Electroporation effect of ZnO nanoarrays under low voltage for water disinfection

1 Introduction

Drinking water safety is closely related to human health [1]. Due to rapid economic development, water pollution has occurred in many parts of the world [2,3]. Waterborne pathogens can lead to various infectious diseases, such as diarrhea, typhoid, and cholera. Most deaths from waterborne pathogen infections occur in poor countries that still do not have access to sanitation and electricity [4,5]. To obtain safe drinking water, water disinfection treatment is required. Therefore, water disinfection treatment technology has always been the focus of attention [3,4,6]. Commonly used chlorination has low cost and is easy to apply, but there is a safety risk of producing carcinogenic by-products [7,8]. Ultraviolet disinfection and ozone disinfection have high energy consumption and limited disinfection capacity [9,10]. Moreover, traditional disinfection methods are not easily applicable in poor areas without sanitation and electricity supply [11]. To better protect human health, it is critical to develop efficient disinfection technology with low safety hazards, low energy consumption, and easy operation as a complement to modern disinfection technology.

A simple and effective application for inactivating different types of pathogens is electrodisinfection [12]. Electrodisinfection is of great interest because of its high efficiency in water treatment without generating products potentially toxics or bacterial resistance [13]. The effect of electrochemical disinfection is based mainly on the efficient generation of reactive oxidants (ROS), which not only inactivate bacteria but also degrade organic matter. In contrast, the effect of electroporation disinfection is based mainly on bacterial lysis and perforation triggered by a strong electric field, resulting in bacterial deactivation. Electroporation disinfection is more rapid compared to electrochemical disinfection [14]. The electroporation phenomenon of cell generally refers to the formation of nanoscale defects or pores in cell membrane under strong electric field, resulting in increased permeability of cell membrane to ions and other non-permeable molecules.
Irreversible electroporation will be triggered when the strength of electric field is high enough \(10^7 \text{ V/m}\), leading to cell death [15]. Traditional electroporation disinfection usually requires high applied voltage (10 kV) to generate strong electric field, which will not only lead to high energy consumption and high cost but also cause safety problems [16].

Since 2010, researchers have developed a bactericidal technique, which is using one-dimensional nanostructures to trigger electroporation for disinfection. Based on the locally enhanced electric field effect of the nanotips, irreversible electroporation of bacteria can achieve at a low applied voltage (<20 V), allowing for safe and low-cost electroporation disinfection [17]. Electroporation disinfection with nanoarray-modified electrodes is considered a reliable disinfection technology due to its ability to maintain good sterilization performance at low energy and low cost [18,19]. Electroporation disinfection is simultaneously performed with multiple disinfection mechanisms, including the production of ROS under electrical stimulation [14]. The contribution of ROS in disinfection depends on the experimental conditions and the nature of the electrode. It was reported that in electroporation disinfection performed at voltages above 10 V, more ROS were produced due to higher voltages, so that the disinfection mechanisms included electroporation and oxidative stress [20,21]. Under electrical stimulation, electrodes with longer contact time with bacteria, ROS became the main disinfection mechanism based on the electron transfer between the material and bacteria [22]. To avoid the problems of unnecessary water decomposition and corrosion occurring at the nanoelectrodes, researchers used a voltage below the typical voltage of electrolytic water (<2 V) for electroporation disinfection, in which case electroporation was the main disinfection mechanism [23–25].

How to improve the disinfection efficiency is the key issue of the electroporation-disinfecting technology [26,27]. Several kinds of nanoarray-modified porous electrodes of metal compounds were developed to achieve efficient disinfection [24,28]. Ag nanoparticle-loaded CuO nanoarray-modified (Ag NPs-CuO NWs) copper foam electrode was reported to exhibit high flow rate disinfection at 10 V applied voltage [29]. Carbon layer-coated Ag NPs-Cu2O NW-modified (C/Cu2O NWs-Ag NPs) copper foam electrode was prepared by coating a carbon layer on Ag NPs-Cu2O NWs, which enhanced the conductivity of the electrode while protecting the nanostructure using the carbon layer [21]. Branch-structured CuO-Co3O4 NWs were constructed on copper foam, and carbon film was coated on NWs to obtain a CuO-Co3O4@C NW-modified copper foam electrode, which further improved the efficiency of electroporation disinfection [30]. However, the use of precious metals, such as Ag, will bring more safety risks in the disinfection process, complex branch-structure construction and carbon layer loading processes will increase the difficulty and economic cost of the electrode preparation, while there are still problems such as hydrolysis during disinfection above 10 V [23,25,31]. These problems limit the practical application of electroporation disinfection technology. Therefore, it is important and necessary to find electroporation electrodes with simple preparation process, low material cost, and excellent disinfection performance to achieve efficient electroporation disinfection at low voltage.

As we all know, the efficiency of electroporation disinfection is closely related to the enhanced electric field generated from the nanotips under applied voltage, which may be determined by the size and morphology of the nanostructure. Therefore, morphology modulation of nanoarrays as a simple and effective means should be explored and applied to the optimization of electroporation electrodes. Up to now, there is few report on the effect of nanoarray size on the efficiency of electroporation disinfection. In this article, four types of ZnO nanoarrays (ZnO NRs) with different tip widths and lengths were prepared on copper foam electrodes. Efficiency for electroporation disinfection of ZnO NRs was realized by morphological modulation, size regulation, and the effect of electroporating disinfection under low voltage. The correlation between morphology of the nanoarray and efficiency of electroporation disinfection has been recognized, which can pave a way to the technology of electroporation disinfection.

2 Materials and methods

2.1 Chemicals and materials

Copper foams (pore size ~200 μm) were cut into 2 cm × 2 cm as electrodes (thickness 1 mm), which were supplied by Shanghai Macklin Biotechnology Co. (China). Chemicals including hexamethylenetetramine (HMTA, (CH2)6N4), zinc nitrate hexahydrate (Zn(NO3)2·6H2O), iron nitrate nonahydrate (Fe(NO3)3·9H2O), and zinc acetate dihydrate (Zn (CH3COO)2·2H2O) were obtained from Aladdin Co. Ltd. (Shanghai). Polyethyleneimine (PEI, Mw 600) was purchased from Sigma-Aldrich. Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 6538) were purchased from the Guang Dong Detection Center of Microbiology (China).
2.2 Fabrication of ZnO nanoarray-modified electrodes

ZnO NRs were prepared by a two-step hydrothermal method, and the fabrication process of the ZnO seed layer referred to the sol–gel method [32] (Figure 1a). The ZnO seed layer was pre-deposited on copper foam by dip-coating from a zinc acetate ethanol colloidal solution (5 mM) and then annealing at 400°C for 40 min. To obtain a certain density of the seed layer, the coating step was selected four times. Then, chemical bath deposition was used to grow ZnO NRs [33]. A piece of copper foam substrate with the ZnO seed layer was immersed into a hydrothermal growth solution containing 50 mM of Zn(NO$_3$)$_2$·6H$_2$O, 50 mM of HMTA, and PEI (1, 4, or 7 mM) at 95°C for 4 h. ZnO nano-prism, ZnO nano-prismoid, and ZnO nano-pyramid nanoarrays were prepared. ZnO nano-needle nanoarrays were grown by pre-oxidation (400°C, 20 min) of copper foam substrate before loading ZnO seed layer, and 7 mM PEI was added in the growth solution. After growth, all samples were washed with deionized water.

2.3 Materials characterization

The morphology of the ZnO NR-modified electrodes was characterized by a scanning microscope (SEM, COXEM EM-30). The crystal structure of the samples was tested.

![Figure 1: Electrode fabrication and construction of electroporation disinfection system. (a) Schematic illustration for the synthesis of ZnO NRs on copper foam electrodes. (b) Schematics showing the configuration of electroporation disinfection process.](image-url)
by X-ray diffraction (XRD, Empyrean), with CuKα radiation in the 2θ range of 5–85°.

2.4 Operation for water disinfection

Two model bacterial suspensions (E. coli [ATCC 25922] and S. aureus [ATCC 6538]) were selected and diluted with 0.9% NaCl solution to 10⁶ colony-forming units (CFU)/mL. Before testing, two prepared ZnO NR-modified electrodes were assembled in an electroporation-disinfecting device in parallel, and the thickness of each electrode is 1 mm. The electroporation-disinfecting device is shown in Figure S1. Considering that the working surface of electroporation is about 1 cm², flow rates were kept in the range of 5–20 mL/min, corresponding to contact times of 2.4–0.6 s.

The disinfection performance was investigated under different flow rates (corresponding to different contact times) and voltages. Among them, the flow rate was controlled by a peristaltic pump, and the voltage was controlled by a DC power supply. First, the electroporation-disinfecting device was connected to the water pipe controlled by the peristaltic pump, and the DC power supply was connected to the positive and negative copper wire. During testing, the electrodes were in the absence of an applied electric field and the presence of 1 and 2 V external voltages (the bacterial concentration is about 10⁶ CFU/mL and the flow rate is 10 mL/min, corresponding to contact time of 1.2 s). Contact time between bacteria and electrode ranged from 0.6 to 2.4 s, which was controlled by the flow rate in the electroporation-disinfecting device (the bacterial concentration is about 10⁶ CFU/mL, and the external voltage is 1 V). Colony counts were used to calculate bacterial removal efficiency. To ensure reproducibility, tests were conducted triple times for each group. The inactivated rate was calculated according to the following equation:

\[ E = \frac{C_1 - C_e}{C_1} \times 100\% , \]

where \( E \) represents the inactivated rate, \( C_1 \) is the microorganism concentration in the influent, and \( C_e \) is the microorganism concentration in the effluent (CFU/mL).

3 Results and discussion

3.1 Characterization of ZnO NR-modified electrodes

ZnO NRs on copper foam were fabricated by a two-step hydrothermal growth method. Among the four kinds of ZnO NRs on copper foam, ZnO nano-prisms, ZnO nano-prismoid, and ZnO nano-pyramids with similar density were obtained by introducing PEI to regulate the tip size of ZnO NRs. In addition, ZnO nano-needle with higher density was obtained by pre-oxidizing the copper foam substrate before growth. SEM images of four different tip widths of ZnO NRs are shown in Figure 2a–d. The PEI molecules tend to absorb on the crystalline surface of ZnO (100), increasing the relative c-axis growth rate, resulting in the formation of NR with uniform tip width [34]. Therefore, the tip width of ZnO NRs gradually decreased with the increase in PEI concentration. In addition, the surface roughness of copper foam increased after pre-oxidized, leading to an increase in the surface roughness of the subsequently prepared ZnO seed layer (Figure S2). The increased surface roughness of the ZnO seed layer led to an increase in the nucleation sites at the initial stage of ZnO NR growth, resulting in smaller tip width and length of nanoarrays [35].

The average value of tip width and length of the nanoarrays in the given area was measured using particle size statistics software, and the results are shown in Figure 3. The average tip width and length of ZnO nano-prism were 465 nm and 4.9 µm, respectively. The average tip width and length of ZnO nano-prismoid were 265 nm and 4.8 µm, respectively. The average tip width and length of ZnO nano-pyramid were 75 nm and 4.9 µm, respectively. The average size and length of ZnO nano-needle were 35 nm and 1.3 µm, respectively. The aspect ratio of nanoarrays was calculated in the order of ZnO nano-pyramid > ZnO nano-needle > ZnO nano-prismoid > ZnO nano-prism.

The XRD patterns of the ZnO NR are shown in Figure 4. It could be seen that the peaks of all four different tip size ZnO NRs are identical. When ZnO grew on the copper foam, the surface of the copper foam was oxidized to form a small amount of Cu₂O, so the diffraction peaks of Cu₂O and ZnO were weak compared with those of Cu. The strong peaks of Cu represented the original Cu substrate and could be designated as Cu (111), (200), and (220) planes (JCPDS card no. 04-0836). The Cu₂O (JCPDS Card no. 05-0667) peaks corresponding to (110), (111), (200), (220), and (311) located at 29.5, 36.4, 42.2, 61.3, and 73.5°, respectively. The diffraction peaks corresponding to (100), (002), (101), (102), (110), (103), and (112) at 31.6, 34.3, 36.2, 47.4, 56.5, 62.8, and 67.9°, respectively, were associated with the hexagonal ZnO (JCPDS Card no. 36-1451), confirming the growth of ZnO on the copper foam.

3.2 Performance for electroporation disinfection of ZnO nanoarrays

Applied voltage and flow rate were important parameters for evaluating the efficiency of electroporation disinfection.
The disinfection efficiency of the four kinds of ZnO NR electrodes under different voltages and flow rates against two model bacterial suspensions was studied. Figure 5 shows the disinfection results of four ZnO NRs under different voltages. It could be seen that the disinfecting effect of all the four samples was not satisfied without added voltage, and the sterilization effect comes from the physical sterilization effect of ZnO NR [33]. Under the condition of applied voltage, the disinfection efficiency of the four kinds of ZnO NRs all increased. ZnO nano-pyramid had the best disinfection effect, which can achieve over 99.9% disinfection efficiency against E. coli under an applied voltage of 1 V. Moreover, the disinfection efficiency of all four kinds of ZnO NR under 2 V was significantly higher than that under 1 V, indicating that the voltage had a large effect on the efficiency of electroporation disinfection. Compared with the intact and smooth membrane of untreated E. coli, treated E. coli had significant damage on the surface (Figure S3).

The voltage value of 1 V was selected for safety and low energy consumption, which is less than 10.5 × 10^-3 J/S, as seen in Table S1. Figure 6 shows the disinfection results of four kinds of ZnO NR electrodes under different contact times. Due to the increase in the treatment flow rate, the contact time between bacteria and electrodes decreased, and the disinfection results show a gradual increase in the number of colonies in the counted Petri dishes (Figure 6a). This was due to the fact that at low flow rates, the bacteria were in contact with the ZnO electrode for a longer time and had a bigger chance for electroporation. We noticed that both ZnO nano-pyramid and ZnO nano-prismoid electrodes could achieve over 99% disinfection efficiency at a contact time of 2.4 s, illustrating the importance of sufficient contact time between bacteria and electrodes for electroporation. 

Figure 2: SEM images of ZnO NRs hydrothermally grown on copper foam with PEI solution as (a) 1, (b) 4, and (c) 7 mM and (d) after pre-oxidation of copper foam, respectively.
disinfection. Meanwhile, the ZnO nano-pyramid had the best disinfection efficiency, which achieved 99.9% disinfection efficiency at a contact time of 1.2–2.4 s (flow rates 5–10 mL/min), maintained over 90% disinfection efficiency at a contact time of 0.6–0.8 s (flow rates 15–20 mL/min).

Figure 7 shows the disinfection results of all four kinds of ZnO NR electrodes against *E. coli* and *S. aureus*. It was obvious from the colony count results that the disinfection efficiency of ZnO NR electrodes against

**E. coli** was better than that against *S. aureus*. Again, ZnO nano-pyramid exhibited the best electroporation disinfection efficiency for both bacteria, achieving 99.9% for *E. coli* and 84.4% for *S. aureus* under flow rate 10 mL/min (contact time of 1.2 s) and applied voltage 1 V, indicating the superiority of this morphological nanoarray in electroporation disinfection. And we found that despite a small amount of detachment and inclination of the nanorods, the surface of the ZnO nano-pyramid electrode still maintained a good morphology (Figure S4).

We know that bacterial inactivation during electroporation of nanoelectrodes may be caused by a strong electric field and oxidative stress [20]. To explore the

![Figure 3: Topographical parameters of the nanoarray: tip width and length, determined by SEM (according to Nano Measurer).](image)

![Figure 4: XRD patterns of ZnO NRs with different morphologies.](image)

![Figure 5: Disinfection efficiency of ZnO nano-pyramid, ZnO nano-needle, ZnO nano-prismoid, and ZnO nano-prism electrodes at an applied voltage of 0–2 V and a flow rate of 10 mL/min (corresponding to contact time of 1.2 s), using 10⁶ CFU/mL *E. coli* solution in a saline environment. (a) Optical images and (b) efficiency for electroporation disinfection of four ZnO NRs under 0–2 V applied voltages.](image)
potential role of oxidative stress in bacterial inactivation, the bacteria were incubated with 10 mM glutathione (reduced form, GSH) as the influent microorganism to remove the contribution of oxidative stress to bacterial deactivation. The results show that, in this work, the disinfection efficiency is mainly attributed to the electroporation bactericidal effect caused by the strong electric field, and the oxidative stress caused by ROS is negligible (Figure S5). By comparing the disinfection performance of four kinds of ZnO NR electrodes, we found that the disinfection efficiency was in the order of ZnO nano-pyramid > ZnO nano-prismoid > ZnO nano-needle > ZnO nano-prism. Among them, the ZnO nano-pyramid with small tip width and the largest nanoarray length had the

Figure 6: Disinfection efficiency of ZnO nano-pyramid, ZnO nano-needle, ZnO nano-prismoid, and ZnO nano-prism electrodes at an applied voltage of 1 V and a contact time of 0.6–2.4 s (corresponding to the flow rate of 5–20 mL/min), using $10^6$ CFU/mL E. coli solution in a saline environment. (a) Optical images and (b) efficiency for electroporation disinfection of four ZnO NRs under 0.6–2.4 s contact time.

Figure 7: Disinfection efficiency of ZnO nano-pyramid, ZnO nano-needle, ZnO nano-prismoid, and ZnO nano-prism electrodes at an applied voltage of 1 V and a flow rate of 10 mL/min (corresponding to a contact time of 1.2 s), against $10^6$ CFU/mL E. coli and S. aureus solutions in a saline environment. (a) Optical images and (b) efficiency for electroporation disinfection of four ZnO NRs against E. coli and S. aureus under 1 V and a contact time of 1.2 s.
highest disinfection efficiency, which can achieve a disinfection efficiency of more than 99.9% for all $10^3$–$10^6$ CFU/mL of *E. coli* (Figure S6). Disinfection efficiency of ZnO nano-needle with minimal tip width was not satisfied. This may be due to the fact that, although the tip width of ZnO nano-needle was minimal, its array length was short and dense, and such a morphology was not conducive to the electric field enhancement of the nanotips. These results indicated that for electroporation disinfection, the size of nanooarray should be pursued not only for the small size of the tip width but also for the suitable length. In addition, the comparison of the ZnO nano-pyramid electrode and other previously reported electrodes for water disinfection is shown in Table S1, further confirming the superior performance of the ZnO nano-pyramid electrode (Table S2).

4 Conclusions

In summary, the most suitable size of ZnO nanoarrays for electroporation disinfection was obtained by investigating the performance for electroporation disinfection of four kinds of ZnO NRs with different tip widths and lengths, and a simple and efficient ZnO electroporation electrode was prepared. The results indicated that the ZnO nano-pyramid with small tip width and proper length exhibited over 99.9% disinfection efficiency against *E. coli* under a low voltage of 1 V and a flow rate of 10 mL/min (a contact time of 1.2 s). Here, the electroporation disinfection performance was mainly derived from the locally enhanced electric field of the nanotip, rather than from the ROS. Lastly, we concluded that the smaller tip width of nanoarray was beneficial for electroporation disinfection within a certain range, but the length of nanoarray was also important. A tip width of below 100 nm and a length of above 4 μm should be a more suitable size of nanoarray for electroporation disinfection. A study on the effect of nanooarray size on the efficiency of electroporation disinfection is important for the development of electroporation-disinfecting technology.

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References


