Multivalent and synergistic chitosan oligosaccharide-Ag nanocomposites for therapy of bacterial infection

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Chitosan oligosaccharide functionalized silver nanoparticles with synergistic bacterial activity were constructed as a multivalent inhibitor of bacteria. Placing the chitosan oligosaccharide on silver nanoparticles can dramatically enhance the adsorption to the bacterial membrane via multivalent binding. The multicomponent nanostructures can cooperate synergistically against gram-positive and gram-negative bacteria. The antibacterial activity was increased via orthogonal array design to optimize the synthesis condition. The synergistic bacterial activity was confirmed by fractional inhibitory concentration and zone of inhibition test. Through studies of antimicrobial action mechanism, it was found that the nanocomposites interacted with the bacteria by binding to Mg2+ ions of the bacterial surface. Then, the nanocomposites disrupted bacterial membrane by increasing the permeability of the outer membrane, resulting in leakage of cytoplasm. This strategy of chitosan oligosaccharide modification can increase the antibacterial activity of silver nanoparticles and accelerate wound healing at the same time. The nanomaterial without cytotoxicity has promising applications in bacteria-infected wound healing therapy.

Human health has been threatened with abuse of antibiotics and the appearance of multiple drug-resistant bacteria1,2. In the European Union, the number of deaths attributable to antibiotic-resistant bacteria is about 25,000 each year3. Therefore, it is crucial to design and develop new antimicrobial materials with high antibacterial activity and low resistance in the field of biomedicine.

Silver nanoparticles (AgNPs) with small size and large specific surface area have a stronger antibacterial effect against different bacteria, virus, and fungi4-6. AgNPs can contact with bacterial cell membranes, penetrate into the cytoplasm, and then inactivate essential respiratory enzymes and proteins, leading to bacterial death7,8. However, these antibacterial action of AgNPs is often dependent on high concentration since their physical collision with bacterial surface is random9. For enhancing interaction of AgNPs and the bacteria, cationic polymers-stabilized AgNPs were designed to bind to the negatively charged cell surfaces of the bacteria by electrostatic interaction10,11. However, the toxicity of cationic polymers obstructed their biomedicine application. Then, to improve their bio-compatibility, collagen-stabilized AgNPs were prepared by a photochemical method12. However, it was found that this AgNPs showed a weaker antibacterial property, comparing with small molecules-stabilized AgNPs (eg. lysine and citrate).

Chitosan oligosaccharide (COS), which is a cationic, non-immunogenic, biocompatible and an FDA-recognized mucoadhesive polymer13, with low molecular and good water-solubility is the positively charged alkaline-amino-oligosaccharide. Several biological activities of COS, such as hemostasis, antibacterial activity and anti-inflammatory effects, have been extensively studied in biomaterials field14. Conjugation with COS to the surface of AgNPs can increase their surface charge and enhance the adsorption to the negatively charged bacterial cytoplasmic membrane through electrostatic interactions. Moreover, COS as antimicrobial agent in multivalent binding can mediate the penetration of AgNPs into the bacteria15,16. To the best of our knowledge, COS-functionalized AgNPs (AgNPs-COS) as multivalent inhibitors of the bacteria has not been reported.

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In this study, a green and simple method has been developed for the preparation of AgNPs-COS as a novel antimicrobial nanomaterial. AgNPs-COS can be achieved via surface-modification in situ by reduction of AgNO₃ in the presence of COS. To achieve highly efficient antimicrobial ability, the synthesis conditions of AgNPs-COS are optimized by orthogonal array design (OAD). The fractional inhibitory concentration (FIC) and zone of inhibition test are used to characterize synergistic antimicrobial activity. Furthermore, effect of metal ions, the outer membrane permeabilization assay and scanning electron microscopy (SEM) is performed to explore antibacterial action mechanism. Finally, we further explore the biocompatibility toward RAW 264.7 cells and the treatment of bacteria-induced wound infection.

Materials and methods

Materials. COS was purchased from TCI Chemical (Tokyo, Japan). Silver nitrate (AgNO₃, 99.995%, metals basis, Ag 63% min), citric acid (CA) and 1-N-phenylnaphthylamine (NPN) was obtained from Aladdin Chemical Reagent (Shanghai, China). E. coli ATCC 25922 and S. aureus ATCC 25923 strains were provided by College of Life Science of Zhengzhou University (Zhengzhou, China). All solutions were prepared by the ultrapure water and stored in the refrigerator at 4 °C.

Synthesis and characterization of AgNPs-COS. Briefly, under vigorous stirring, 500μL of 1.0% (w/w) AgNO₃ and 500μL of 1.0% (w/w) COS were added into 48 mL ultrapure water and then heated to 80°C. 900μL of 1.0% (w/w) CA solution was rapidly added and kept stirring for 30 min. The obtained solution (dark yellow) was dialyzed (8−14 kDa M.W. cutoff) by the ultrapure water for 12 h and stored at 4 °C for use.

Zone of inhibition test. After overnight incubation at 37 °C, the bacterial suspensions were diluted with LB broth to approximately 2.0 × 10⁸ CFU/mL. The bacterial suspension was centrifuged at 6000 rpm for 5 min and washed with phosphate buffer solution (PBS, 0.01 mol/L, pH 7.4) three times. The supernatant was removed and the remaining bacteria were resuspended in PBS. 20μL of MgCl₂ solution was added to 1.5 mL of bacterial suspensions (approximate 4.0 × 10⁷ CFU/mL) to a final concentration of 0.1 mmol/L. Then, 100μL of AgNPs-COS solution (the final concentration of 8.0, 4.0, 2.0, and 1.0 μg/mL) was added to the suspensions and incubated at 37 °C for 24 h. The OD₆₀₀ was measured using UV-vis spectrophotometer.

Preparation of bacterial sample for SEM. The bacterial suspension (approximate 4.0 × 10⁷ CFU/mL) was washed with PBS and resuspended in 1.5 mL PBS (approximate 1.0 × 10⁶ CFU/mL). Then 50 μL of 1.0 mg/mL NPN and AgNPs-COS solution with different final concentrations were added into the above bacterial suspension. The fluorescence intensity was determined by fluorescence spectrometer (Hitachi, F-7100). An excitation wavelength was 350 nm and an emission wavelength was 370–550 nm. The control assay was performed by adding PBS instead of AgNPs-COS.

Cytotoxicity test. The RAW 264.7 cells were obtained from Henan University of Chinese Medicine (Zhengzhou, China). The cells were cultured in DMEM medium (Gibco) supplemented with 5% fetal bovine serum in 5% CO₂/95% air at 37 °C. The cells were seeded into 96-well plates at a density of 1.0 × 10⁴ cells per well and further incubated for 24 h. Then the medium in the wells was replaced with the different concentrations of AgNPs-COS solution. After incubation for 24 h, 10μL of 5% MTT solution was added into each well and further incubated for 4 h. After removing the medium containing MTT, 100 μL of DMSO was added into each
well to dissolve the formazan crystals with low-speed shaking for 15 min. The OD_{570} value was measured using a microplate reader. The untreated cells were used as positive control.

**In vivo tests.** Male Wistar rats (weighing 190–210 g) were offered from Henan University of Chinese Medicine (Zhengzhou, China). Animal welfare and experimental procedures were strictly performed in accordance with the Guidelines for Animal Experimentation of Henan University of Chinese Medicine (Approval Number: DWLL2018030038) and the protocol was approved by the Animal Ethics Committee of Henan University of Chinese Medicine.

The rats were anaesthetized with intraperitoneal injection of chloral hydrate and then shaved the hair on the backs. A full thickness cutaneous wound within a defined 1.0 cm i.d. circular area was made and then injected with 50 μL bacterial suspension (S. aureus, approximate 2.0 × 10⁶ CFU/mL) by a pipette tip. The bacterial suspension was evenly applied to the wound surface to ensure that the bacteria was only spread on the wound. After modeling success, there were the secretions of yellowish pus from infected tissue. The rats were divided into four groups and each group had six rats. Daily therapy was administered 50 μL 3% (w/w) COS solution and 600 μL 1.0% (w/w) CA solution at 300, 600, or 900 μL, respectively. Under this condition, synthesized AgNPs-COS achieved the desired antimicrobial activities toward both gram-positive and gram-negative bacteria.

**Characterization of AgNPs-COS.** UV-vis absorption spectrum was used to confirm the formation of AgNPs-COS. The spectra of AgNPs and AgNPs-COS were shown in Fig. 1. Compared with the absorption spectrum of AgNPs, the maximum absorption wavelength of AgNPs-COS showed a blue shift from 400 to 391 nm.

### Table 1. Factors and levels for L₉(3⁴) OAD experiments and MIC for AgNPs-COS against *S. aureus* and *E. coli*.

| No. | Amount of AgNO₃ (μL) | Amount of COS (μL) | Amount of CA (μL) | MIC against *S. aureus* (μg/mL) | MIC against *E. coli* (μg/mL) |
|-----|---------------------|-------------------|------------------|---------------------------------|-------------------------------|
| 1   | 300                 | 200               | 500              | 2.56 ± 0.49                     | 1.83 ± 0.65                   |
| 2   | 300                 | 500               | 600              | 1.98 ± 0.12                     | 1.64 ± 0.35                   |
| 3   | 300                 | 800               | 900              | 1.42 ± 0.22                     | 0.96 ± 0.24                   |
| 4   | 500                 | 200               | 600              | 1.56 ± 0.55                     | 1.11 ± 0.40                   |
| 5   | 500                 | 500               | 900              | 0.78 ± 0.26                     | 0.51 ± 0.18                   |
| 6   | 500                 | 800               | 300              | 1.21 ± 0.43                     | 0.82 ± 0.26                   |
| 7   | 700                 | 200               | 900              | 1.78 ± 0.47                     | 1.35 ± 0.27                   |
| 8   | 700                 | 500               | 300              | 1.82 ± 0.53                     | 1.37 ± 0.45                   |
| 9   | 700                 | 800               | 600              | 1.19 ± 0.30                     | 0.97 ± 0.33                   |

**Results and discussion**

**Orthogonal array design for optimizing the synthesis conditions of AgNPs-COS.** AgNPs-COS were synthesized via reduction of AgNO₃ by CA at 80 °C in the presence of COS in water. The amine groups of COS can complex with silver cations and then conjugate on the growing AgNPs surface following reduction process. The antibacterial activity of AgNPs-COS was related to the amount of AgNO₃, COS, and CA in synthesis process. The amount of AgNO₃ and CA was found to be related to the size of AgNPs-COS and then can affect the antibacterial activity of AgNPs-COS. COS as capping groups to the AgNPs surface can stabilize AgNPs and then affect the surface plasmon resonance absorbance of AgNPs. OAD can quickly generate useful information on key variable by arranging different factors within a single experiment. The results of OAD can then be analyzed by analysis of variance. Optimization of synthesis condition via an OAD would reduce the amount of experiments and costs⁷,¹⁸. Therefore, OAD was used to optimize experimental conditions of AgNPs-COS. In this study, the synthesis conditions include three factors: the amount of AgNO₃, COS, and CA. Therefore, an OAD L₉(3⁴) was used to evaluate effects of these factors. Each factor was evaluated in three levels. Experiments were carried out with 1.0% (w/w) AgNO₃ solution at 300, 500, or 700 μL, 1.0% (w/w) COS solution at 200, 500, or 800 μL, and 1.0% (w/w) CA solution at 300, 600, or 900 μL, respectively. The OAD experiments were performed according to Table 1. Subsequently, the MIC values against *S. aureus* and *E. coli* were determined to evaluate the antibacterial activity of the nine synthesized AgNPs-COS and the results were shown in Tab. 1. Clearly, AgNPs-COS synthesized by the optimum synthesis conditions (No.5 in Table 1) can obtain the lowest MIC value and act synergistic antibacterial functions against gram-positive (*S. aureus*) and gram-negative bacteria (*E. coli*). Therefore, the optimum conditions were 500 μL of 1.0% (w/w) AgNO₃ solution, 500 μL of 1.0% (w/w) COS solution, and 900 μL of 1.0% (w/w) CA solution, respectively. Under this condition, synthesized AgNPs-COS achieved the desired antimicrobial activities toward both gram-positive and gram-negative bacteria.
Moreover, the peak intensity of AgNPs-COS was higher than that of AgNPs. COS as stabilizers was beneficial to the nucleation and growth of nanoparticles and avoided the formation of these large nanoparticles. In addition, the surface charge analysis was performed. The zeta potential of AgNPs was $-20.3 \text{ mV}$. Although AgNPs with a large number of negative charges can maintain stability in aqueous solution, the interaction between AgNPs and the bacteria is impeded by electrostatic repulsion. However, the zeta potential of AgNPs-COS was $11.3 \text{ mV}$, which could enhance their adsorption to negatively charged bacterial membranes by electrostatic interaction. This result indicated that COS molecules successfully binding to the AgNPs surface.

The morphology of AgNPs-COS was observed by TEM (Fig. 1, inset A). It can be found that the obtained nanoparticles were spherical with good dispersibility and COS coated on AgNPs surface in the core-shell module. The average diameter of AgNPs-COS was $36.7 \text{ nm}$ (Fig. 1, inset B). Because of conjugation of COS onto AgNPs surface, the edge of AgNPs-COS emerged light corona (Fig. 1, inset A) compared with AgNPs (see Supplementary Fig. S1 online).

Study of synergistic antimicrobial activity. The FIC index was employed to detect any synergistic antimicrobial effect between AgNPs and COS by a two-dimensional microdilution assay. The FIC was calculated as follows:

$$FIC = \frac{\text{MIC of compound A in combination}}{\text{MIC of compound A alone}} + \frac{\text{MIC of compound B in combination}}{\text{MIC of compound B alone}}$$

If the FIC index was less than 0.5, the interaction was defined as synergistic effect$^{19}$. The results showed that the FIC indices were 0.34 and 0.29 against $S. \text{ aureus}$ and $E. \text{ coli}$, respectively, indicating that COS binding to the AgNPs can obtain synergistic antimicrobial effect.

The synergistic effects between AgNPs and COS was also verified by zone of the inhibition test. For this purpose, COS, AgNPs, and AgNPs-COS at a final concentration of 100, 64, and $64 \mu\text{g/mL}$, respectively, was prepared. From Fig. 2, the diameters of zone of inhibition of AgNPs-COS were significantly larger than those of COS and AgNPs, indicating that AgNPs-COS had better antimicrobial performance compared with COS and AgNPs. The outstanding synergistic activity of COS and AgNPs was also confirmed against both gram-negative and gram-positive bacteria. Moreover, COS was used as stabilizers to protect AgNPs against agglomeration for retaining their diffusivity and enhancing antimicrobial property.

Effect of Mg$^{2+}$ ions on the antibacterial activity of AgNPs-COS. To explore the binding sites of AgNPs-COS on the bacterial surface, the effects of Mg$^{2+}$ ion on the bacterial growth inhibition were examined. From Fig. 3, OD$_{600}$ value of $S. \text{ aureus}$ and $E. \text{ coli}$ were obviously increased when Mg$^{2+}$ ions were added to the bacterial suspensions in the presence of AgNPs-COS with different concentration. It was indicated that the antimicrobial activity of the nanoparticles decreased. It has been reported that the lipopolysaccharide (on the surface of the gram-negative bacteria, e.g. $E. \text{ coli}$) linked with Mg$^{2+}$ ions via electrostatic interaction to form a stable structure on the surface of the bacterial membrane$^{20,21}$. However, amino group of COS can chelate Mg$^{2+}$ ions by metal-to-ligand $\pi$-bonding. Then the lipopolysaccharide was isolated and dispersed to the medium, result in the damage of the outer membrane of the bacteria. When Mg$^{2+}$ ions were added to the bacterial suspension, the COS of the nanoparticle surface would chelate Mg$^{2+}$ ions in the medium, thereby avoiding replacement of these ions from their binding sites in lipopolysaccharide. Similarly, in gram-positive bacteria (e.g. $S. \text{ aureus}$), the teichoic acids of the bacterial cell wall can attract Mg$^{2+}$ ions to provide rigidity to the cell wall$^{22}$. When COS of the nanoparticle surface chelated Mg$^{2+}$ ions from these original sites on the bacterial surface, teichoic acid would be...
dispersed to the medium and the bacterial cell wall would be damaged. Therefore, AgNPs-COS can interact with the bacteria by binding to Mg2+ of the bacterial surface.

**The permeability of outer membrane.** The interaction of AgNPs-COS with outer membrane of *E. coli* cells was studied using the hydrophobic fluorescent probe NPN, which has strong fluorescence in hydrophobic environments and weak fluorescence in aqueous environments. When AgNPs-COS disorganized outer membrane of bacterial cell, NPN could partition into the phospholipid layer of the outer membrane, which can increase NPN fluorescence intensity. As shown in Fig. 4, NPN fluorescent intensity in *E. coli* suspensions was increased with the increase in the nanoparticles concentration and interaction time. The fluorescent intensity reached a plateau after approximately 20 min, which indicated that AgNPs-COS can permeabilize the cell membrane and destroy the integrality of bacterial cell.

**Morphological change of the bacteria by SEM observation.** The morphological change of *S. aureus* and *E. coli* before and after incubation with AgNPs-COS were investigated via SEM observations. Untreated *S. aureus* and *E. coli* had a smooth surface with the integrity of membrane structure (Fig. 5A,C). In contrast, the morphology of the treated bacteria is altered significantly after incubation with 4.0 μg/mL AgNPs-COS for 10 min (Fig. 5B,D). The cell walls of *S. aureus* had formed a large number of vesicles (Fig. 5B). Furthermore, the leakage
of large cytoplasmic components can be observed on the cytomembrane of *E. coli* (Fig. 5D). Similar SEM images of various bacteria had been also observed in that of the treated bacteria. The thickness of the peptidoglycan layer of gram-positive and gram-negative bacteria was different. When the bacteria interacted with AgNPs-COS for 10 min, *S. aureus* with the thick peptidoglycan layer formed the vesicles (small leakage of cytoplasmic components) and *E. coli* with the thin peptidoglycan layer represented large leakage of cytoplasmic components. It indicates that AgNPs-COS can disrupt the bacterial membrane, leading to leakage of cytoplasm. Then the damaged membrane can destroy the structural integrity and the membrane ability, resulting in bacterial death.

The antimicrobial mechanisms of AgNPs-COS are possibly as follows. AgNPs-COS interact with the bacteria by binding to Mg$^{2+}$ ions of the bacterial surface. Then, the nanoparticles disrupt bacterial membrane via increasing the permeability of the outer membrane, resulting in leakage of cytoplasm. These are possibly reasons causing bacterial cell death.

**Cytotoxicity assay.** The cytotoxicity of AgNPs-COS was investigated in Raw246.7 cells by MTT assay. As shown in Fig. 6, no obvious cytotoxicity was observed at a concentration up to 128 $\mu$g/mL, which had exceeded...
160-fold and 250-fold MIC against *S. aureus* and *E. coli*, respectively (Table 1, No.5). Moreover, the toxicity of AgNPs can be improved by binding to the COS with good biocompatibility. Therefore, the nanoparticles had the potential for *in vivo* use.

**In vivo study.** To explore the healing status of the wound infection with *S. aureus*, 8.0 µg/mL AgNPs-COS, 75% ethanol or 0.9% NaCl solution were administered to the wounds of every group for daily therapy, respectively. The physical measurement of wound area before the treatment and during the treatment were evaluated by wound photographs. From Fig. 7, the wounds of AgNPs-COS group and ethanol group were healed after 12 days. However, the wounds treated with NaCl and without treatment were not healed. Histological evaluation of rat dermal wound was performed at the 12th days after treatment and representative optical micrographs by H&E staining were showed in Fig. 8. The epithelialization underlying wound connective tissues were observed by the therapy of AgNPs-COS or ethanol. Meanwhile, a few inflammatory cells emerged from the 75% ethanol-treated wounds. However, massive inflammatory cells appear from the 0.9% NaCl solution-treated and untreated wounds. From immunohistochemical staining of rat epidermal tissues, NaCl group and blank control group had more collagen I (brown parts or dots) than ethanol group and AgNPs-COS group. Moreover, as shown in Fig. 9, relative expression levels of collagen I were significantly increased in the rat skin tissues of NaCl group and blank control group, indicated inflammation still exists. Meanwhile, the results of relative expression levels of
keratin, fibronectin and laminin illustrated that AgNPs-COS can promote wound healing of bacterial infection without inflammation28–31.

Conclusions
AgNPs-COS was synthesized as a new antimicrobial material via surface-modification in situ. These antibacterial activity could be improved via OAD optimization of synthesis condition. This nanoparticles with synergistic antimicrobial activities could efficiently inhibit the growth of gram-positive and gram-negative bacteria. The studies of antimicrobial mechanism indicated that AgNPs-COS could combine with Mg2+ ions of the bacterial surface, interact with the bacterial membrane and increase the permeability of the outer membrane. These interactions will lead to the damage of bacterial membrane and the death of the bacteria. This antimicrobial material has a great potential for the wound healing of damaged skin tissues accompanied with bacterial infection, and will be widely applicable for combating multiple bacteria-induced infectious diseases in medical field.

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References
1. Zgurskaya, H. I., López, C. A. & Gnanakaran, S. Permeability barrier of Gram-negative cell envelopes and approaches to bypass it. ACS Infect. Dis. 1, 512–522 (2015).
2. Panáček, A. et al. Bacterial resistance to silver nanoparticles and how to overcome it. Nat. Nanotech. 13, 65–71 (2018).
3. Lim, C., et al. Epidemiology and burden of multidrugresistant bacterial infection in a developing country. Elife v5, (2016).
4. Yuan, L. et al. Stress responses of aquatic plants to silver nanoparticles. Environ. Sci. Technol. 52, 2558–2565 (2018).
5. McGillicuddy, E. et al. Silver nanoparticles in the environment: Sources, detection and ecotoxicology. Sci. Total Environ. 575, 231–246 (2017).
6. Miller, K. P., Wang, L., Benicewicz, B. C. & Decho, A. W. Inorganic nanoparticles engineered to attack bacteria. Chem. Soc. Rev. 44, 7787–7807 (2015).
7. Mei, L. et al. Bioconjugated nanoparticles for attachment and penetration into pathogenic bacteria. *Biomaterials* **34**, 10328–10337 (2013).
8. Zhao, Y. et al. Small molecule-capped gold nanoparticles as potent antibacterial agents that target gram-negative bacteria. *J. Am. Chem. Soc.* **132**, 12349–12356 (2010).
9. Zhang, Y. et al. Hyperbranched poly (amidoamine) as the stabilizer and reductant to prepare colloidal silver nanoparticles in situ and their antibacterial activity. *J. Phys. Chem. C* **112**, 2330–2336 (2008).
10. Song, J., Kang, H., Lee, C., Hwang, S. H. & Jang, J. Aqueous synthesis of silver nanoparticle embedded cationic polymer nanofibers and their antibacterial activity. *ACS Appl. Mater. Inter.* **4**, 460–465 (2011).
11. Mei, L., Lu, Z., Zhang, X., Li, C. & Jia, Y. Polymer-Ag nanocomposites with enhanced antimicrobial activity against bacterial infection. *ACS Appl. Mater. Inter.* **6**, 15813–15821 (2014).
12. Alarcon, E. I. et al. The biocompatibility and antibacterial properties of collagen-stabilized, photochemically prepared silver nanoparticles. *Biomaterials* **33**, 4947–4956 (2012).
13. Peniche, C. et al. Chitosan: an attractive biocompatible polymer for microencapsulation. *Macromol Biosci.* **3**, 511–520 (2003).
14. Yang, N. & Li, W. H. Facile one-pot synthesis of chitosan oligosaccharide/silver nanocomposites and their antimicrobial properties. *Mater. Lett.* **132**, 145–148 (2014).
15. Moon, J. S. et al. The antibacterial and immunostimulative effect of chitosan oligosaccharides against infection by Staphylococcus aureus isolated from bovine mastitis. *Appl. Microbiol. Biotechnol.* **75**, 989–998 (2007).
16. Benhabiles, M. S. et al. Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste. *Food Hydrocolloids* **29**, 48–56 (2012).
17. Liu, Y. et al. Sensitive chemiluminescence immunoassay by capillary electrophoresis with gold nanoparticles. *Anal. Chem.* **83**, 1137–1143 (2011).
18. Zhang, Y., Li, X., Yuan, Z. & Lu, Y. Orthogonal array design experiments for optimizing the separation of nine pesticides by micellar electrokinetic chromatography. *Microchem. J.* **73**, 307–315 (2002).
19. Kumar, S. N., Siji, J. V., Nambisan, B. & Mohandas, C. Activity and synergistic interactions of stilbenes and antibiotic combinations against bacteria in vitro. *World J. Microbiol. Biotechnol.* **28**, 3143–3150 (2012).
20. Vaara, M. Agents that increase the permeability of the outer membrane. *Microbiol. Rev.* **56**, 395–411 (1992).
21. Nikaido, H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev.* **67**, 593–656 (2003).
22. Wickham, J. R., Halve, J. L., Kashbanov, S., Khandogin, I. & Rice, C. V. Recrystallizing magnesium chelation by trichoic acid with phosphorus solid-state NMR and theoretical calculations. *J. Phys. Chem.* **B113**, 2177–2183 (2009).
23. Loh, B., Grant, C. & Hancock, R. E. Use of the fluorescent probe 1-N-phenylnaphthylamine to study the interactions of aminoglycoside antibiotics with the outer membrane of *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **26**, 546–551 (1984).
24. Ibrahim, H. R., Sugimoto, Y. & Aoki, T. Ovotransferrin antimicrobial peptide (OTAP-92) kills bacteria through a membrane damage mechanism. *Biochem. Bioph. Res. Co.* **314**, 196–205 (2000).
25. Mei, L. et al. Multivalent polymer–Au nanocomposites with cationic surfaces displaying enhanced antibacterial activity. *Polym. Chem.* **5**, 3038–3044 (2014).
26. Mangoni, M. L. et al. Effects of the antimicrobial peptide temporin L on cell morphology, membrane permeability and viability of Escherichia coli. *Biochem. J.* **380**, 859–865 (2004).
27. Dai, X. et al. All-in-one NIR-activated nanoplat formers for enhanced bacterial biofilm eradication. *Nanoscale* **10**, 18520–18530 (2018).
28. Hertle, M. D. et al. Aberrant integrin expression during epidermal wound healing and in psoriatic epidermis. *J. Clin. Invest.* **89**, 1892–1901 (1992).
29. Saarialho-Kere, U. K. et al. Cell-matrix interactions modulate interstitial collagenase expression by human keratinocytes actively involved in wound healing. *J. Clin. Invest.* **92**, 2858–2866 (1993).
30. Swamy, S. M. K. et al. “Role of phenytoin in wound healing: microarray analysis of early transcriptional responses in human dermal fibroblasts.” *Biochem. Bioph. Res. Co.* **314**, 661–666 (2004).
31. Aukhil, I. Biology of wound healing. *Periodontology* **2000**(22), 44–50 (2000).

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Designed the experiments and wrote the manuscript: Lin Mei; performed the experiments: Zhenlong Xu, Lijun Zhang and Pengxu Li; supervised the cytotoxicity and animal experiment: Yanmei Shi; analyzed experimental data: Chunlei Lin and Shuyan Jiao.

Competing interests
The authors declare no competing interests.

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