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The chemodynamic antibacterial effect of MnO$_x$ nanosheets decorated silicon nanowire arrays

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Bacteria exist everywhere and are threatening human health. Reactive oxygen species (ROS), as a highly reactive chemical substance, can effectively oxidize and break lipids, DNA, protein and other bioactive molecules, leading to the death of bacteria. Modification of manganese oxide on the surface of silicon nanowire arrays deposited with polydopamine (SN@PDA@MnO$_x$) can significantly increase the yield of ROS in a short period of time, which provides SN@PDA@MnO$_x$ with a strong chemodynamic antibacterial effect. Both manganese ions and manganese oxides in the reaction system promote the generation of ROS, and their mechanisms of action can be synergistic with each other. The results show that SN@PDA@MnO$_x$ can achieve 99.99% antibacterial effect by using the ROS generated by chemodynamic reactions within 1 hour. SN@PDA@MnO$_x$ has great potential as a novel antibacterial material.

1. Introduction

Bacterium is one of the most abundant type of all organisms, widely distributed in soil and water, or symbiosis with other organisms. Bacteria are the pathogens of many diseases. They can spread diseases between humans in various ways, seriously threatening human life and health, and hindering the development of society. In the long struggle against bacteria, humans have already possessed a variety of weapons, such as the applications of physical methods (temperature, radiation, and electrical stimulation), chemical methods (antibiotics, metals, and metal oxides), and biological methods (bacteriophage, and biological control bacteria). However, physical methods are often limited by the lack of exogenous stimuli. Chemical methods may cause bacterial resistance and inevitable biological toxicity. Biological methods are difficult to be developed due to their low antibacterial efficiencies. Therefore, it is particularly important to develop new antibacterial materials as soon as possible to protect human health.

Reactive oxygen species (ROS) are one of the main reasons for the bactericidal effect of many natural substances. ROS are single-electron reduction product of oxygen. They have high chemical activity due to their structure containing unpaired electrons and can oxidize and break lipids, DNA, protein and other bioactive molecules. ROS are widely used in antibacterial application, tumor treatment, immune regulation, and other biomedical fields. ROS can be produced through the catalysis of metals and metal oxides, electron transfer in redox reactions, and normal mitochondrial metabolism, etc. Excitation conditions include chemodynamics, photodynamics, and acoustic dynamics. The generation of ROS by metal oxide nanosheets often requires external energy stimulation. Thomas et al. used the ROS generated on the AuPd catalyst in the process of synthesizing hydrogen peroxide from hydrogen and air to achieve efficient sterilization and disinfection, providing a new idea for water disinfection. Liu et al. developed a catecholchitosan film that could continuously produce ROS. This film can inhibit the growth of bacteria at the wound site in the presence of ascorbic acid, reduce inflammation and promote wound healing. Although these materials can produce ROS effectively, they must rely on a large amount of reducing agent to achieve excellent antibacterial performance, which limits their wide applications.

Manganese is a transition metal element abundant in nature. Manganese oxides (oxides, hydroxides and oxyhydroxides) produced by spontaneous oxidation have the advantages of low cost, simple preparation, and low biological toxicity. Because of its multiple valence states and multiple structural morphologies, and the rapid transfer of electrons and oxygen, manganese oxide is considered to be a potential catalyst. By interacting with various peroxides, manganese oxide can catalyze the production of ROS, and manganese oxide can functionalize the microgel with catechol and heme. The material uses heme to react with H$_2$O$_2$ to produce hydroxyl radicals with obvious antibacterial effects. However, due to the limited autooxidation of dopamine, the generation of H$_2$O$_2$ is still in a low level. Therefore, the overall production...
efficiency of ROS is unsatisfied, which restricts the sterilization effect of related materials.

In order to solve these problems, based on the efficient promotion of manganese oxide on the generation of ROS, we modified the silicon nanowire arrays deposited with polydopamine in-situ reduced manganese oxide nanosheets (SN@PDA@MnO₂). Silicon nanowires were good candidates for energy conversion and were selected as substrates because of their good biocompatibility. The silicon nanowire arrays have strong support for the adhesion of dopamine and dispersion for manganese oxide nanomaterials, which is beneficial to increase the specific surface area. Polydopamine was widely used as a material with excellent biocompatibility and degradability. The excepted adhesion of polydopamine allows the nanomaterials to adhere closely to the substrate, ensuring the stability of the material[39]. At the same time, the reducibility of polydopamine due to its own structure also provided convenience for the production of manganese oxide[40,41]. SN@PDA@MnO₂ uses chemodynamic, only in the presence of water and oxygen without adding external stimuli, pathway to significantly generate the amount of ROS in a short period of time. It can release high concentration of ROS equivalent to more than 115 mM H₂O₂ within 2 h, which can achieve 99.99 % antibacterial effect, greatly improving the antibacterial efficiency.

2. Experimental

2.1. Materials

Silicon wafers were from Guangzhou Institute of Semiconductor Materials (Guangzhou, China), and the specific model is (1,0,0)-oriented, 0.56 mm thickness, 100 mm diameter, n-dopes, one side polished. Tris(hydroxymethyl)-aminomethane hydrochloride (Tris-HCl) were bought from Beijing Solarbio Science & Technology (China). Dopamine hydrochloride (DA), hydrofluoric acid (HF), nitric acid (HNO₃), hydrochloric acid (HCl), silver nitrate (AgNO₃), acetone, potassium permanganate (KMnO₄), hydrogen peroxide (H₂O₂), manganese dichloride tetrahydrate (MnCl₂·4H₂O), and manganese dioxide (MnO₂) were purchased from Sinopharm Chemical Reagent (China). Bacterial culture medium (tryptone, yeast extract and agar) were from Oxoid (England). The ultrapure water was minimum resistivity of 18.2 MΩ cm.

2.2. Fabrication of silicon nanowire arrays (SN)

The pre-cut silicon wafers (0.5 cm×0.5 cm) were ultrasonically cleaned alternately with ultrapure water and acetone for 3 times, each for 2 min. Prepare the reaction solution (5M HF, 0.05 M AgNO₃) in a polytetrafluoroethylene container soaked in aqua regia (HCl: HNO₃=3:1) for more than 4 h, and it was preheated in an oven at 50°C for 5 min. The silicon wafer was place evenly in the polytetrafluoroethylene reaction dish, and the preheated reaction solution slowly was poured into the reaction dish to completely immerse the silicon wafer. After reacting at 50°C for 15 min, the reaction solution was sucked out, and 20% HNO₃ was added to terminate the reaction. When there are no more bubbles on the surface of the silicon wafer, the solution was sucked out, and another 20% HNO₃ was added to clean it. After being washed with ultrapure water, the wafer was put in an oven at 50°C for drying.

2.3. Depositing polydopamine on the surface of silicon nanowire arrays (SN@PDA)

Weigh 20 mg of dopamine hydrochloride and dissolve it in 10 ml Tris-HCl solution (10 mM, pH=8.5), and the silicon nanowire arrays were quickly immersed in the reaction solution for 3 h. After the reaction is finished, SN@PDA was washed three times with ultrapure water, then dried naturally at room temperature.

2.4. Surface modification of polydopamine-deposited silicon nanowire arrays with MnO₂ (SN@PDA@MnO₂)

The polydopamine-deposited silicon nanowire arrays were put into the prepared KMnO₄ solution (10 μg/ml), and reacted for 12 h. Then SN@PDA@MnO₂ was rinsed with ultrapure water, and dried at room temperature.

2.5. Modified MnO₂ on the surface of silicon nanowire array after reduction treatment (SN@MnO₂)

After immersing the etched silicon nanowires in HF solution (5%, V/V) for 5 min, the reduced silicon nanowire arrays were cleaned with ultrapure water. The processed SN was put into the prepared KMnO₄ solution (10 μg/ml) and reacted for 12 h. Then SN@MnO₂ was taken out and rinsed with ultrapure water. Finally it was dried at room temperature.

2.6. Characterization of material structure

SEM (FESEM, S4700, Hitachi, Japan) is used to characterize the morphology of the material surface, and XPS (EscaLab 250Xi, Thermo Scientific, USA) is used to qualitatively, quantitatively and chemically analyze the chemical composition of the material surface.

2.7. The release of reactive oxygen species (ROS) of SN, SN@PDA, SN@PDA@MnO₂ (converted to H₂O₂ amount)

Non-fluorescent DCFH can be oxidized by ROS to produce fluorescent DCF, and the concentration of the generated ROS can be analyzed by detecting the intensity of the fluorescence signal[42]. As a kind of ROS, hydrogen peroxide is often used to quantify the concentration of ROS produced by materials due to its relatively stable chemical properties.

2.7.1. Standard curve of H₂O₂

H₂O₂ was diluted into different concentrations. Equal volumes of H₂O₂ solution and active oxygen detection reagent (DCFH-DA, Beyotime, China) were mixed well, and incubated at 37°C for 30 min. The fluorescence of the mixture was detected at 488 nm (excitation) and 525 nm (emission) with a microplate reader (Varioskan Flash, Thermo Fisher Scientific, USA).

2.7.2. ROS detection
Mix the material with 700 μl of a 1000-fold diluted reactive oxygen species detection reagent. After a period of incubation at 37 °C, three parallel samples are taken to test the fluorescence values at EX488/EM525 with a microplate reader (Varioskan Flash, Thermo Fisher Scientific, USA). Concentrations of ROS released from different samples were calculated from the corresponding fluorescence values.

2.8. Release of manganese ions from SN@PDA@MnOx

SN@PDA@MnOx was soaked in 500 μl PBS for 0.5 h, 1 h, and 2 h respectively, and the solution was diluted to 5 ml with a volume fraction of 2% hydrochloric acid solution. The manganese ion was measured by atomic absorption spectroscopy (AA240FS+GTA120, Varian, USA).

2.9. Antibacterial test

E. coli strain (E. coli MG1655) was inoculated into the liquid medium with a pipette tip, and cultivated overnight in a constant temperature (37 °C) shaking incubator. After centrifugation, the supernatant was discarded, and PBS was added into it. The centrifugation was repeated twice. Then the material was put into an OD600 value of 0.01, and dropped on the surface of the material. Then the material was put into an OD600 value of 0.01, and dropped on the surface of the material. After the incubation, the bacteria were diluted, and the killing efficiency for the antibacterial effect of the material was calculated by the colony forming unit (CFU) with the equation as:

\[
\text{Killing Efficiency} (\%) = \left(1 - \frac{\text{CFU}_{\text{experimental\ group}}}{\text{CFU}_{\text{control\ group}}}\right) \times 100\%
\]

2.10. Observation of the morphology of bacteria

Drop 100 μl of bacterial solution (OD600=0.01) on SN@PDA@MnOx, and incubate it at 37 °C for 2 h. Add 400 μl of 2.5% glutaraldehyde solution and stand for 1.5 h. Then all the liquid was sucked out, and different concentrations of ethanol (30%-100%) were added step by step according to the concentration from low to high. The morphological changes of bacteria after being treated were observed by SN@PDA@MnOx with FESEM (FESEM, S4700, Hitachi, Japan).

3. Results and discussion

3.1. Characterization of SN@PDA@MnOx

The preparation process of SN@PDA@MnOx is shown in Fig. 1. First, we fabricated silicon nanowire arrays (SN) by chemical etching. The cross-sectional analysis of SEM shows that the silicon nanowires are uniformly and vertically distributed (Fig. 2a). Dopamine uses its excellent adhesion to form polydopamine to be fixed and attached to the surface of silicon nanowires. At the same time, it can also use its own reducibility to reduce KMnO4 to MnOx and bond to silicon nanowires. According to Fig. 2b, a large number of MnOx nanosheet structures appear on the surface of the silicon nanowires. In order to know the chemical properties of the achieved material, SN@PDA@MnOx was analyzed by XPS (Fig. 2c, 2d). The result showed that MN@PDA@MnOx has two specific peaks at 642.4 eV and 654.1 eV, which correspond to Mn2p3/2 and Mn2p1/2 respectively. The energy difference between the two peaks is 11.7 eV, indicating that Mn4+ in MnOx is the main oxidation state of Mn. MnO3 can induce the production of Mn2p3/2 and Mn2p1/2, respectively. The energy difference between the two peaks is 11.7 eV, indicating that Mn4+ in MnOx is the main oxidation state of Mn. MnO3 can induce the production of Mn2p3/2 and Mn2p1/2, respectively.
ROS, increase the activity of ATPase and cause the leakage of electrolyte and protein content, leading to the death of bacteria. 

3.2. Antibacterial effect of SN@PDA@MnOₓ

The antibacterial properties of SN, SN@PDA, and SN@PDA@MnOₓ were tested by the colony formation assay.

![Diagram](https://example.com/diagram.png)

Fig. 2 (a) SEM image of SN (cross section). (b) SEM image of SN@PDA@MnOₓ (cross section). (c) XPS full spectrum of SN@PDA@MnOₓ. (d) XPS narrow scan of SN@PDA@MnOₓ for Mn.

![Diagram](https://example.com/diagram.png)

Fig. 3 Antibacterial effect of SN, SN@PDA and SN@PDA@MnOₓ. (a) Colony formation (agar plate) of E. coli treated by different nanomaterials. (b) Colony formation unit of E. coli. (c) Colony formation (agar plate) of S. aureus treated by different nanomaterials. (d) Colony formation unit of S. aureus. Data were expressed as mean ± SD, n=3, ***p<0.001, SN(b) and 0 h(d) were used as a control group for significant difference analysis.
(Fig. 3a, 3b). After the bacteria were incubated on the SN for 0.5 h, 1 h and 2 h, the number of bacteria did not decrease significantly, indicating that SN has no inhibitory effect on the growth of bacteria. When SN is modified by PDA, it could be found that the number of bacteria on SN@PDA decreased to a certain extent, with an antibacterial rate of less than 10%. However, SN@PDA@MnO₂ showed a very strong antibacterial effect. After 0.5 h of incubation, the number of bacteria can be reduced by 70%. And almost 99.99% of the antibacterial effect could be achieved when the incubation time was prolonged to 20%. After more than 1 h of incubation, the antibacterial effect of 99.99% can be achieved. These results show that SN@PDA@MnO₂ itself can achieve excellent antibacterial effects against Gram-negative bacteria and Gram-positive bacteria, independent of the addition of any reducing agent.

It can be seen from the above results that the presence of MnO₂ may be the main reason for the enhanced antibacterial effect of the material. In order to verify the role of the SN and PDA components in SN@PDA@MnO₂ in the antibacterial process, we compared their antibacterial performance differences with Si@PDA@MnO₂ and SN@MnO₂. According to Fig. 4a and 4b, there was no significant difference between the number of bacteria after 2 h incubation on the silicon chip and the number of bacteria after 0.5 h incubation, only a reduction of less than 10%. But after the bacteria were incubated on Si@PDA@MnO₂ for 0.5 h, 1 h and 2 h, the number of bacteria decreased only slightly, and the antibacterial rate was about 10%. The huge difference in antibacterial effect with SN@PDA@MnO₂ highlights the advantages of SN in the system. The silicon nanowire array can deposit more dopamine due to its huge specific surface area, so that it can load more MnO₂ nanosheets and promote the antibacterial effect (Fig.S1). According to Fig. 4c and 4d, after the bacteria were incubated on SN@MnO₂ for a period of time, the number of bacteria decreased to a certain extent, and the antibacterial rate was calculated to be about 15%. Compared with the 99.99% antibacterial effect of SN@PDA@MnO₂, the antibacterial performance of SN@MnO₂ is greatly reduced due to the lack of PDA. The adhesion of polydopamine makes it form a thin film on the surface of the silicon nanowire array, which is conducive to the dispersion of MnO₂ nanosheets. At the same time, the H₂O₂ produced by polydopamine in the self-circulating redox process might also be one of the key reasons that SN@PDA@MnO₂ can produce ROS.

3.3. the generation of Reactive oxygen species (ROS) by SN@PDA@MnO₂

ROS is a one-electron reduction product of oxygen, including one-electron reduction product of oxygen superoxide anion (O₂⁻), two-electron reduction product hydrogen peroxide (H₂O₂), three-electron reduction product hydroxyl radical (·OH), etc. ROS has high reactivity and short lifespan, which can cause damage to biological molecules such as bacterial cell walls, protein peptide chains and nucleic acids, and ultimately produce an antibacterial effect. After SN and SN@PDA were

Fig.4 Antibacterial effect of Si@PDA@MnO₂, SN@MnO₂ and SN@PDA@MnO₂. (a) Colony formation (agar plate) of E. coli on Si and Si@PDA@MnO₂. (b) Colony formation unit of E. coli on Si, Si@PDA@MnO₂ and SN@PDA@MnO₂. (c) Colony formation (agar plate) of E. coli on control and SN@MnO₂. (d) Colony formation unit of E. coli on control, SN@MnO₂, and SN@PDA@MnO₂. Data were expressed as mean ± SD, n=3, **p<0.05, ***p<0.001, Si (b) and control (d) were used as a control group for significant difference analysis.
incubated in PBS for a period of time, the concentration of ROS generated was about 2 mM (Fig. 5a). There is little difference between SN and SN@PDA in the amount of ROS released. However, SN@PDA@MnO$_X$ had a strong activity in generating ROS through its specific chemodynamic property. It was found that ROS produced by SN@PDA@MnO$_X$ was 7 mM after just 0.5 h incubation in PBS (Fig. 5a), which was higher than that produced by the catechol reaction for 50 h. And surprisingly, the concentrations of ROS generated by SN@PDA@MnO$_X$ increased significantly with time, reaching 115 mM within 2 h. This result corresponds to the enhancement of the antibacterial effect with the prolonged reaction time with the bacteria, indicating that the antibacterial performance of SN@PDA@MnO$_X$ might be directly related to the continuous production of ROS through chemical reactions catalyzed by MnO$_X$ sheets.

Since MnO$_2$ was the important component in MnO$_X$ sheets on SN@PDA@MnO$_X$ and Mn$^{2+}$ could be released from MnO$_X$ materials$^{46,47}$, Mn$^{2+}$ and MnO$_2$ might be the key responsible for the production of ROS. In order to explore the influence of different components in the system on the generation of ROS, we added Mn$^{2+}$ and MnO$_2$ to the PBS solution soaked with SN@PDA. It can be seen from Fig. 5b that the ROS released by both SN@PDA and Mn$^{2+}$ treated SN@PDA were less than 1 mM even after 2 h. While the addition of MnO$_2$ could effectively increase the production of ROS, so that the production of reactive oxygen species could reach 21 mM within 2 h. When SN@PDA was treated by both Mn$^{2+}$ and MnO$_2$, it could generate the concentration of active oxygen to as high as 37 mM. Generally, manganese oxides such as manganese dioxide achieve catalysis through the mechanism of adsorption of oxygen and the mechanism of lattice oxygen$^{48,49}$. Manganese ions achieve catalysis through the formation of complexes and other intermediates$^{50,51}$. Therefore, Mn$^{2+}$ and MnO$_2$ might be synergistic with each other in promoting ROS generation on SN@PDA, which can effectively increase the catalytic efficiency of manganese oxide is higher.

Transition metal ions are important for the formation of ROS. The ability of these ions to remove electrons is the basis for the formation and expansion of many highly toxic ROS reactions. Because of the presence of polydopamine, manganese oxide can be reduced and dissolved in the solution, thereby leaching trace manganese ions to catalyze the production of ROS. Actually, the release of manganese ions was observed from SN@PDA@MnO$_X$ (Fig. 5c). The result showed that there was about 0.3 μg of manganese ions released from a chip of SN@PDA@MnO$_X$ (0.5 cm×0.5 cm) in 0.5 h. With the prolongation of the immersion time in PBS, more manganese ions released from the material. However, even after 2 h, the amount of manganese ions released from SN@PDA@MnO$_X$ was just as low as around 0.7 μg. It may be due to both the oxidation reaction of polydopamine and the reduction process of manganese oxide in the solution were slow, leading to a low amount of manganese ions released.
Considering that the antibacterial effects of metal ions (such as Ag⁺) might come from the disturbance of bacterial metabolism, the effect of Mn²⁺ in inhibiting bacterial growth was investigated. It was observed that the killing efficiency of Mn²⁺ in the amount of 0.1-1μg was only about 10 %. This result further proved that manganese ions were not the main reason of the antibacterial effect of SN@PDA@MnO₂. However, the addition of manganese ions will inhibit the activity of enzymes in the microorganisms, resulting in a decrease in the activity of microorganisms, which may be favorable for killing bacteria.

3.4. Research on the mechanism of bacterial death

E. coli is a gram-negative bacterium. Under normal conditions, both ends of E. coli are blunt round and full in shape (Fig. 6c). ROS can enter the bacteria and react with biological macromolecules, such as protein, lipid and DNA, etc. They will destroy the antioxidant capacity of the intracellular protective enzymes, and cause changes in the permeability of the bacterial cell membrane, leading to the death of the bacteria. It can be seen from Fig. 6a and 6b that the bacteria have a very low tolerance to hydrogen peroxide, and 15 mM hydrogen peroxide can achieve a 99.99% killing efficiency of E. coli. The concentration of ROS released by SN@PDA@MnO₂ catalyzed by chemodynamic action is calculated by the equivalent of hydrogen peroxide, which was 115 mM, far exceeds this value. Therefore, the generation of ROS might be the main reason for the excellent sterilization performance of SN@PDA@MnO₂. Such high concentrations of ROS released from SN@PDA@MnO₂ had a significant effect on changing the morphology of the bacteria. From the SEM image (Fig. 6d), it can be found that E. coli was obviously shrunk, indicating that the permeability of the bacterial cell membrane has changed, which also confirms that SN@PDA@MnO₂ achieves the antibacterial effect through ROS.

Conclusions

In the present study, the specific adhesion property and weak reducibility of polydopamine (PDA) were used to modify manganese oxide (MnO₂) on the surface of the silicon nanowire arrays (SN) to prepare SN@PDA@MnO₂. SN@PDA@MnO₂ has excellent antibacterial effect, which reaches 99.99 % killing efficiency in 1 h treatment. Mn²⁺ and MnO₂ from SN@PDA@MnO₂ have a synergistic effect in generating ROS through chemodynamic reaction. The concentration of ROS increased to as high as 115 mM, which leading to bacterial death. These results demonstrate that SN@PDA@MnO₂ has great potential in antibacterial application.

Conflicts of interest

There are no conflicts to declare.

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