Bioinspired nanostructured spiderweb for high-efficiency capturing and killing of bacteria

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ABSTRACT Antibacterial nanoagents with broad-spectrum antibacterial activities have emerged as an evolution of antibiotics. However, their bacteria-capturing capability and bacteria-killing efficiency remain insufficient for commercial implementation. Inspired by the hunting behavior of spiders, here, a novel spiderweb-like nanoagent based on silver-adenine nanowires is designed to achieve high-efficiency capture and killing of bacteria. By virtue of the initiative bacteria-capturing functionality, this spiderweb-like nanoagent could effectively attract and trap bacteria through electrostatic interaction and its reticular morphology feature. Furthermore, its synergetic antibacterial mechanism combining physical membranolysis with reactive oxygen species (ROS) release allows such nanoagent to harvest a promoted bactericidal activity. Importantly, the woundplast employing the spiderweb-like nanoagent exhibits a superior antibacterial efficacy against drug-resistant Gram-negative *Escherichia coli* and Gram-positive methicillin-resistant *Staphylococcus aureus* compared with commercially available woundplasts. This work may pave a new way for rational design of new generation bactericidal agents facing the future super-bacteria booming.

Keywords: bioinspired nanoagent, antibacterial activity, bacteria capturing, nanostructured spiderweb

INTRODUCTION

The infection issues caused by pathogenic bacteria, causing hundred thousand of deaths worldwide annually, have attracted increasing public attention as a medical challenge [1–5]. Up to date, treatment paradigms rely heavily on antibiotics in most instances of daily healthcare and clinic therapy [6–8]. Unfortunately, as the result of long-term abuse and overuse of antibiotics, the emergence of the multidrug-resistant bacteria decreases the therapeutic efficacy of the conventional antibiotics or even makes them useless under many circumstances [9,10,11,12]. Therefore, novel potent antibacterial materials are highly desirable to reduce the extensive uses of antibiotics [13,14].

Antibacterial nanoagents have emerged as an evolution of antibiotics with broad-spectrum antibacterial activities and are

expected to take the place of antibiotics these years [15,16]. However, their bacteria-capturing capability and bacteria-killing efficiency remain insufficient for clinical implementation [17–21]. Typically, nanoagents are presumed to perform the antibacterial effect *via* two dominant mechanisms: the first involves generating strong interactions with bacteria to disrupt the cell membrane [22–24] and the second involves producing poison species, such as reactive oxygen species (ROS), to disturb the cell metabolism [1,6,25,26]. Nevertheless, not only the inadequate probability of contact between nanoagents and bacteria causes difficulty to exert the abundant potency, but also the bacterial movement makes nanoagents be not able to act on the pathogens persistently, ultimately resulting in a moderate antimicrobial effect. On the other hand, the inherent drawbacks of poison species, such as short lifespan and limited diffusion distance of ROS, usually lead to an unsatisfactory infection eradication [20,27,28]. Thus, high-efficiency capturing of bacteria to shorten their acting distance and enhance the contact efficiency are pivotal prerequisite to improve bactericidal activity of nanoagents [29].

Web-weaving spiders, neither with wings nor agile mobility, provide a fascinating example of hunting insects *via* spiderweb by which they can effectively capture flying insects and kill them with venom quickly. Inspired by this hunting behavior, an efficient antibacterial nanoagent could be designed by integrating both “spiderweb” and “venom” functional moieties. Here, we demonstrate the feasibility of a novel spiderweb-like nanoagent based on silver-adenine nanowires as a high-efficiency bactericide. Up to date, silver (Ag) and its derivatives are the most commonly used bactericidal nanoagents and have been approved as additives in many commodities [30–35]. However, despite the wide spread, the application of Ag-based antibacterial materials remains a controversial subject due to their insufficient potency and the potential toxicity of released Ag⁺ ions towards mammal cells [19,31,36,37]. Here, to fabricate Ag-based spiderweb-like antibacterial nanoagents, the biocompatible adenine is introduced as the linker. Adenine, one kind of nucleotides containing several nitrogen (N) sites in the molecular structure [38], has been reported to present strong coordination effect towards Ag⁺ ions and thus could facilitate the formation of a crosslinked spiderweb-like structure [39]. Moreover, the strong coordination between adenine and Ag⁺

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could lead to a remarkable retardation effect towards Ag^+ ion release, and thus diminish the toxicity towards normal tissues. Additionally, in the physiological environment, the N sites tend to protonate and thus endow the nanomaterial with a positive surface charge, which would provide additional “venom” potency besides Ag^+ via the electrostatic adsorption towards negative-charged bacteria and a membrane-lytic antibacterial process [21,22,40,41]. In this study, the as-designed spiderweb-like antibacterial nanoagents (Ag-adenine nano-spiderwebs (Ag-A NSWs)) present excellent capturing and killing efficacy towards both Gram-positive and Gram-negative bacteria. Excitingly, Ag-A NSWs also present higher potency towards resistant bacteria species compared with common antibiotics. Moreover, wound healing experiment results confirm that the woundplast employing Ag-A NSWs exhibits outstanding performance in killing bacteria, eliminating inflammation and promoting wound healing.

EXPERIMENTAL SECTION

Materials

Adenine was purchased from Aladdin Reagent. Silver nitrate (AgNO_3 , $\geq 99.0\%$) was purchased from Sigma-Aldrich. Terephthalic acid (TA) was purchased from Beijing Chemicals. 2,7-Dichlorodi-hydrofluorescein diacetate (DCFH-DA) was purchased from Beyotime Inst. Biotech. LIVE/DEAD[®] BacLight[™] Bacterial Viability Kits (SYTO 9 & propidium iodide (PI)) were obtained from Invitrogen Detection Technologies. All the reagents were used without further purification.

Synthesis of Ag-A NSWs

Adenine (1 mmol) was dissolved in *N,N*-dimethylformamide (DMF) (80 mL) in a 250-mL round-bottom flask, followed by heating at 140°C to form a transparent solution. Then, AgNO_3 (1 mmol) was added into DMF to form a 20 mL solution avoiding light, and the above solution was mixed under 1000 r min^{-1} stirring. The obtained solution was stirred at 140°C for 2 h without light irradiation. After cooling down to room temperature, the Ag-A NSWs were collected by centrifugation, and then washed with water and ethanol for several times. Finally, the samples were dried at 80°C overnight.

·OH detection assay

TA was selected to indicate the generation of ·OH. Five groups of samples including TA (0.5 mmol L^{-1}), Ag-A NSWs (0.0625 mg mL^{-1}) + TA, Ag-A NSWs (0.125 mg mL^{-1}) + TA, and Ag-A NSWs (0.25 mg mL^{-1}) + TA, Ag-A NSWs (0.5 mg mL^{-1}) + TA were investigated. All groups were incubated at room temperature for 2 h. Whereafter, the fluorescence of the samples was measured by using 312 nm as excitation wavelength, and the emission at a wavelength of 425 nm is related with ·OH production.

Preparation of bacterial samples for SEM

The bacteria-nanospiderweb samples were washed with phosphate buffer saline (PBS), fixed with 2.5% glutaraldehyde for 1 h, and then washed with PBS for three times, followed by dehydration with sequential treatment of 25%, 50%, 75% and 100% $\text{C}_2\text{H}_5\text{OH}$ for 5 min. Finally, a drop of bacterial suspension was put onto the silicon wafer and observed by scanning electron microscope (SEM).

Evaluation of Ag divulgation in PBS

The Ag-A NSWs (2 mL) solution with a concentration of $100\text{ }\mu\text{g mL}^{-1}$ was added in a dialysis tube (molecular weight cut-off: 1500), which was then placed in 400 mL of PBS under stirring. One milliliter of PBS solution was sampled and supplemented with the same volume of fresh PBS daily for 24 h. The concentration of Ag was measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES).

Live/dead fluorescent staining

Both methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* (*E. coli*) bacterial cells were firstly treated with Ag-A NSWs for 1 h. Afterwards, SYTO 9 and PI were added to incubate for 15 min at room temperature in the dark, and subsequently washed with PBS. Finally, the live/dead bacterial cells were then observed by confocal laser scanning microscope (CLSM).

Determination of ROS generation in cells

The intracellular ROS generation was monitored by using DCFH-DA. *E. coli* cells were firstly cultured overnight. Afterwards, Ag-A NSWs (1 mg mL^{-1}) and DCFH-DA ($10\text{ }\mu\text{mol L}^{-1}$) were added to incubate for 30 min, while the unloaded probe was subsequently washed by PBS. Finally, the generation of intracellular ROS was observed by CLSM.

Evaluation of antibacterial activity against drug-resistant bacteria

The spread plate method was employed to evaluate the antibacterial activity of Ag-A NSWs compared with chloramphenicol. After treatment with the same concentration (0.05 mg mL^{-1}) of the proposed material and chloramphenicol as well as PBS, respectively, the *E. coli* K-12 strains were cultured on agar plate for 24 h at 37°C .

Woundplasts model antibacterial experiments

The bacteria were diluted to $\sim 10^6\text{ CFU mL}^{-1}$ and then inoculated on each group of woundplast cotton and treated for 6 h at 37°C . After treatment, the woundplast cotton was cultured in 3 mL of Luria-Bertani (LB) medium under shaking at 180 r min^{-1} and 37°C over 18 h, and 50 μL of the obtained solution was added onto the agar plate and cultured for 24 h at 37°C , respectively.

In vivo treatment of MRSA-infected wounds

Firstly, the mice (ICR, 6–8 weeks, 18–22 g) were slashed for a $\sim 12\text{ mm}^2$ ($d = 4\text{ mm}$) wound on the back and injected with $1 \times 10^8\text{ CFU mL}^{-1}$ of MRSA bacteria. The mice with bacteria-infected wounds were separated and treated by different groups of woundplast containing the proposed model and three commercial woundplasts for seven days, respectively. Meanwhile, clean cotton was used as the control group. The wounds were observed and photographed, and the woundplasts were replaced every day. All the animal experiments were conducted in agreement with the guidelines of the Animal Study Committee of Changchun Institute of Applied Chemistry, Chinese Academy of Sciences. All efforts were made to minimize suffering of the mice.

Evaluation of Ag divulgation on the wound tissue

After seven days of therapy, the mice in different groups were sacrificed and the wound tissues were harvested. The sample was decomposed, and then analyzed by the ICP.

RESULTS AND DISCUSSION

Synthesis and characterization of Ag-A NSWs

As illustrated in Scheme 1, the designed antibacterial material was fabricated by one-pot complexing of adenine and AgNO_3 in DMF. Transmission electron microscopy (TEM) images showed that the as-synthesized product was composed of cross-linked nanofibers with a diameter of 5–10 nm (Fig. 1a, b). The formation of cross-linked structure could be attributed to the coordination interaction between Ag^+ ions and adenine that provided several nitrogen sites for the coordination effect [39]. Additionally, in the physiological environment, the N sites tended to protonate and thus endowed the nanomaterial with a positive surface charge, which would promote the electrostatic adsorption towards negative-charged bacteria and a membrane-lytic antibacterial process [22,40,41]. ζ -potential result presented that Ag-A NSWs displayed a positive charge of +30.1 mV in accordance with the above prediction (Fig. 1d). As this tangled structure was similar to that of spiderweb, the generated product

was termed as Ag-A NSW in the rest text. Then, the Ag-A NSW was carefully characterized by element mapping, Fourier transform infrared spectra (FT-IR), X-ray photoelectron spectra (XPS) and ICP-OES (Fig. 1e, f and Figs S1–S4, see details in the Supplementary information). As measured with ICP-OES, the mass ratio of Ag contents in the Ag-A NSWs was 17.39 wt%. The obvious elemental distribution of Ag, C and N on the mapping image verified the successful synthesis of coordination nanoweb (Fig. 1c).

Antibacterial performance of Ag-A NSWs

To evaluate the antibacterial performance of Ag-A NSWs, a minimum inhibitory concentration (MIC) assay was conducted towards *E. coli* with a double broth dilution method. As shown in Fig. S5, Ag-A NSWs presented a low MIC value in the range of 5–10 $\mu\text{g mL}^{-1}$, which was comparable to that of reported Ag nanoparticles (diameter: 7, 29, and 89 nm) [34]. Moreover, a significant decrease of optical density at 600 nm (OD_{600}) was found when the bacteria cells were treated with Ag-A NSWs for

Scheme 1 Schematic illustration for construction of the Ag-A NSW antibacterial system inspired by the spiders and its capturing and killing properties for bacteria.

Figure 1 (a) TEM image of a single Ag-A NW. Scale bar: 100 nm. (b) TEM image of Ag-A NSWs. Scale bar: 200 nm. (c) High-angle annular dark-field scanning TEM image and the corresponding energy dispersive X-ray spectroscopy (EDS) mapping of Ag-A NSWs. (d) ζ -potential and (e, f) XPS spectra of adenine, AgNO_3 and Ag-A NSWs.

6 h (Fig. S6) [42]. To investigate the bactericidal process, SEM was used to analyze the morphological change. As shown in Fig. 2a, Ag-A NSWs presented a time-dependent bactericidal manner. After 2 h of incubation, the typical rod-shaped structure with intact and smooth membrane turned wrinkled and collapsed. As the incubation time extended to 4 h, large holes could be observed on the surface of *E. coli* cells, and the bacterial membrane and structure got severely ruptured. During this process, cytoplasm may leak out and become a major causal factor for bacterial death. Thus, a membrane-lytic antibacterial mechanism was proposed for Ag-A NSWs. Of additional note is that, the bacterial cells tended to aggregate during the bactericidal process. This phenomenon demonstrated the anticipatory adsorption effect between the positive-charged Ag-A NSWs and the negative-charged bacterial membrane.

To extend the evaluation of antibacterial capability of Ag-A NSWs, antibacterial assessment was also conducted towards a representative Gram-positive bacterial, *Staphylococcus aureus*. As a result, similar structural deformation could be observed with the increasing incubation time and concentration of Ag-A NSWs (Fig. 2b). The MIC value towards *Staphylococcus aureus* was measured to be 25–50 $\mu\text{g mL}^{-1}$ (Fig. S5). The relatively higher MIC compared with that towards *E. coli* could be ascribed to the difference in the composition of cell external membrane between Gram-positive and Gram-negative bacteria. The difference in MIC between Gram-positive and Gram-negative bacteria has been commonly reported for several kinds of Ag-based bactericidal agents [34]. All these results, taken together, demonstrated a potent and broad-spectrum antimicrobial capacity of Ag-A NSWs towards both Gram-positive and Gram-negative bacteria.

In order to gain further insight into the membrane rupture-based antibacterial process, fluorescence-based Live/Dead assays were applied by employing the mixture of green-fluorescent

STYO 9 and red-fluorescent PI. STYO 9 generally labels both the bacteria with intact membranes and damaged membranes. In contrast, PI only penetrates bacteria with damaged membranes, resulting in a severe reduction in the STYO 9 staining and thus an integral red fluorescence. As shown in Fig. 2c, untreated *E. coli* cells exhibit dominant green fluorescence, proving that the cells mainly stayed alive. Then, the red fluorescence gradually became prominent with the increasing concentration of Ag-A NSWs, verifying that serious membrane rupture happened. Moreover, this concentration-dependent antibacterial manner elucidated that the bacterial inactivation was directly related with the presence of Ag-A NSWs. Additionally, it was worth noting that these two fluorescence regions distributed in non-uniform manners, especially when high concentrations of Ag-A NSWs were used. As shown in Fig. 2c, dead bacterial cells accumulated seriously and formed a large red fluorescence-stained region. The accumulation of dead cells was consistent with that observed in SEM images, and both of these results suggested a correlation between cell death and bacterial fixation. In contrast, lived cells (green fluorescence-stained) tended to disperse randomly in the image except the red-stained region. The distinct distribution manner for dead and lived cells implied that bacterial capture and adhesion played important roles in the antibacterial process.

Antibacterial mechanism of Ag-A NSWs

To analyze the mechanism underlying the efficient antibacterial capability of Ag-A NSWs, the release of Ag^+ ions was firstly monitored, because it was usually the main mechanism for Ag-based antibacterial projects. Interestingly, it was found here that there was hardly any released Ag^+ in the first 6 h. Importantly, there was only 1.65% Ag leaked from the initial Ag-A NSWs solution even after incubation for 24 h. This result implied that

Figure 2 (a) Typical SEM images of the *E. coli* cells exposed to Ag-A NSW treatment for different durations. (b) SEM images of the MRSA cells exposed to Ag-A NSW treatment for different durations. Yellow circles mark the deformation of the bacteria cells. (c) CLSM images of *E. coli* treated with different concentrations of Ag-A NSWs. The red fluorescence (stained by PI) represents dead bacterial cells, and the green fluorescence (stained by STYO 9) represents live bacterial cells. Scale bars: 50 μm .

the Ag-A NSWs present strong Ag^+ fixation capacity (Fig. S7). The promising retardation effects towards Ag^+ ion release could be ascribed to the strong interaction between Ag^+ ions and adenine which contains several N sites for coordination with Ag^+ ions. Moreover, it is reported that Ag^+ ions at low concentrations are safe for mammalian cells [43]. Based on our results and previously reported literatures, we speculated that such a low dosage of released Ag^+ could hardly present any assistant for the bactericidal process, and thus ruled out the Ag^+ ion release-based antibacterial mechanism. Even though the antibacterial capacity decreased, the relative side toxicity originating from released Ag^+ ions could simultaneously be expected to diminish.

Next, as another antibacterial pathway of Ag, the capacity in ROS production of Ag-A NSWs was evaluated. Firstly, TA was employed to analyze the generation of $\cdot\text{OH}$ by monitoring their fluorescent product, 2-hydroxy TA (TAOH) [44]. As shown in Fig. 3a, the fluorescence intensity increased gradually with the concentration of Ag-A NSWs, suggesting that the $\cdot\text{OH}$ species were mainly generated from Ag-A NSWs. Then, we further evaluated the intracellular ROS generation by using DCFH-DA staining. It was found that, the intracellular ROS level (green fluorescence) was much higher in bacteria treated with Ag-A NSWs than in untreated bacteria (Fig. 3b), and escalation of green fluorescence was related with the concentration of Ag-A NSWs (Fig. S8). Based on all the above results, the antibacterial mechanism for Ag-A NSWs could be concluded to a combination of bacterial capture, membrane rupture and ROS release.

Bacterial resistance evaluation of Ag-A NSWs

Up to date, in response to living stress, various bacterial resistance mechanisms have been discovered in pathogenic bacteria against antibiotics. The increasing number of deaths caused by resistant bacteria infection has posed a great challenge to public health [45–47]. Thus, the antibacterial capacity towards resistant bacteria becomes one of the most important requirements for newly developed antibacterial materials. Impressively, both the *Staphylococcus aureus* and *E. coli* used here are resistant strains with high tolerance towards methicillin and chloramphenicol, respectively. Thus, the obtained low MIC presented a potentially potent antibacterial efficiency of Ag-A NSWs towards resistant strains. In order to gain further evidence, antibacterial capacity against huge amount of resistant *E. coli* infection was parallelly examined towards three agar-plates spread with Ag-A NSWs, chloramphenicol, and saline solution. As shown in Fig. 4, barely any difference in the number of *E. coli* colonies could be identified between the saline and chloramphenicol groups, suggesting inappreciable inhibition effect of chloramphenicol. In contrast, distinct decrease of bacteria could be observed when incubated with Ag-A NSWs, suggesting a potent antibacterial efficiency towards resistant bacteria.

Apart from the bacterial resistance towards traditional antibiotics, the resistance against new generational nano-agents has also been widely investigated. For instance, to eliminate the toxicity of Ag^+ ions, an active efflux mechanism was developed in mutant strain and reduced the toxicity from intracellular Ag^+

Figure 3 (a) The fluorescent intensity for TA detection of hydroxyl radicals from different groups of Ag-A NSW addition. (b) Intracellular ROS assay of *E. coli* treated with Ag-A NSWs. Scale bars: 50 μm .

Figure 4 Images of drug-resistant *E. coli* colonies treated with chloramphenicol and Ag-A NSWs on the LB agar plates.

ions. Recently, it has been reported that, bacteria could present resistance against Ag nanoparticles through a phenotypic change. During the repetitive administration of nonfatal concentration of Ag nanoparticles, bacterial flagellin was produced, which caused the aggregation of Ag nanoparticles and thus increased their tolerance to Ag nanoparticles [19]. Therefore, we further evaluated whether Ag-A NSWs could induce bacterial resistance by a proof-of-concept experiment. Interestingly, the MIC towards *E. coli* maintained almost steady at $5\text{--}10\ \mu\text{g mL}^{-1}$ in five generations, indicating that no bacterial resistance was stimulated. This excellent performance could be ascribed to special combining antibacterial mechanisms. Firstly, the membrane-lytic antibacterial process was a physical mechanism which was reported to have less possibility in stimulating bacterial resistance [48]. Secondly, because of the strong coordination between Ag^+ and adenine in the Ag-A NSWs, neither the Ag^+ ions nor the Ag nanoparticles could leach out and trigger the bacterial resistance. This property is of great importance, especially in facing with the current challenge of super-bacteria booming.

Antibacterial application of Ag-A NSWs

Encouraged by the promising antibacterial properties of Ag-A NSWs, we then evaluated their performance in a practical application model, woundplast. Woundplast is the most convenient antibacterial commodity and has been widely used as treatment for cutaneous wounds. Apart from the traditional function of preventing unexpected pollution, there is an increasing appeal of bacterial inhibiting in the infected wound, especially in those wounds infected with resistant strains. Here,

to examine the capability of Ag-A NSWs in inhibition of bacterial proliferation, a woundplast based on Ag-A NSWs was primarily fabricated by using cotton as the antibacterial material carrier. The woundplast was firstly inoculated with Gram-negative strain *E. coli* in a concentration of 10^6 CFU, and allowed to incubate at 37°C overnight. Then, the *E. coli* cells on the woundplast were subsequently rinsed and inoculated on an LB-agar plate. Finally, the bacteriostatic potency could be represented by the number of the colony in the plate. As expected, in stark contrast to the untreated woundplast, hardly any *E. coli* colony could be observed on the agar plates of the Ag-A NSW-based woundplast group, suggesting a satisfactory bacteriostatic potency. Simultaneously, three commercial woundplast as a comparison were also evaluated with the same procedure (Fig. 5a, b). As a result, all these commercial woundplast exhibited inappreciable antibacterial potency against *E. coli*. Moreover, these commercial woundplast presented nearly no antibacterial efficiency when faced with intractable resistant MRSA (Fig. 5c). On the contrary, barely any MRSA colony could be observed in the Ag-A NSW-based woundplast group. The superior inactivation efficiency against both *E. coli* and MRSA clearly verified the broad-spectrum antibacterial effect of the Ag-A NSW woundplast compared with those commercial woundplast. Most importantly, the Ag-A NSW-based woundplast maintained its high bacteriostatic potency towards intractable resistant MRSA. Thus, the Ag-A NSW-based woundplast provided a promising antibacterial alternative, especially facing with superbugs in the near future. Combining with the property of being free of bacterial resistance stimula-

Figure 5 (a) The novel Ag-A NSW woundplast model and the widely used commercial woundplast (1–3). (b, c) The evaluation of *E. coli* and MRSA growth separated from different groups of woundplast and cultured on agar plates.

Figure 6 (a) Photographs of MRSA-infected wounds on mice after different durations of therapeutic process. (b) H&E staining of normal skin tissue slices. (c) H&E staining of skin tissue slices treated with the Ag-A NSW woundplast model at 7th day. (d) H&E staining of skin tissue slices from the control group at 7th day. Black arrows indicate the epidermis of the injured tissue was significantly thinned. Yellow arrows indicate the neutrophils infiltrated. Insets in (b–d) show the wound tissue.

tion, the wide application of Ag-A NSWs could be expected.

To examine the capacity of the Ag-A NSW woundplast to improve wound healing *in vivo*, a conceptual wound model infected with resistant MRSA was built on mice. Before this evaluation, the stability of Ag-A NSWs in physiological environment was ensured by TEM images after the stability test in PBS solution for 24 h (Figs S9 and S10) [49]. Further, the systemic influence from Ag-A NSW woundplast was assessed with routine blood test (Fig. S11). The results presented that all the main components in the blood from the Ag-A NSW woundplast-treated group maintained similar levels with that of normal mice, implying acceptable biosafety. This could be partly ascribed to the neglectable Ag⁺ ion release (Fig. S12). Then, the mice were separated into three groups: treated with the saline-soaked cotton (i), Ag-A NSW woundplast (ii), and commercial woundplasts (iii: 1#, 2#, 3#). The wound healing process was monitored during a week. As a result, the group treated with the Ag-A NSW woundplast presented an accelerated wound healing process compared with those treated with saline-soaked cotton and commercial woundplasts. Newly formed scab appeared even in the first day of treatment, and significant improvement in wound healing could be observed after five days of treatment with the Ag-A NSW woundplast by the evidence of scab peeling and cambium appearing (Fig. 6a). In contrast, the wounds treated with both the saline-soaked cotton and commercial woundplasts maintained uncovered and presented inflammatory swollen at the same time (Fig. S13). All these results suggested a potent efficacy of the Ag-A NSW woundplast in promoting the wound healing process. Impressively, hair coverage was observed on the healed wound after seven days of treatment, implying that the function of skin tissue had recovered to initial condition. To further evaluate the wound healing process, histological analysis was conducted by hematoxylin and eosin (H&E) staining and Masson staining (Fig. 6b–d and Fig. S14). At

the 7th day post-treatment, there were still plenty of inflammatory cells remaining in the wound treated with saline-soaked cotton (Fig. 6d). In contrast, intact epidermal layer could be observed in the Ag-A NSW woundplast group, as well as less infiltrated inflammatory cells in the wound site. All these results, taken together, demonstrated the important effect of the Ag-A NSW woundplast in eliminating inflammation and promoting wound healing.

CONCLUSIONS

In summary, we have fabricated a novel bioinspired spiderweb-like nanoagent (Ag-A NSWs) with high antibacterial efficiency based on the coordination effect between silver and adenine. Taking advantage of the positive surface charge derived from the protonated N sites, Ag-A NSWs could effectively attract and trap bacteria through electrostatic interactions and decrease the interaction distance between bacteria and the poison species. As a result, Ag-A NSWs presented significantly higher antibacterial potency and superior broad-spectrum antimicrobial activities compared with widely-used antibiotics. Furthermore, novel antibacterial woundplast was fabricated based on Ag-A NSWs, which exhibited preferable therapeutic efficacy for bacteria-infected wounds in mice compared with commercial woundplasts. All these results indicate that the proposed system provides a new strategy for developing antibacterial nanoagents and possesses broad prospects for application in commercial implementation and clinic therapeutics.

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Supplementary information Supporting data are available in the online version of the paper.

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生物启发的纳米蜘蛛网结构用于细菌的高效捕获和杀伤

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摘要 具有广谱抗菌活性的纳米抗菌剂的出现是抗生素的一次革命性进化, 然而其细菌捕获能力及杀菌效率低致使它仍然无法达到商品化应用的要求. 受到蜘蛛捕虫行为的启发, 本研究设计了一种基于银和腺嘌呤配合物合成的蜘蛛网状的纳米抗菌材料, 从而实现高效的细菌捕获和杀伤. 这种纳米蛛网材料能够通过静电相互作用并利用其网状的形态特征有效地吸附并捕获细菌, 此外, 其物理溶膜机制和活性氧杀菌机制协同作用也使其具备更优异的抗菌活性. 由该纳米蛛网材料制成的创可贴与市售的商品化创可贴相比, 对耐药革兰氏阴性的*大肠杆菌*和耐药革兰氏阳性的金黄色葡萄球菌均表现出更优异的抗菌效果.