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Preparation of ZnO Array Nanoflowers and Their Antibacterial Action against Escherichia Coli and Staphylococcus Aureus

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***Abstract*—In order to develop a new antibacterial material for the treatment of bacterial infection, a ZnO array nanoflower (ZnO@NRF) was designed and synthesized by a simple and efficient hydrothermal method without template, seed or substrate. Through SEM test found that ZnO nanoflowers are composed of many well arranged ZnO nanorods with typical conical characteristics. Besides, ZnO@NRF can inhibit the growth of bacteria such as Escherichia coli and Staphylococcus. ZnO@NRF has a good killing performance for bacteria, which makes it a promising antibacterial nanomaterial in biomedical field.**

***Index Terms*- ZnO array nanoflowers, bacteria, Drug resistance, Antibacterial**

I INTRODUCTION

At present, bacterial infection has become the main problem faced by all countries in the world. Bacterial infection has been manifested as the main pathogenicity of high pathogenicity and high mortality. Bacterial infections can seriously damage human health, increase patient trauma and medical costs[1], [2]. Research shows that different species of bacteria use different techniques to approach and attach to surfaces, colonize them, grow and multiply, and eventually form biofilms. Mature biofilms are highly resistant to antibiotics and other chemical fungicides and are difficult to remove once formed.

The use of antibiotics has brought hope to infected people and saved millions of lives in the past decades. However, with the widespread abuse of antibiotics, multidrug resistant bacteria (MDR) have emerged, resulting in low or even ineffective antibacterial effect[3]-[5]. Many strategies have been proposed to solve severe drug-resistant bacterial infections, and the introduction of antibacterial materials includes cationic polymers[6], [7], antimicrobial peptides[8], [9] and quaternary ammonium compounds[10], [11]. However, their practical use is hampered by bacterial resistance and potential cytotoxicity, leading to adverse reactions such as inflammation. Therefore, biomaterials with better antibacterial properties are urgently needed.

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The development of novel antibacterial nanostructured surfaces shows promising applications in medicine as next-generation biomaterials. The advantage of mechanical bactericidal nanostructures over traditional chemical-based antibacterial agents is due to their physico-mechanical interactions with bacteria. These nanostructures and bacteria on the surface of the physical and mechanical interactions lead to bacteria to kill or prevent the bacteria and the subsequent biofilm formation, thus to avoid bacterial infections. Insect wings was the inspiration for this technology found on the surface of natural column array, it can be mechanically inactivate cells to effectively kill bacteria[12]-[16]. When bacteria attach to the nanolcolumnar material surface, adhesion forces acting on the bacterial membrane cause the membrane between adjacent nanolcolumnar to stretch, causing the cells to suspend. This suspension exerts enough pressure on the bacterial membrane that the membrane eventually ruptures, resulting in cell death. [17]

In this experiment, nano materials like flowers were synthesized by simple hydrothermal method. The successful synthesis of ZnO array nanoflower (ZnO@NRF) material was characterized by SEM. The antibacterial effect of nano materials on bacteria was evaluated by plate counting method.

II. EXPERIMENTAL AND METHODS

A Materials

Silicon wafer, Titanium wafer, Hexa-methylenetetramine (HMTA, $C_6H_{12}N_4$), Chemical Reagent Zinc Nitrate Hexahydrate ($Zn(NO_3)_2$ $6H_2O$), All aqueous solutions were prepared with sterile distilled water. Luria-Bertani (LB) broth and LB agar were purchased from BD Difco, USA.

B Synthesis of the ZnO@NRF

Due to the very low tendency of divalent metal ions to precipitate in aqueous solution by hydrolysis-condensation in neutral or acidic medium (compared to metal ions with higher oxidation state such as Fe^{3+} or Cr^{3+}), the synthesis was conducted by aqueous thermal decomposition of Zn^{2+} amino complex with reagent-grade chemicals. A regular stopped flask containing, for instance, a polycrystalline F-SnO_2 glass substrate, a Silicon wafer or Titanium wafer, a bare piece of glass or a conducting plastic substrate, and an equimolar (0.1 M) aqueous solution (MilliQ, 18.2 MΩ) of zinc nitrate, $Zn(NO_3)_2{\cdot}6H_2O$, and methenamine, $C_6H_{12}N_4$, is placed in a regular laboratory oven and heated

at 95 °C for 10 h, depending on the required microrod length. Subsequently, the thin films are thoroughly washed with water to remove any residual salt or amino complex and allowed to dry in air at room temperature.

C Characterization of the ZnO@NRF

Scanning electron microscopy (SEM, s-4800, Hitachi, Japan) was applied to observe the surface morphology and structure of the nanmaterials. Water contact angle instrument (DSA MK2, Germany) was performed to evaluate the hydrophilicity/hydrophobicity of the nanmaterial surface.

Table 1 Static contact angle measurement of uncoated and ZnO array nanoflowers-coated Silicon wafer surfaces

	Silicon wafer	ZnO@NRF-Silicon wafer
Contact angle	23.6 ± 1.0	27.8 ± 4.0

D Testing for antimicrobial activity of ZnO@NRF

With use of the so-called antibacterial drop-test, the bactericidal activities of all the samples were investigated against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) bacteria as Gram-negative and Gram-positive models, respectively. Before each microbiological experiment, all the samples and glassware were sterilized by autoclaving at 120 ℃ for 10 min. The bacteria were cultured on a nutrient agar plate at 37 ℃ for 24 h. Then, the cultured bacteria were added in 10 mL of saline solution to reach the concentration of bacteria of -10^8 colony forming units per milliliter (CFU/mL). A portion of the bacterial suspension was diluted to 10^6 CFU/mL. For the antibacterial drop-test, each sample was placed into a sterilized Petri dish. Then 100 µl of the diluted saline solution containing bacteria was spread on the surface of the sample. After incubation for 30 min, 60 min, 120 min with dark environment, the bacteria were washed from the surface of the sample using 5 ml phosphate buffer solution in the sterilized Petri dish. Then 10 µl of each bacteria suspension was spread on a nutrient agar plate and incubated at 37 ℃ for 14 h for counting the surviving bacterial colonies using an optical microscope. The reported data were the average values of three separate runs or the values showed the best fitting for an exponential reduction. The average dispersion of each data point was in the range of 45-55% of that point which is not so considerable in the analysis of a logarithmic diagram.

III RESULTS AND DISCUSSION

A. Morphology characterization of the ZnO@NRF

As shown in Fig 1, we tested Silicon wafer and Titanium wafer by SEM ZnO@NRF Structural pattern. From the figure, we can clearly see the successful synthesis of nano materials, which are uniformly attached to the surface of our substrate, and the nano patterns seen after local amplification show the structure of flowers, the petals are not all scattered, and there is a certain interval between each flower. In addition, the petals in each flower are similar to columns and have a certain structural similarity with nano arrays.

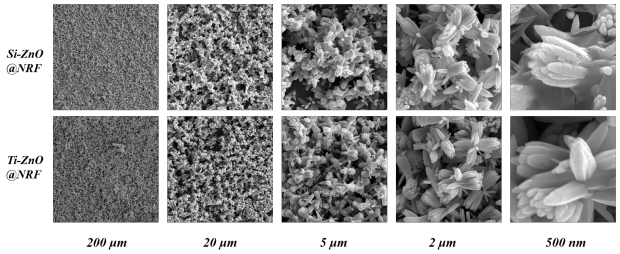

Fig 1. SEM images of ZnO@NRF of different sizes on silicon wafer and Ti wafer(The size range is 500nm-200µm)

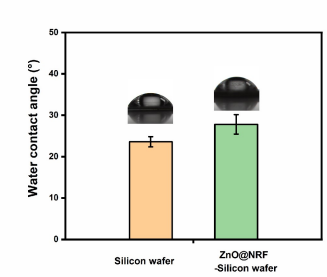

Fig 2. Water contact angle measurement of Control silicon wafer and ZnO@NRF on silicon wafer

B. Water contact angle characterization of the ZnO@NRF

The hydrophilicity of nanoflower materials was tested by SEM, as shown in Fig. 2. Besides，table 1 lists the contact angle data of blank Si sheet and nano materials. The contact angle of nano flowers is 27.8 ± 4.0, which is not different from that of blank Silicon wafer and has good hydrophilicity.

C. Growth principle of ZnO@NRF formation

In a simple hydrothermal method, ZnO grew and formed a three-dimensional flower structure. The zinc ion precursor first decomposes to form Zn^{2+} ions, and then reacts with OH^- ions to form Zn $(OH)_2$ refer to "(1) of Fig. 3,". This $Zn(OH)_2$ further reacts with OH^- to give $[Zn(OH)_4]^{2-}$ refer to "(2) of Fig. 3,". Then $[Zn\ (OH)_4]^{2-}$ was used as the precursor of ZnO crystal growth to form nano flower structure.

D. Antibacterial test

From the results of SEM, we can know that nanoflowers and nano array structures have similar rod structure. We speculate that nanoflowers also have certain anti-bacterial activity. Therefore, we designed and studied the anti-bacterial test of nanoflowers. As shown in Fig. 4 and Fig. 5, we use the method of coating plate to study the anti-bacterial test on the surface with nano flower structure pattern. From the results, we can know that for the control group of blank Silicon wafer, the number of bacteria increases with time, but the Silicon wafer with nano flowers have obvious antibacterial effect compared with the blank control.

$$Zn^{2+} + 2\,OH^- \longrightarrow Zn(OH)_2 \quad (1)$$

$$Zn(OH)_2 + 2\,OH^- \longrightarrow Zn(OH)_4^{2-} \quad (2)$$

$$Zn(OH)_4^{2-} \longrightarrow ZnO + H_2O + 2\,OH^- \quad (3)$$

Fig 3. ZnO@NRF Schematic diagram of chemical equation formed

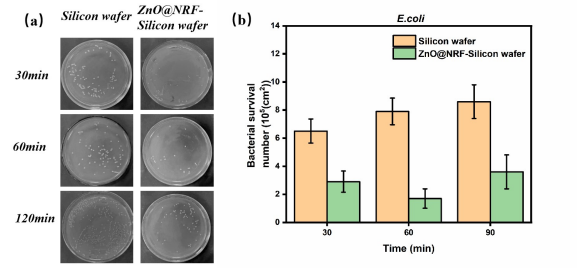

Fig 4. Photos of the bactericidal test on *E. coli*, the left figure (a) of the control silicon wafer is the picture of the coated plate, and the right figure (b) is the data statistical diagram

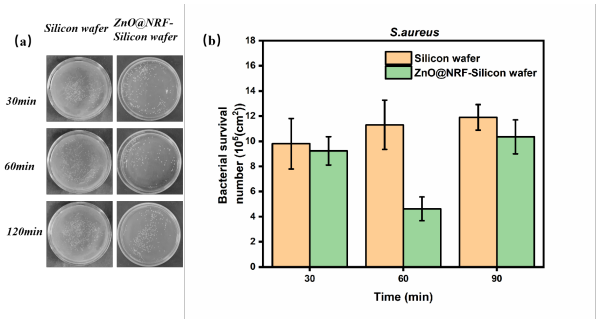

Fig 5. Photos of the bactericidal test on *S. aureus,* the left figure (a) of the control silicon wafer is the picture of the coated plate, and the right figure (b) is the data statistical diagram

When bacteria contact and adhere to the surface of nanoflower materials, the adhesion force acting on the bacterial membrane stretches the membrane between adjacent nanocolumnar materials, resulting in cell suspension. This suspension exerts enough pressure on the bacterial membrane, which eventually leads to the rupture of the bacterial membrane and cell death. The antibacterial activity of *E. coli* is stronger than that of *S. aureus*, which may be due to the rod-shaped *E. coli*, which can have more contact area with nano materials. In addition, among the anti-bacteria in different time periods, it has the best killing effect at 60 minutes. The antibacterial efficiency of *E. coli* and *S. aureus* bacteria are 78.48% and 59.12% respectively.

IV CONCLUSION

In this study, a simple hydrothermal method was successfully used to prepare ZnO@NRF. The morphology of the prepared nano materials was characterized by SEM, the hydrophilicity of the materials was also tested by contact angle, and finally the antibacterial activity of the nano materials against common bacteria was studied.

Nanoflower materials have not yet been used in practical applications. Although there are many problems to be considered, the project provides a new way to solve the problem of bacterial infection. Its advantage is that it will not produce drug resistance. In the future, more similar nanostructures can be used to resist bacterial infection.

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