfaces to inactivate most of the bacteria, which greatly limits their application in the fields favoring rapid bactericidal performance. Besides, the accumulated bacteria debris left on these surfaces is rarely discussed in the previous reports. Herein we report the nanotip-engineered ZnO nanoarrays (NAs) with ultrafast physical bactericidal rate and the ability to photocatalytically remove the bacteria debris. Neither chemical (Zn2+ or reactive oxygen species) nor photocatalytic effect leads to the ultrafast bactericidal rate, where 97.5% of E. coli and 94.9% of S. aureus are inactivated within only 1 min. The simulation analysis further supported our proposed mechanism attributing the ultrafast bactericidal activity to the great stress enabled by the uneven topography. Moreover, the re-exposure of the ZnO NAs nanotips can be achieved in only 10 min under a mild UV light source. This study not only presents an ultrafast physical bactericidal activity, but also demonstrates the potential of the recyclable and photocatalytic self-cleaning functions of these surfaces for applications that desire rapid and sustainable bactericidal performance.

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1. Introduction

Up till now, the rapid global spread of the Coronavirus Disease 2019 (COVID-19) has caused great concerns in many countries (Holshue et al., 2020; Wang et al., 2020; Zhu et al., 2020). However, just like COVID-19, many pathogenic microorganisms could also spread via droplets (Bowen et al., 2017; Huijbers et al., 2015) and surfaces (Gedik et al., 2013; Morgan et al., 2012). In the absence of specific medicine, controlling the rapidly spreading threat of pathogenic microorganisms has become the most important means of epidemic prevention (Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020). Due to the rapid spread of pathogenic microorganisms, it is of the urgent need to develop alternative bactericidal approaches for applications that need rapid inactivation of bacteria, such as clinical treatment, water purification and air filtration (Favre and Tazy-Pain, 1993; Hasan et al., 2013a; Jung et al., 2011; Mortin et al., 2007; Zhou et al., 2020). Since the abuse of chemical bactericides can bring pollution (Ma et al., 2006), toxicity (Dix-Cooper and Kosatsky, 2019; Martinez-Paz, 2018) and drug-resistant bacteria (Jutkina et al., 2013), it is highly desired to develop new and environmentally friendly ways to kill bacteria. Ivanova et al. first reported that cica wing surfaces (Hasan et al., 2013b; Ivanova et al., 2012) and dragonfly wings (Ivanova et al., 2013) displayed a rapid mechanical bactericidal behavior against Gram-negative bacteria due to their nanoray topography, followed by a series of artificial physical bactericidal surfaces, such as black silicon (Ivanova et al., 2013), etc. These surfaces are based on physico-mechanical mechanisms and independent of chemical composition, which offer an efficient and safe alternative solution to prevent the bacterial contamination and transmission (Elbourne et al., 2017; Meng et al., 2014; Tripathy et al., 2018b).

However, previous efforts were primarily focused on the development of bactericidal nanostructured surfaces with high bactericidal efficacy, instead of improving the bactericidal speed (Dickson et al., 2015; Diu et al., 2014; Fisher et al., 2016; Green et al., 2017; Pham et al., 2015). Although it was found that 80% of the germ transmission is via surface contact (Yoo et al., 2013), little is known about how fast the contaminated surfaces spread (Lei et al., 2017). It was found that for the high-touch environmental surfaces, the number of contaminated surfaces grows logarithically (Lei et al., 2017), which could lead to the possible rapid transmission of infection. In other words, the contaminated surfaces are still under the risk of spreading bacteria if the attached ones were not inactivated soon enough. However, to the best of our knowledge, there are only a few reports involving rapid antibacterial performance of physical bactericidal surfaces to date (Hasan et al., 2013a; Ivanova et al., 2013; Michalska et al., 2018; Xie et al., 2019b; Yi et al., 2018), while most physical bactericidal surfaces in literature need up to several hours to inactive bacteria (Table S1) (Ivanova et al., 2013; Linklater et al., 2018; Yi et al., 2018). Therefore, developing physical bactericidal surfaces that can inactive bacteria both effectively and rapidly is little focused previously but highly crucial for practical applications.

In order to design a bactericidal surface that can rapidly inactivate bacteria, it is necessary to get a clear understanding of the physical bactericidal mechanism. Unfortunately, it is yet to be conclusively determined due to the complex nature (Elbourne et al., 2017; Tripathy et al., 2018a, 2017a). Among the many mechanisms proposed, the most popular ones contribute the cell death either to the direct puncture of the nanostructures (Crawford et al., 2015; Ivanova et al., 2013, 2012), or to the rupture after stretching between valleys of nanostructures (Li, 2015; Li and Chen, 2016; Pogodin et al., 2013; Wu et al., 2016; Xue et al., 2015). Although the precise physico-mechanism is still under substantial debate (Bandara et al., 2017; Diu et al., 2014; Elbourne et al., 2017), the applied stress induced by the deformation of cell in response to the nanostructured surface is considered the leading factor for cell death (Bandara et al., 2017; Diu et al., 2014; Elbourne et al., 2017; Li, 2015; Li and Chen, 2016; Nowlin et al., 2015; Pogodin et al., 2013; Xue et al., 2015). However, previous studies fail to pay enough attention on the significance of stress but instead were keen on the fabrication of nanostructures with various features such as density (Dickson et al., 2015; Hazell et al., 2018), height (Dickson et al., 2015; Jeong et al., 2020), aspect-ratio (Green et al., 2017; Hazell et al., 2018; Nowlin et al., 2015), size (Dickson et al., 2015; Fisher et al., 2016; Hazell et al., 2018; Xie et al., 2019b), multi-scale order (Fisher et al., 2016; Yi et al., 2018) and feature angle (Table S1) (Elbourne et al., 2019). It can be summarized from these previous results that the multi- hierarchical and sharp nano-pillars are more likely to kill bacteria efficiently (Elbourne et al., 2017). From the perspective of deformation-induced stress, it is very likely that these nanostructures were able to apply greater stress that leads to cell death. The recent discovery by J. Jenkins et al. that nanorods achieve bactericidal effects by inducing cell membrane deformation and affecting cell membrane permeability also supports this conclusion (Jenkins et al., 2020). Therefore, the key to design surfaces that can rapidly inactivate bacteria might hide underneath the nanostructured surfaces that can induce greater stress and cell membrane deformation.

Meanwhile, the accumulated bacteria debris left on the surface covers the nanostructured surface and then has a great negative impact on physical bactericidal performance (Xie et al., 2019a). Therefore, the effective removal of the bacteria debris is the key to the persistent and fast bacteria killing. Semiconductors have been well studied for organic pollutant degradation (Iqbal et al., 2014; Rajamanickam and Shanthi, 2016; Xiang et al., 2012), where nanoarrays were found to exhibit better catalytic performance due to the multiple light scattering and increase in optical path length enabled by the columnar hierarchical structures (Gao et al., 2015; Iqbal et al., 2014; Kong et al., 2017; Park et al., 2013). Although TiO2 nanosial structures has been proved feasible in the complete removal of bacteria debris after several hours (Meng et al., 2015), it has not been explored yet how the nanostructured semiconductor surface can endow the physical bactericidal surfaces with self-cleaning features for ultrafast bacteria inactivation.

Here we report the ultrafast physical bactericidal performance of the nanotip-engineered ZnO NAs, designed to enhance the stress at the interface between each nanorod tip and the bacteria cell via lowering the contact area and the number of nanorods in contact with the bacteria cell. The specific design aimed at faster bacteria killing rate is proved quite successful to both Gram-negative Escherichia coli (E. coli) and Gram-positive Staphylococcus aureus (S. aureus) when compared with the previously reported natural and artificial bactericidal surfaces. In contrast to the previous reports with relatively ordered topography, we showed the uneven NA topography with fewer number of nanotips in contact with the bacteria cell contributes to the enhanced stress at the interface. Similarly, the NAs in a similar density but with smaller tip-width also improve the bactericidal rate due to the increased stress at the interface. Finally, the nanotip-engineered ZnO NAs endow the nanostructured surfaces with rapid photocatalytic self-cleaning features, where the nanotips can be re-exposed even after a short time under UV irradiation. This study provides both experimental and simulation support for the ultrafast antibacterial activity of ZnO NAs, which represents the fastest reported physical bactericidal surfaces and demonstrates the potential for applications that need rapid and persistent bacteria inactivation.

2. Experimental procedure

2.1. Materials

Stainless steel sheets (SSS, 304 austenitic) were used as the substrate. Chemicals including pure ethanol, dimethylamineborane, and monoethanolamine were analytical grade and purchased from Chang Zheng Co. Ltd. (Chengdu). Precursors such as hexamethylenetetramine (HMTA, CH₆N₂), zinc nitrate hexahydrate (Zn(NO₃)₂·6H₂O) and Zinc acetate dihydrate (Zn(CH₃COO)₂·2H₂O) were obtained from Aladdin
Co. Ltd. (Shanghai). Polyethylenimine (PEI, Mw 600) was used to regulate crystal growth and purchased from Sigma-Aldrich. Deionized water (resistivity: 18 MΩ·cm) was used in all our experiments.

2.2. Hydrothermal growth of ZnO NAs

In this article, ZnO nanoarrays were prepared by two-step hydrothermal method, and the fabrication process of ZnO seed layer referred to the sol-gel method previously used (Xie et al., 2019b). The ZnO seed layer was pre-deposited on SSS substrate (3 cm × 3 cm) by dip-coating from a zinc acetate ethanol colloidal solution (5 mM) and then annealing at 300 °C for 10 min. To obtain a certain density of the seed layer, the coating-annaling step was selected four times. Then chemical bath deposition (CBD) was used to construct ZnO NAs on SSS (Xu and Wang, 2011; Yu et al., 2005; Zhu et al., 2013). A piece of SSS substrate with the ZnO seed layer was first immersed into a 60 mL hydrothermal growth solution which contained equimolar (30 mL, 50 mM) Zn(NO₃)₂·H₂O and HMTA in aqueous solution at 90 °C for 1 h. Then the substrate was submerged into a fresh solution which contained PEI (0, 3, or 6 mM) and grown at 90 °C for another 1 h. Hexagonal prism, hexagonal prismoid, and hexagonal pyramid ZnO NAs were prepared. After growth, all samples were washed with deionized water and annealed at 500 °C to remove any organic residue, such as PEI, HMTA and their decomposition product.

2.3. Fabrication of ZnO film

As a comparison group, the zinc oxide film was also fabricated by dip-coating-drying method (Ohyama et al., 1997; Xie et al., 2019b). The precursor concentration of Zinc acetate in the 2-methoxyethanol (MEA) solution was controlled at 300 mmol·L⁻¹ and aged at room temperature for 24 h. The SSS substrates (2 cm × 5 cm) was dipped into the precursor solution and withdrawing at a speed of 5 cm·min⁻¹. Then the wet film samples were dried at 90 °C for 10 min. In order to produce a uniform ZnO film, the dip-coating-drying process were repeated ten times and annealed at 500 °C for 1 h then cooled to room temperature.

2.4. Characterization

Images were taken with a field emission scanning electron microscopy (FE-SEM, JSM-7001F, JEOL) using a 20 kV voltage. The ZnO nanorods scraped from the substrate were dispersed by ethanol first and then characterized by transmission electron microscopy (TEM, JEM-2100, JEOL). The crystal structures of the nanorods were investigated using X-ray diffractometer (XRD, Philips X’ Pert PRO, Cu Kα radiation, λ = 1.5418 Å).

2.5. Determination of ZnO NAs morphology parameters

In our experiment, 5 regions were randomly selected on a ZnO NAs sample for SEM observation, then the average height, average tip-width and the number of nanorods in a given area was measured by particle size statistics software (Image J). Grubbs test was used to remove the outliers and then the average morphology parameters of each sample were calculated statistically.

2.6. Bactericidal properties of ZnO NAs

Referring to the JIS Z 2801 (Japanese Industrial Standard) (“Japanese Industrial Standard: JIS Z 2801: 2000”, 2000; Yi et al., 2018), the sticking membrane method were utilized to evaluate the bactericidal properties of the samples. The steps of bacterial activation and proliferation refer to our previous work (Xie et al., 2019b, 2019a). Two model bacterial suspension (E. coli stain [ATCC 25922] and S. aureus stain [ATCC 6538], Guang Dong Detection Center of Microbiology, China) were selected and amplified in a nutrient broth medium (AOBOX Co., Ltd. Beijing) at 37 °C for 4 h. Then broth medium was used to dilute bacterial cell suspension to proper concentration (E. coli, 1.4 × 10⁶ CFU·mL⁻¹ – 1.7 × 10⁶ CFU·mL⁻¹ and S. aureus, 1.3 × 10⁷ CFU·mL⁻¹ – 1.4 × 10⁷ CFU·mL⁻¹) to afford a standardized suspension. All samples were pre-cut into 1 cm × 1 cm, then soaked in 75% ethanol for 1 min and dried in a sterile environment to use. Each sample surface was dropped with standardized bacteria suspension (100 μL) and incubated at 37 °C in dark for 1 min, 0.5 h, and 2 h, respectively. All the height of the drops is accuracy controlled to avoid the interference of initial droplet rate. After the samples were contacted with bacteria for a certain period of time, 10 mL sterile saline was used to wash them thoroughly, and the resulting bacterial suspension was diluted with sterile physiological saline for 100 times for E. coli, and 10 times for S. aureus. Then 100 μL diluent was transferred onto agar plate for plate counting. After incubation at 37 °C for 18 h, the colony-forming units (CFU) were counted, and the survival ratio was calculated. All the process is carried out in dark conditions and repeated three times. The bactericidal ratio was calculated by Eq. (1).

\[
\text{Bactericidal ratio} = \frac{N_0 - N}{N_0} \times 100\%
\]  

where \(N_0\) is the number of living bacteria at the original time on the SSS control sample surfaces and \(N\) is the number of living bacteria on the test sample surfaces. All the experiments are carried out in dark conditions.

2.7. Interactions between bacterial cells and ZnO NAs

The E. coli stain was activated on an agar culture medium at 37 °C overnight, followed by proliferation in 30 mL of nutrient broth medium at 37 °C for 3 h. 100 μL of bacterial suspension was then added onto each surface. After incubation for 1 min, samples were fixed in 2.5% glutaraldehyde for 12 h at 4 °C, followed by ethanol gradient dehydration. The samples were soaked for 15 min in each step. After drying overnight at room temperature, bacterial cell topographies were observed by FE-SEM.

2.8. Bacterial cell integrity on ZnO NAs

Confocal laser scanning microscope (CLSM 510 Meta laser scanning confocal microscope, Carl Zeiss) was used to observe bacterial cells on the ZnO NA surfaces. E. coli cells were stained with the fluorescent nucleic acid dye kit containing SYTO-9 and PI (LIVE/DEAD BacLight Bacterial Viability Kit, Invitrogen). Prior to microscopic observation, 1 mL of bacterial cell suspension was stained with 1.5 μL of a PI and SYTO-9 mixture (1:1, V/V), followed by incubation for 15 min. Then the testing samples were immersed into stained bacterial cell suspension and flat on the aseptic bench, followed by covering with a sterilized cover slip. After incubating for another 1 min, the samples were observed under CLSM. All the steps were proceeded in dark at room temperature.

2.9. Pressure stress between cell wall and various ZnO NAs

The difference in cell integrity damage performance of various ZnO NAs was estimated using finite element modeling (FEM, ANSYS 17.1). Specifically, a 2D FEM model with cell wall and cytoplasm was implemented. The material properties of the cell wall were assumed to be a linear elastic material conforming to generalized Hooke’s law (Young’s modulus = 10 MPa, Poisson’s ratio = 0.3) (Chen et al., 2012; Gumbart et al., 2014; Smith et al., 1998; Thwaites and Mendelson, 1989). An incompressible fluid model with low Young’s modulus (1e⁻³ MPa) and high Poisson’s ratio (0.499) was implemented in cytoplasm (Pitt and Davis, 1984). Considering the various ZnO NAs as rigid bodies model to reduce calculation time. For correlating the FEM results with the experimental results, the size of FEM model corresponds to the
experimental. To ensure the simulation result, the cell model was divided into 18,348 elements (PLANE 182) and 18,449 nodes by free meshing. In the area where the cell wall was contact with the NAs, contact analysis was performed using CONTA 172 and TARGE 169 element based on Augmented Lagrange method. All DOF constraints were applied to the top of the cell. According to apply a certain displacement (Y-axis) to the NAs to simulate the cell destroy process.

2.10. Surface tension between cell wall and various ZnO NAs

In order to consider the effect of surface tension and capillarity on bactericidal effect, a simplified finite element model is proposed. The models include bacteria cell, nanoarrays and the fluid between them. In the simulation process, it is assumed that the cell is at the hot side and the liquid is at the cold side. The capillary effect and cell surface tension are taken into account by using the contraction effect of the liquid. The specific parameters and assumptions in the simulation are as follows:

1) The cell was simplified into two parts: cell wall and cytoplasm. The cell wall was a linear elastic material conforming to Hooke’s law, where elastic modulus $E = 10$ MPa, Poisson’s ratio $\nu = 0.3$, thermal expansion coefficient $Î» = 0.3 \text{ w} \cdot \text{mm}^{-2} \cdot \text{k}^{-1}$. The cytoplasm is an incompressible fluid with elastic modulus $E = 10^{-3}$ MPa, Poisson ratio $\nu = 0.499$, thermal expansion coefficient $Î» = 10^{-9} \text{ k}^{-1}$, thermal conductivity $\kappa = 0.0003 \text{ w} \cdot \text{mm}^{-2} \cdot \text{k}^{-1}$.

2) The flow of liquid was ignored. The material parameters of the liquid between the nanorods are: Young’s modulus $E = 10^{-3}$ MPa, Poisson’s ratio $\nu = 0.499$, Thermal expansion coefficient $Î» = 10^{-5} \text{ k}^{-1}$, Thermal conductivity $\kappa = 0.3 \text{ w} \cdot \text{mm}^{-2} \cdot \text{k}^{-1}$.

3) In the process of initial simulation process, assuming that the cell temperature is 100 °C, liquid temperature is 20 °C, and the reference temperature is 100 °C, temperature of nanoarray is 100 °C, the liquid will pull on cell wall during the contraction.

4) Ignoring the difference in the width of bacteria, the model was simplified into a two-dimensional model. The whole model was divided into 26,419 elements, including 26,943 nodes.

The NAs were assumed to be rigid and fixed in the simulation process. The interface between cells, liquid and nanorods is glued together in the finite element model.

3. Results and discussion

3.1. Fabrication and topography characterization of ZnO NAs

Unlike the nanostructured surfaces with a relatively ordered topography fabricated by techniques such as reactive ion etching (Ivanova et al., 2013; Tripathy et al., 2017b), chemical etching (Hu et al., 2017) etc., the ZnO NAs in this study were fabricated by a two-step hydrothermal growth method (Yu et al., 2005; Zhu et al., 2013), so that the tip-width, density and height can be controlled. In order to grow ZnO NAs with similar density, uniform and equidensity ZnO seeds were first prepared on stainless steel sheet (SSS) for all three samples by a certain dip-coating-annealing process (Fig. S1b) and then hydrothermally grown to form ZnO NAs from each seeds. The distribution of the NA structures on the SSS was assessed using scanning electron microscopy (SEM) and uniform NAs distribution across the substrate surfaces was observed (Fig. S2). EDS mapping was used to analysis of composition of NAs showed that the surface was ZnO component (Fig. S3).

To regulate NAs with various tip-sizes, polyethyleneimine (PEI) was introduced into the hydrothermal system as a topographical adjusting agent (Hu et al., 2017). Three different types of topographies (hexagonal prism, hexagonal prismoid, and hexagonal pyramid) with similar density were fabricated on the SSS substrates as shown in Fig. 1a-c. SEM images indicated that hexagonal prism NAs were obtained from the solution without PEI. These ZnO NAs had flat rod tips and an average...
tip-width of 118.1 nm. Comparatively, NAs with smaller average tip-widths were obtained in the presence of PEI. Hexagonal prismoid NAs and hexagonal pyramid NAs, with the average tip-width of 75.3 and 28.5 nm, were prepared with increasing PEI concentrations of 3 and 6 mM (Table S1). It is likely that the PEI molecules absorbed on the ZnO (100) crystal face and increased the relative c-axis growth rate to form NAs with smaller tip-widths (Chen et al., 2011). As shown in Fig. 1d and Table S1, the densities of these NAs were quite similar due to the same density of ZnO seed layer. Since the certain roughness of the stainless steel substrate, the orientation of the seed crystal is not perfectly aligned, resulting in the differences in the growth environment of ZnO NAs (Chen et al., 2011). In the end, there is a variance about 200 nm in height of the grown NAs, which leads to the uneven topography.

The X-ray diffraction (XRD) patterns of three ZnO NAs show diffraction peaks at 31.8°, 34.4°, and 36.5°, which were indexed to a typical hexagonal wurtzite structure (JCPDS card, No. 001-1136, Fig. 1g) (Ohyama et al., 1997; Xu and Wang, 2011). The peak at 34.4°, corresponding to the (001) plane, is much higher than those at 31.8° (100) and 36.5° (101), which indicates it is the preferred crystal orientation [001] of the as-prepared samples. The peaks at 42.9, 49.9, and 73.4° are characteristic of the stainless steel (JCPDS card, No. 65-4150) austenite structure (Zhu et al., 2013). Based on high resolution transmission electron microscopy (HR-TEM) analysis of a single nanorod scraped from three different NAs (Fig. 1g), all the ZnO crystal plane spacing was calculated to be 0.52 nm, corresponding to the interplanar spacing of the (001) planes of the wurtzite structured ZnO. This is in good agreement with the XRD data, with both confirming the preferred growth direction of the ZnO crystal along [001] orientation (Ohyama et al., 1997; Xu and Wang, 2011). Therefore, the characterization results showed that the crystal structure of the three morphologies of ZnO NAs were the same.

3.2. Tip-size dependent ultrafast bactericidal properties of ZnO NAs

In order to evaluate the bactericidal performance of the as-prepared ZnO NAs, two model bacterial standardized suspensions were dropped onto the substrates and incubated for 1 min to 2 h at 37 °C in dark conditions. Besides, the concentration of photocatalytic products and Zn2+ were also tested to determine their role in bactericidal activity. Photographs of the E. coli colony forming units (CFU) are presented in Fig. 2a–d. No bactericidal effect was observed on the pristine SSS surface as the control sample, since the number of bacteria settled on the pristine SSS increased with increasing incubation time (Fig. 2a). Furthermore, a flat ZnO film surface was also prepared as another control sample showing weak bactericidal performance with survival ratio of 99.34 ± 0.21% against E. coli after 1 min of bacterial cell contact (Fig. S4b). However, the number of living bacteria reached a relatively high level after incubation for 2 h, which suggests the ZnO film fails to provide a long-lasting bactericidal surface (Fig. S4b). In contrast, all the NAs in this work showed an almost complete bacteria inactivation after 2 h (Fig. 2), which is among the best antibacterial performance reported in the literature (Hasan et al., 2013a; Ivanova et al., 2013; Yi et al., 2018). More importantly, the NAs with similar density demonstrate a positive relationship between the survival ratio and tip-width. The pyramid NAs with the smallest tip-width exhibited the highest E. coli reduction of $1.5 \times 10^8$ CFU·mL$^{-1}$ and S. aureus reduction of $1.3 \times 10^7$ CFU·mL$^{-1}$ within 1 min, with the survival ratio of 2.5% and
5.1%, respectively (Fig. 2i–j). The prismoid NAs with larger tip-width showed lower bacteria reduction rate and the prism NAs with the largest tip-width were found to display the lowest bacteria reduction rate for E. coli and S. aureus. Moreover, the rapid bactericidal behavior of ZnO NAs was found stronger against E. coli than S. aureus, which was attributed to the different cell wall structures. Compared with S. aureus, E. coli has a thinner peptidoglycan layer, therefore more vulnerable to mechanical damage (Perry et al., 2009). The ultra-rapid bactericidal rate is \(1.5 \times 10^7 \text{ per-cm}^{-2} \cdot \text{min}^{-1}\) against E. coli and \(1.3 \times 10^6 \text{ per-cm}^{-2} \cdot \text{min}^{-1}\) against S. aureus, which is the highest reported to date. Furthermore, in contrast with previous reports that conducted on relatively ordered surfaces, where a higher density of nanopillars was found to inactivate bacteria more effectively (Dickson et al., 2015), we showed that a much lower density can achieve the same level of bactericidal efficacy and at a much faster speed. Since the wettability of different surface morphology might alter the attachment rate of bacteria, which will affect the accurate measurement of the bacteria inactivation rate, both the droplet spreading rate (Fig. S5) and contact angle (Fig. S6b–d) was measured on all of the samples. In the measurement of droplet spreading rate, the small droplet of bacterial suspension completed the spreading on all of the samples within 250–500 ms, which indicates that average time required for bacteria to reach the surface is similar. On the other hand, the contact angle results show that the contact angle of these NAs with bacterial solution is also quite similar, which further confirms that the difference of the array morphology has little effect on the wettability.

Although these results indicate that the ultra-rapid bactericidal behavior of ZnO NAs against E. coli and S. aureus is related to the size of tip-width, it is not confirmative yet the bactericidal performance is primarily caused by physical rather than chemical mechanism. Therefore we further investigate two commonly accepted chemical bactericidal mechanisms, the generation of reactive oxygen species (ROS) and the release of Zn\(^{2+}\) ions (Joe et al., 2017; Sirelkhatim et al., 2015), for these ZnO NAs. In terms of ROS, superoxide anion radical (\(\cdot\text{O}_2^-\)), hydrogen peroxide (\(\text{H}_2\text{O}_2\)), and hydroxyl radical (\(\cdot\text{OH}\)) via ZnO were believed to disrupt cell membranes and kill bacteria (Brayner et al., 2006; Li et al., 2012). Furthermore, between the radicals, a recent study reported that it is \(\cdot\text{O}_2^-\) rather than \(\cdot\text{OH}\) that should be responsible for the bactericidal properties ZnO pillars (Yi et al., 2018). We found that the generation of \(\cdot\text{O}_2^-\) for these ZnO NAs exhibits lower concentration of \(\cdot\text{O}_2^-\) than the ZnO film as the control sample (Fig. S7), which suggests \(\cdot\text{O}_2^-\) has a trivial influence on the cell death in this study. On the other hand, the concentration of \(\text{H}_2\text{O}_2\) generated by ZnO NAs was measured through a Hydrogen peroxide kit detection (Bai et al., 2013), and it turns out to be even lower than that of the ZnO film control sample (Fig. S8). Therefore, the bactericidal activities of these ZnO NAs were proved to be irrelevant to the generation of ROS. In terms of the Zn\(^{2+}\) ions release from the ZnO NAs that were previously found to injure the bacteria membrane (Suwalsky et al., 2009), the concentration of Zn\(^{2+}\) measured for the ZnO NAs in this study (Table S2) was far lower than the minimum bactericidal concentration (MBC, 1024 mg/L) of Zn\(^{2+}\) (Du et al., 2009), the bactericidal performance is not attributed to Zn\(^{2+}\). Therefore, since neither of the chemical bactericidal mechanisms applies in the present study, it can be speculated that it is the physical bactericidal mechanism that plays the key factor influencing the rapid bactericidal performance. A recent report by Jeong et al. also confirms this conclusion (Jeong et al., 2020). Although it was reported that the aspect ratio of the NAs dominates the bactericidal efficiency (Kelleher et al., 2016), given the similar height of these ZnO NAs, the term ‘aspect ratio’ is replaced by the term ‘tip size’ in this manuscript. Also, since the chemical composition and crystalline features of these three ZnO NA surfaces were the same (Fig. 1g), it can be deduced that the rapid bactericidal activities observed can be associated with the tip size of the ZnO NAs.

### 3.3. The interaction between bacteria cell and ZnO NAs

In order to investigate the direct interaction between the bacteria and ZnO NAs under such a rapid condition, we designed a series of bactericidal cell observation experiments to explore the interaction between E. coli cells and ZnO NAs after 1 min contact time (Elbourne et al., 2019). All of these experiments were finished in darkness so as to avoid the influence of photocatalysis on the bactericidal activity.

Based on the confocal laser microscopy (CLSM) results shown in Fig. 3a, bacterial cells on pristine SSS displayed only green fluorescence within the allotted time-frame, showing no bactericidal effects against E. coli for pristine SSS at all. Although the cells on ZnO film showed a few red fluorescence signals, it is still considered rather weak bactericidal activity under the same condition (Fig. S9a). However, there are both green and red fluorescence signals, which represent the presence of SYTO 9-DNA and PI-DNA emanating from cells, observed on the hexagonal prism ZnO NA surfaces, clearly indicating the damage of cell. In fact, the damaged E. coli cells are known to self-recover and avoid cell death once the leakage pressure stimulus is eliminated (Pagán and Mackey, 2000). Thus combined with the 2 h bactericidal results shown in Fig. 2, the damage brought by these ZnO NAs is beyond the ability of E. coli cell to self-recover in a short time. For hexagonal prismoid NA surfaces, there are more cells with red fluorescence signals were observed, indicating a higher level of cell damage (Fig. 3a). And the highest level of bacteria death was observed on the hexagonal pyramid NA surfaces with almost all of the bacteria showing strong red fluorescence signals. These results show that ZnO NAs with smaller tip size are more likely to kill bacteria within such a short time, which further confirms the assumption that the rapid sterilization performance is associated with the tip size of the ZnO NAs.

To further explore the interaction between cells and ZnO NAs, the morphologies of the E. coli cells were captured after 1 min contact. It is clearly shown in Fig. 3a that there are varied cell-responses to the surface attachment between the pristine SSS and three ZnO NA surfaces. The cells on the pristine SSS as well as on ZnO film surfaces demonstrated a natural rod morphology (Figs. 3b and 59b). Rod-shaped cells were observed on the hexagonal prism NA surfaces, which is similar to that on the pristine SSS and ZnO film, indicating a similar cell response to the attachment of varied surface topography. However, a higher level of deformation of bacteria was observed on the hexagonal

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**Fig. 3.** Experimental results and schematic illustration of the bactericidal behavior of the ZnO NAs. a) CLSM images of E. coli cells on SSS pristine and different ZnO NA surfaces after 1 min contact, cells stained with SYTO 9 are green and those stained with PI are red; b) SEM images of E. coli cells on SSS pristine and different ZnO NAs after 1 min contact; c) Schematic illustration of the speculated bactericidal mechanism of three ZnO NAs. All experiments were operated in darkness.
prismoid surfaces, where a number of dead bacterial cells were greatly deformed by the ZnO NAs but still maintaining the rod-like shape. Notably, the dead bacterial cells on hexagonal pyramid NAs showed the highest level of deformation where the morphology of cells was found irregular and spreading over the NA surfaces, which indicates the sharp tips of the hexagonal pyramid ZnO NAs completely damaged the cell in such a short time. Therefore, it is speculated that the stress applied between the bacteria cell and the hexagonal pyramid NAs is so large that the E. coli cells were no longer able to hold its structural integrity in the rod-like shape and then killed compared with the other samples (Fig. 3c).

3.4. Physical bactericidal mechanism of ZnO NAs

Although there were a few theoretical studies on the bactericidal nature of nanopatterned surfaces (Li, 2015; Li and Chen, 2016; Pogodin et al., 2013; Wu et al., 2016; Xue et al., 2015), these models were built within the framework of the “stretching” theory and more suitable for longer-time inactivation of bacteria, taken their bactericidal rate into consideration. In fact, there is not yet any report on the ultrafast physical bactericidal surface nor any theoretical study suitable for such rapid bacteria inactivation. From the above results, we suspect that it might help explain the rapid bactericidal mechanism from the perspective of stress, which has not been involved in previous theoretical reports. In order to verify the assumption above, we sought finite element simulation for help as follows. First, we built three models with different tip-sized nanorods aligned in similar density and height. Then, we applied a cell upon these nanorods to witness the maximum stress at the interface. Since the mechanical properties of bacteria cells are attributed to peptidoglycan layers in the cell wall (Chapot-Chartier and Kulakauskas, 2014; Yao et al., 2002), the mechanical parameters of cell wall were selected as described in the Experimental procedure (Chen et al., 2012; Gumbart et al., 2014; Smith et al., 1998; Thwaites and Mendelson, 1989).

The simulation result shows that the max stress on the cell wall increases with the decrease of the diameter of the tip of the nanorods (Fig. S10a), where the maximum stress, 0.74 MPa is applied by the hexagonal pyramid tip. Combined with the morphological results of cells on the NAs that the number of contact sites on pyramid NAs was higher than that of prismoid NAs (Fig. 3b), it can be inferred that the higher stress applied by the NAs with smaller tip-width might rupture cells more easily. Moreover, around the contact region, the simulation result shows that for all three NAs, the stress applied on the inner side of the cell wall is higher than that on the outer side of the cell wall (Fig. S10), which suggests if cell rupture were to happen at the contact region, it should start from the inner side of the cell wall. Apart from the contact region, the equivalent stress diagram also demonstrates the cell is subjected to tensile stress at many regions upon contact with the NAs (Fig. S10b). The stress near the contact region of the nanorod tip displays different distribution patterns for three NAs. For hexagonal pyramid NAs with smallest tip-width, the stress applied on the contact region (I) is much higher than that on the valley region (II), indicating that the stress is primarily concentrated around the contact region. However, for hexagonal prism NAs with largest tip-width, the stress applied on the contact region (I) is quite similar with that on the valley region (II), which indicates if the cell rupture were to happen, it could start at either of these two regions (Fig. S10c).

Notably, these results not only support the “stretching” theory, the most popular mechanisms for physical bactericidal surfaces, where the rupture happens in the valleys between contact regions (Li, 2015; Li and Chen, 2016; Pogodin et al., 2013; Xue et al., 2015), as follows: if the stress applied at the contact region is similar to that of the valley region, as the cell stretches along the time, eventually exhibiting the rupture in the valleys between contact regions, but also suggests if the stress applied at the contact region is large enough, the rupture is likely to happen from the inner side of the cell wall and might lead to the final cell death.

3.5. Ultra-rapid bactericidal mechanism of pyramid ZnO NA surface

Although we have shown that NAs with smaller tip-width could apply higher stress at the contact region that could rapidly lead to the cell death, however, it might not be enough to explain the whole rapid bactericidal mechanism. The ultra-rapid bactericidal rate of the pyramid NA surface is 35 times higher than the fastest value ever reported in the literature (Fig. S11c), i.e. the black-silicon surface (Ivanova et al., 2013), where both of these surfaces have a similar density (around 3 × 10⁹ rod·cm⁻²). Moreover, the black-silicon NAs have a smaller tip-width (20 nm) than that of the pyramid NAs (28.5 nm), which should have been able to apply higher stress to the bacterial cells and achieve faster bactericidal rate. Thus, there should be other factors that contribute to the overall stress that leads to ultra-rapid sterilization.

Since surface tension is essential in bacterial inactivation and depends on capillary pressure in the “stretching” theory, a finite simulation analysis of the tensile stress on cell wall was carried out so as to determine whether the “stretching” theory applies in this ultrafast bactericidal case. The simulation of the tensile stress on cell wall was carried out by filling collapsible liquid between nanorods and then shrinking the liquid. On one hand with the ordered surfaces, the simulation results show that the tensile stress of the region between the nanorods (Fig. S12, Region II), increases with the decreasing tip-width (Fig. S12a–c), possibly due to the increased capillary force and the results are in accordance with previous longer-time results (Dickson et al., 2015; Ivanova et al., 2013). On the other hand, with the uneven surfaces, it is subject to smaller tensile stress when compared with the ordered surface, which leads to a contradictory conclusion compared with the experimental results. In fact, the increased spacing between the nanorods caused by the uneven topography might weaken the capillary force. Therefore, the surface tension is not the dominating factor for their ultrafast bactericidal activity.

Enlightened by a careful examination of the bacterial morphology on the NA surface, where the bacteria debris was destroyed by NAs in a non-uniform way (Fig. 3b), it is speculated that the uneven topography may be involved. Compared with the NAs with the relatively ordered topography reported previously (Hasan et al., 2013a; Ivanova et al., 2013; Yi et al., 2018) the uneven topography of ZnO NAs in this study could allow a fewer number of nanotips contacting the bacteria with an uneven height distribution, which might increase the stress per contact region of the NAs. However, this is not to say the fastest way to kill bacteria is by infinitely increasing the distance between the nanoscale columns so that the bacteria have as little contacted point with the columns as possible. Because an appropriate density of nanorods is necessary for bacteria to meet the nanorods first and then get killed (Dickson et al., 2015; Fisher et al., 2016). In order to verify the assumption, a simplified finite element simulation analysis was conducted to mimic the complex real case. The simulation result shows the stress concentrated on both inner and outer sides of the cell wall were significantly increased by NAs with uneven topography, which could synergistically damage the cell wall (Fig. S11a, b, Fig. 4). Besides, the uneven topography could provide more space for cell subsidence and therefore generate greater stress to the cell wall and more severe deformation of the cell. Unlike the ordered topography fabricated by nanoimprint lithography which allows the stress to be distributed equally upon each nanorod, the uneven topography enhances the stress applied by the spatially uneven nanotips. Combined with the results from a recent study that demonstrated multi-directional NAs as more efficient antibacterial surface than the conventional highly-ordered nanostructures (Fisher et al., 2016), it can be assumed that the hierarchical nanostructure brought by the uneven topography contributes to the rapid bactericidal activity of these ZnO NAs. Besides, there were both theoretical (Li, 2015; Pogodin et al., 2013; Xue et al., 2015) and experimental studies (Nowlin et al., 2015) showing the cellular adhesion to the surfaces due to gravity and other physical interactions contributes to the bacteria
rupture. Thus, in this study, the ZnO NAs with uneven topography might cause the hierarchical cellular adhesion that both increase the stress and accelerate the cell rupture.

Instead of challenging the prevailing “stretching” theory of bacteria being ruptured between nanorods (Li, 2015; Li and Chen, 2016; Pogodin et al., 2013; Wu et al., 2016; Xue et al., 2015), our results make an excellent complement to the existing physical bactericidal mechanism. Since the surface tension can’t explain the ultrafast bactericidal performance, but it is essential in bacterial inactivation in previous “slower bactericidal” studies, there might have been two different mechanisms for the ultrafast and the “slower” bactericidal performance. Thus, we proposed a two-stage theory for the bactericidal performance, including the arriving stage and the resting stage (Fig. 4). At the arriving stage, the bacteria get close to the surface either actively or passively by the movement of surrounding liquid. Thus, the initial kinetic energy carried by the bacteria will be then transferred into stress at the contact region between the cell wall and the nanorods. If the stress is large enough for the bacteria to lose its structure integrity, the bacteria will be then inactivated at the arriving stage. However, if the stress at the contact region is no greater than the region between the nanorods even when the kinetic energy of the cell is consumed, then the resting stage steps in, where the stretching of the bacteria between the nanorods caused by the capillary force and other effects leads to the final death after some time, which matches the direct observation in the previous report (Linklater et al., 2017).

Thus, from the perspective of stress, the rapid bactericidal mechanism of pyramid NAs can be explained as follows: when the bacteria cell contacted these ZnO NAs, the sharp nanotip, the uneven topography, and the cellular adhesion together contributed to the high applied stress and membrane deformation, which went beyond its ability to keep the integrity of the cell and then led to the following rupture of the cell. Compared with other physical bactericidal surfaces, the ZnO NAs fabricated in this work can inactivate bacteria at a much faster speed, where the pyramid NAs exhibited about 35 times higher than the fastest record ever reported in the literature (Fig. S14), the 365 nm UV light source was selected for the photocatalytic degradation of the bacteria debris on the ZnO NAs, with a mild irradiation intensity of 10 mW/cm². The bacterial debris distribution was examined by SEM after UV exposure to verify the photocatalytic self-cleaning performance of the NAs surfaces (Figs. 5a, S16). After 10 min of incubation on the ZnO hexagonal pyramid NAs without UV exposure, most of the presumably dead E. coli cells still remained on the NA surface. However, after 10 min UV irradiation, there are splintery bacteria debris observed on the NAs and, notably, the nanotips of ZnO NAs were re-exposed (Fig. 5a).

The EDX mapping results also confirmed the findings above. The original sample has high carbon residuals on the surface, which come

### 3.6. Photocatalytic debris removal and recyclable bactericidal behavior of ZnO NA surfaces

The above results have shown the ultrafast bactericidal behavior on the nano-tip engineered ZnO NA surfaces. However, the accumulation of bacteria debris greatly impedes the bactericidal efficacy as shown in Fig. S13a, as the nano-tips gradually cover by the debris. In order to address this issue and endow a persistent ultrafast bactericidal performance to the ZnO NAs, the photocatalytic activity of the nanotip-engineered ZnO is applied.

The hexagonal pyramid ZnO NA surfaces are chosen due to the superior bactericidal speed and efficacy. Based on the UV–Vis absorption spectrum of the ZnO hexagonal pyramid NAs (Fig. S14), the 365 nm UV light source was selected for the photocatalytic degradation of the bacteria debris on the ZnO NAs, with a mild irradiation intensity of 10 mW/cm². The bacterial debris distribution was examined by SEM after UV exposure to verify the photocatalytic self-cleaning performance of the NAs surfaces (Figs. 5a, S16). After 10 min of incubation on the ZnO hexagonal pyramid NAs without UV exposure, most of the presumably dead E. coli cells still remained on the NA surface. However, after 10 min UV irradiation, there are splintery bacteria debris observed on the NAs and, notably, the nanotips of ZnO NAs were re-exposed (Fig. 5a).

The EDX mapping results also confirmed the findings above. The original sample has high carbon residuals on the surface, which come...
from the organic compound of the bacteria debris. After turning on the UV light, the carbon content on the surfaces keeps decreasing as the irradiation time increases (Fig. S15a–d), supporting the degradation of bacteria debris under UV light. Remarkably, after irradiation for 720 min, the bacteria debris on the ZnO NA surface is almost gone (Fig. S15d) and the surface was quite similar to the original ZnO NAs (Fig. 1a), which again confirmed that the surface was capable of photocatalytic self-cleaning under even mild irradiation conditions. This effect is related to the photocatalytic generation of reactive oxygen species (ROS) such as -OH, -O2•-, H2O2 on the surface of the ZnO under UV light (Kuo et al., 2007), which will contribute to the decomposition of the organic compounds (Gao et al., 2014; Lyu et al., 2017; Peternel et al., 2007; Ye et al., 2015). Besides, the short lifetime of ROS (Jang et al., 2006) will not interfere with the subsequent bactericidal experiments in dark.

Cyclic bactericidal experiments have been carried out to verify the proposed sustainable ultrafast bactericidal activity of the hexagonal pyramid ZnO NA surfaces. In the second repeated bactericidal experiment, the bactericidal ratio of the surface without UV photocatalytic self-cleaning decreased rapidly to 45.8%, indicating that the physical bactericidal performance was seriously affected. However, the surface lost its bactericidal performance in the third bactericidal experiment, the surface has no sustainable bactericidal performance. Although a much cleaner surface can be achieved after a long irradiation (720 min) of UV light, it is found that a short time irradiation of 10 min is long enough to maintain the ultrafast bactericidal activity as shown in Figs. 5b and S16. The bactericidal rate of ZnO NAs can be maintained long enough to maintain the ultrafast bactericidal activity as shown in Figs. 5b and S16. The bactericidal rate of ZnO NAs can be maintained at a high level (×107 per cm2•min), which is similar to the original rate, even after 4 cycles of bactericidal experiments, which demonstrates the recyclable and photocatalytic self-cleaning functions of the ultrafast bactericidal surfaces of ZnO NAs.

4. Conclusions

In conclusion, in this work we have shown the ultrafast physical bactericidal activities of a series of ZnO NAs with an uneven topography and different tip-width, fabricated by a hydrothermal growth method. These ZnO NAs exhibit ultrafast physical bactericidal activities against both Gram-negative (E. coli) and Gram-positive (S. aureus) bacterial cells. The pyramid NAs with the smallest tip-width achieved the highest bactericidal efficacy in 1 min, which is 35 times faster than the fastest physical bactericidal surfaces reported in the literature. The experimental results were further supported by a finite element modeling analysis that the small tip-width of the nanorods with an uneven topography greatly enhance the stress applied on the cell wall and lead to the rapid bacteria inactivation. However, it is not clearly yet whether there were other morphological factors that also contribute to the ultrafast bactericidal performance. Recently, Elbourne et al. showed that the orientation of the multi-directional tips plays an important role in promoting the bactericidal efficacy (Elbourne et al., 2019). It is very intriguing to explore the relationship between the tip orientation and the bactericidal speed in further studies. Lastly, in order to address the sustainable applications, the photocatalytic self-cleaning and recycling action of physically bactericidal activity was demonstrated by the intrinsic photocatalysis of ZnO crystals. Thus, this work provides a strategy for the design and fabrication of recyclable and efficient ultrafast bactericidal surfaces, which not only broadens the horizon for the bactericidal surface technology towards applications that required rapid and sustainable bacteria inactivation, but also provide insights into the rapid physical bactericidal mechanism that can be used to guide the design of the next-generation antibacterial materials.

CRedit authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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