The use of natural substance to ward off microbial infections has a long history. However, the large-scale production of natural extracts often reduces antibacterial potency, thus limiting practical applications. Here we present a strategy for converting natural organosulfur compounds into nano-iron sulfides that exhibit enhanced antibacterial activity. We show that compared to garlic-derived organosulfur compounds nano-iron sulfides exhibit an over 500-fold increase in antibacterial efficacy to kill several pathogenic and drug-resistant bacteria. Furthermore, our analysis reveals that hydrogen polysulfanes released from nano-iron sulfides possess potent bactericidal activity and the release of polysulfanes can be accelerated by the enzyme-like activity of nano-iron sulfides. Finally, we demonstrate that topical applications of nano-iron sulfides can effectively disrupt pathogenic biofilms on human teeth and accelerate infected-wound healing. Together, our approach to convert organosulfur compounds into inorganic polysulfides potentially provides an antibacterial alternative to combat bacterial infections.
N atural substances have been used to prevent and treat microbial diseases since ancient times. For instance, the use of bulbs of plants in Allium genus, such as garlic and onion for their antimicrobial activities has been described as early as the 16th century BC1-3. Like the traditional Welsh rhyme which says, ’Eat leeks in March and wild garlic in May, and all the year after the physicians may play’4. Currently, most medicinal garlic products are manufactured from the extracts (e.g., garlic oil) which contain organosulfur compounds5,6,7,8. These organosulfur compounds contain one or more sulfur atoms bonded with carbon, which is the basis for their biological activities, including antimicrobial, antioxidant, antitumor and antiasthmatic activities6,8.

The main antibacterial component present in garlic bulbs is allicin9,10, which is converted by the enzyme allinase from alliin (a derivative of amino acid cysteine)11. Allicin is unstable under physiological conditions and might quickly transform into alkyl sulfides, such as diallyl sulfide (DADS), diallyl trisulfide (DATS) and diallyl sulfoxide (DAS) (Fig. 1a). Transformed products are assumed to be the active ingredients in plants responsible for suppressing bacterial growth. However, these purified components often exhibit weak antibacterial effects, while a small number of these components are devoid of any bioactivity12,13. In addition, many organosulfur compounds are volatile and thus exhibit unpleasant odors. Furthermore, they are characterized by poor water solubility, further limiting wider biomedical and clinical applications. However, recent studies have demonstrated that inorganic sulfur in the form of nanoscale metal sulfides, such as copper sulfide (CuS) and molybdenum disulfide (MoS2), exhibits high antibacterial activity14,15. Thus, converting organosulfur compounds into nanoscale inorganic sulfides represents a promising route to improve the efficacy of existing antibacterial products.

Bacterial infections pose a considerable threat to public health as many pathogenic bacteria readily develop resistance to multiple antibiotics and form biofilms with additional protection from antibiotic treatment16. Therefore, it is urgently required to develop new antibacterial agents17. Here we develop an approach to convert natural organosulfur compounds into nanometer-sized iron sulfides (nFeSs). We find that compared with organosulfur compounds, the nFeSs exhibit enhanced antibacterial activity to pathogenic bacteria with drug resistance or associated with biofilms.

Results
Conversion of natural organosulfur compounds into nFeSs. To convert organosulfur compounds into inorganic sulfides, we employed a solvothermal method that produces inorganic crystals in a sealed autoclave operated at high temperature and high vapor pressure. We previously used this method to synthesize ferromagnetic nanocrystals (Fe3O4) by reducing ferric iron (Fe3+) into Fe2O3 nanocrystal in ethylene glycol (EG) solvent containing sodium acetate (NaOAc) at 200 °C18. The presence of an elemental sulfur in this reaction preferentially causes the formation of iron sulfide19. As shown in Fig. 1b, we directly introduced the natural organosulfur compound into the solvothermal reaction and found that sheet-like and hexagonal nanostructures were formed rather than the typical nanoparticles for Fe3O4. The hexagonal plates exhibited a length of up to 1 μm and a thicknesses of up to 20–30 nm. Using energy dispersive spectrometry (EDS), we found that these nanostructures consisted of iron and sulfide (Supplementary Fig. 1a). X-ray diffraction (XRD) showed that pyrrhotite (Fe1–x,S, 29-0724) and gregite (Fe5S3, 16-0713), two phases of iron sulfides were present in the product (Fig. 1c). High-resolution transmission electron microscope (HRTEM) images taken from the large irregular thin sheet exhibited clear lattice fringes with a d-spacing of 0.298 nm. In addition, the selected-area electron diffraction (SAED) patterns showed that the nanosheets displayed a single crystalline structure with (311) lattice planes of Fe3S4. In comparison, HRTEM images taken from the hexagonal plate showed that lattice fringes were formed, with a d-spacing at 0.15 nm, which can be assigned to the (400) plane of Fe1+xS. However, the SAED patterns taken from the edge of the hexagonal plate showed that Fe1+xS was polycrystalline (Fig. 1d and Supplementary Fig. 1b). We observed these features in the products generated from the additive organosulfur compounds including DADS, DATS, cystine, and its derivatives, such as cystine and glutathione (GSH). The resulting products were denoted DADS-nFeS, DATS-nFeS, Cyss-nFeS, Cyss-nFeS and GSH-nFeS (Supplementary Fig. 2). However, diallyl sulfide (DAS) showed negligible nFeS formation; also, allyl methyl sulfide (AMS) and methionine failed to form any nFeS in the solvothermal reaction (Supplementary Fig. 2 and Supplementary Fig. 3). The successful synthesis of nFeS by solvothermal reduction of Fe3+ with cysteine and GSH was presumably achieved due to the presence of highly chemically reductive thiol groups in these two sulfides. The high conversion rate of di- and tri-sulfide into nFeS is presumably due to their relative weak covalent bonds connecting their S atoms. As shown in Fig. 1b, the calculated values of bond dissociation energy (BDE) for C–S in DADS, DATS, and cysteine were 35.32, 38.68, and 51.89 kcal mol−1, respectively, which were smaller than those in DAS (69.358/50.69 kcal mol−1) and methionine (66.90/70.69 kcal mol−1). The low BDE of C–S may favor thermal degradation to release sulfur which further reacts with iron to form nFeS in the solvothermal reaction. These results indicate that two reactions may occur in the synthetic process: sulfur extraction from organosulfur compounds via pyrolysis, followed by the reaction of nFeS formation (Fig. 1e).

We then investigated the correlation between nFeS formation and the amount of added organosulfur compound. We chose cysteine as a standard molecule because it is a general precursor for many natural organosulfur compounds. In addition, compared to garlic-derived organosulfides (e.g., DADS or DATS) (Supplementary Fig. 4), cysteine is odorless, soluble in many solvents, thus it is more suitable for both solvothermal conversion and antibacterial testing. Cysteine was added to the solvothermal reaction (total volume was 50 mL) in various amounts of 0.1, 0.25, 0.5, 0.75, and 1.0 g (the products are denoted Cyss1.0-nFeS, Cyss0.75-nFeS, Cyss0.5-nFeS, Cyss0.25-nFeS, and Cyss1.0-nFeS, respectively). As shown in Supplementary Fig. 5, the formation of nFeS was dependent on the amount of cysteine added to the solvothermal reaction. Consequently, the atomic ratio of sulfide to iron increased from Cyss1.0-nFeS to Cyss1.0-nFeS in which the ratio of Fe/S was up to 48.75/51.25, while that for oxygen to iron decreased correspondingly (Supplementary Fig. 5a and Supplementary Table 1). The Cyss1.0-nFeS product only contained nanosheets and hexagonal structures whereas Fe3O4 nanoparticles were present in the Cyss1.0-nFeS product. Simultaneously, with increasing concentrations of cysteine in the reaction, the degree of magnetism in the nFeS products decreased. The Cyss1.0-nFeS product exhibited much lower levels of magnetism compared to the Cyss1.0-nFeS product (Supplementary Fig. 6b). It is well established that while the metastable Fe3S4 phase exhibits paramagnetism, the stable Fe1+xS phase fails to exhibit magnetism. Thus, the formation of Fe1+xS may be dominant when sufficient amounts of organosulfur are present in the solvothermal reaction (Fig. 1e). Taken together, adding natural organosulfur compounds to the solvothermal process led to the formation of nFeS, and the type and amount of added organosulfur compounds determined the crystalline phase and nanostructure of nFeS products.

NATURE COMMUNICATIONS | DOI: 10.1038/s41467-018-06164-7 | www.nature.com/naturecommunications

2 NATURE COMMUNICATIONS | (2018) 9:3713 | DOI: 10.1038/s41467-018-06164-7 | www.nature.com/naturecommunications
Antibacterial properties of nFeS. In order to evaluate the antibacterial activity of our nFeS products, we performed quantitative analysis on bacterial viability using colony-forming unit (CFU) method. As shown in Fig. 2a, nFeS produced from different organosulfur compounds exhibited similar antibacterial activity against *Streptococcus mutans* (*S. mutans*), a virulent oral pathogen and well-characterized biofilm-forming organism. In comparison, iron oxide (*Fe*$_3$O$_4$) nanoparticles failed to exert antibacterial activity, while the corresponding organosulfur compounds exhibited negligible antibacterial activity (Fig. 2b). For instance, DADS-nFeS showed a more than 500-fold increase in bacterial killing (*S. mutans*) activity compared with pure DADS (normalized to the sulfur amount used in DADS-nFeS) (Supplementary Fig. 7). In particular, nFeS derived from cysteine demonstrated dose-dependent antibacterial activity based on the cysteine amount, whereby Cys$_{0.5}$-nFeS showed the highest biocidal activity (Fig. 2c). Cys$_{0.5}$-nFeS at 0.5 mg mL$^{-1}$ was able to kill bacteria with 3-log reduction of viability (from $10^7$ to $10^4$ CFU mL$^{-1}$) within 10 min of treatment (Supplementary Fig. 8). In addition, the antibacterial efficacy was dependent on the dosage and time.

Our antibacterial tests also showed that Cys$_{0.5}$-nFeS displayed a broad antibacterial activity against Gram-negative bacteria, including *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella enteritidis* (*S. enteritidis*) and Gram-positive bacteria such as *S. mutans*, *Staphylococcus aureus* (*S. aureus*) as well as drug-resistant strains of *S. aureus* (Fig. 2d–h and Supplementary Fig. 9).

To further assess the antibacterial mechanism of nFeS, we investigated the oxidative state and impact on both bacterial...
surfaces and intracellular molecules. The level of reactive oxygen species (ROS) increased by >50%, while lipid peroxidation was measured using a MDA assay increased threefold in the treated species (ROS) increased by >50%, while lipid peroxidation as a result of nFeS treatment.

**nFeS releases polysulfanes for antibacterial activity.** To determine the active component in nFeS product, we analyzed the physicochemical changes our nFeS underwent during the antibacterial process. As shown in Fig. 2i, small grain-like nanoparticles, rather than nanosheets or hexagonal plates, surrounded the bacteria following their treatment with Cysteine-nFeS (Fig. 2k). This observation indicates that nFeS is structurally transformed during incubation with bacterial cells. To verify this assumption, Cysteine-nFeS was directly dissolved in water, and the physical characteristics and nanostructural changes were analyzed over time. As shown in Fig. 3a, the solution containing Cysteine-nFeS gradually changed color from black to orange with increasing time. This phenomenon is common for nFeS (Supplementary Fig. 12). Further- more, the typical sheet structure disappeared, together with the hexagonal plates. Also, grain-like nanoparticles were formed after 96 h of incubation. The collected nanoparticles only contained iron and oxygen, as identified by EDS (Supplementary Fig. 13).
values and error bars were discussed. The significance was assessed using unpaired Student’s two-sided t-test compared to the control group. **p < 0.01, ***p < 0.001 and ****p < 0.0001. Mean values and error bars were defined as mean and s.d., respectively. All experiments were performed in triplicate, and representative images are shown.
donor, lacked the antibacterial activity. Thus, we excluded H₂S as the possible antibacterial agent. Previously, the antibacterial activity of garlic oil had been associated with the presence of organosulfur compounds, especially in the form of polysulfides. The antibacterial activity might therefore have been derived from hydrogen polysulfanes (such as H₂S₂ and H₂S₃). To confirm this hypothesis, polysulfanes in the supernatant of Cys₀.₅-nFeS were eliminated by reducing disulfide bonds in the presence of tris(2-carboxyethyl)phosphine (TCEP). As shown in Fig. 3e, the antibacterial effects disappeared when TCEP was added to both the original mixture as well as into the supernatant of nFeS. This finding provides direct evidence that the polysulfanes released from nFeS are critical factors in reducing bacterial viability.

Interestingly, nFeS also exhibited enzyme-mimicking properties to boost the release of polysulfanes. We speculated that nFeS may be a nanomzome with peroxidase-like and catalase-like activities, which decomposes H₂O₂ into free radicals and oxygen, respectively²³–²⁵. We found that compared to Fe₃O₄ nanoparticles, nFeS possesses enhanced peroxidase-like and catalase-like activities (Supplementary Figs. 16, 17, Supplementary Tables 3 and 4). More specifically, Cys₀.₅-nFeS performed the highest specific activity which was almost 10-fold higher than that of Fe₃O₄ nanoparticles (Supplementary Table 5). Importantly, we found that during the enzyme-like catalytic process, the color of the nFeS solution (1.0 mg mL⁻¹) became orange within 30 min in the presence of 50 mM H₂O₂ (Supplementary Fig. 18), which was much faster than the color change shown in Fig. 3a. To quantitatively measure the correlation between sulfide release and H₂O₂ concentration, the absorbance spectrum was scanned using NaHS as a standard donor of free sulfides, and 340 nm was chosen as the specific absorbance (A₃₄₀) for free sulfide in aqueous solution (Supplementary Fig. 19a). When we measured absorbance for the nFeS supernatant, we found that its absorbance spectrum was similar to that of NaHS (Supplementary Fig. 19b). Therefore, we chose A₃₄₀ to characterize free sulfides release from nFeS. In addition, the value of A₃₄₀ from the CyS₀.₅-nFeS (1 mg mL⁻¹) supernatant reached a plateau in the first 30 min, indicating that the release of sulfide is fairly fast (Supplementary Fig. 19c). The correlation curve between A₃₄₀ and the H₂O₂ concentration showed that the release kinetics was similar to those in enzyme-like activities of nFeS, in which 80.25 mM H₂O₂ led to the release of half the amount of maximum free sulfides, indicating that free sulfide release was driven by catalysis (Fig. 3f). Following the release of free sulfides, the precipitate from the nFeS exhibited low enzyme-like activity (Supplementary Fig. 20), which is consistent with the catalytic performance of iron oxide. Based on these results, we deduced that in the presence of H₂O₂, oxidation may quickly occur on the surface of nFeS, leading to accelerated release of free sulfides.

On the basis of these observations, we propose that the mechanism for the antibacterial activity of nFeS involves the release of polysulfanes during the oxidation of nFeS to iron oxide (Fig. 3g). In addition to CyS-nFeS, nFeS products derived from other organosulfur compounds, such as DADS, also possess these enzyme-like activities (Supplementary Fig. 21) and thus are able to quickly release polysulfanes when exposed to H₂O₂. As the release is enhanced by the intrinsic enzyme-like activities of nFeS, we named this phenomenon catalysis-accelerated release (CAR). Taken together, these results indicate that the antibacterial activity of nFeS originates from hydrogen polysulfanes released in the process of nanostructure transformation. The release might be improved by the addition of H₂O₂, which is ascribed to the CAR effect of nFeS.

*S. mutans* biofilm elimination using nFeS. The unique multifunctional properties and enhanced antibacterial activity suggest that nFeS is a suitable agent for effective therapeutic approaches that prevent or treat bacterial infections. To evaluate the ability of nFeS for cariogenic biofilm elimination, we used two distinct infection models and topical treatment regimen akin of clinical situation. First, we examined whether nFeS can disrupt the capacity of cariogenic pathogen *S. mutans* to form biofilms on saliva-coated hydroxyapatite (sHA) surface, a tooth enamel-like material commonly used for dental biofilm experiments (Supplementary Fig. 22a, b)¹³,²⁶–²⁸. To simulate topical exposure, CyS₀.₅-nFeS was applied three times (0, 19 and 29 h, 10 min time−¹) by immersing the sHA discs into CyS₀.₅-nFeS solutions during the course of biofilm growth. As shown in Supplementary Fig. 23a, b, we found that biofilm biomass was markedly disrupted (dry weight, 90% reduction), with bacteria viability considerably reduced (<6-log reduction of viable bacteria) in nFeS-treated biofilms, demonstrating that nFeS effectively disrupted *S. mutans* biofilms. In contrast, neither commercial bulk iron sulfide (bFeS) nor cysteine exhibited any detectable anti-biofilm activity.

In addition, SEM imaging showed that when topically treated with CyS₀.₅-nFeS, no biofilm formation was formed on the sHA surface (Supplementary Fig. 23c). Importantly, while DADS alone failed to reduce the biofilm biomass and cell viability, DADS-nFeS successfully prevented biofilm formation (Supplementary Fig. 24). Together, these results demonstrate that nFeS is the principle agent for biofilm disruption. In addition, the nFeS was able to kill bacteria embedded in a biofilm matrix, indicating that the released polysulfanes can permeate into bacterial biofilm.

To evaluate the impact of nFeS treatment on biofilm integrity, the 3D structure of the biofilm was analyzed by staining the bacterial cells with Syto 9 (Green) and labeling the extracellular polymeric matrix with Alexa Fluor 647 (Red) prior to confocal laser scanning microscopy (CLSM) analysis (Supplementary Fig. 25)²⁹. As shown in Fig. 4a, only small and sparsely distributed bacterial clusters and negligible extracellular matrix were observed following nFeS topical treatment, resulting in highly disrupted biofilm structure. These findings clearly demonstrate that nFeS is a suitable agent for disrupting biofilm development.

We also evaluated the anti-biofilm efficacy of CyS₀.₅-nFeS using a dental biofilm model on human tooth surface (Fig. 4b). Similar topical treatments with CyS₀.₅-nFeS were employed (at 0, 19, 29, 43, and 53 h) during biofilm formation for 67 h. As shown in Fig. 4c, *S. mutans* UA159 readily bound and colonized the dental surface, forming a typical biofilm structure with bacterial clusters and the presence of extracellular matrix (see red arrows). However, when treated with CyS₀.₅-nFeS biofilm accumulation and structural organization was markedly disrupted. This result is consistent with processes of bacterial killing and polymeric matrix inhibition. Using a biochemical assays measuring biomass and viability, we found that the biofilms were reduced by 50% in dry weight and 2-log of bacterial viability (CFU biofilm⁻¹) in the treated group (Fig. 4d, e).

Since, the formation of biofilm matrix was strongly impaired, we assessed whether the activity of glucosyltransferase B (GtfB) exoenzymes, which is responsible for extracellular glucan matrix synthesis³⁰,³¹, was affected. Using established glucan synthesis assays, we found that CyS-nFeS significantly reduced the GtfB activity and polysaccharides synthesis (Fig. 4f). Considering that nFeS is also catalytically active, we examined whether the antibiofilm efficacy could be boosted in the presence of H₂O₂. To this end, biofilms were topically treated with CyS₀.₅-nFeS (administered at 19, 29, 43, and 53 h) in combination with 0.5% H₂O₂ (administered at 19 and 43 h). We observed a strong reduction of both dry weight and bacterial viability compared to that treated with CyS₀.₅-nFeS or H₂O₂ alone (Supplementary Fig. 26). This synergistic effect can be ascribed to three properties, namely releasing polysulfanes from nFeS, enhancing H₂O₂ killing...
efficacy by enzyme-like activity and the CAR effect as described above. Importantly, Cys-nFeS and the released polysulfanes present in the supernatant showed negligible cytotoxic effects towards human oral keratinocytes (HOK) (Supplementary Fig. 27). Altogether, these results indicate that nFeS is effectively able to disrupt the formation of oral biofilm without any visible toxic side effects.

Infect-wound healing using topical nFeS treatment. To further assess its potential as a topical antibacterial alternative, we evaluated the effect of our nFeS on wound healing using an injury-infection model. A model of an infected wound was developed by cutting the back of Balb/c mice to create a wound that was infected with *P. aeruginosa*, a common biofilm-forming pathogen found in chronic wound infections. The nFeS has shown efficient antibacterial activity on *P. aeruginosa* in an in vitro assay (Fig. 2d). SEM characterization showed that the cell morphology of *P. aeruginosa* was changed. In addition, the bacterial flagellum was abrogated, indicating that nFeS damaged the cell integrity of *P. aeruginosa* (Fig. 5a).

Furthermore, the nFeS regimen demonstrated promotion on wound healing in an in vivo model following topical treatment of *P. aeruginosa* infected wound. As shown in Fig. 5b, both Cys0.5-nFeS alone or in combination with H2O2 facilitated wound healing compared to H2O2 alone. Histological analysis showed that minor or no scabbing occurred in the group treated with Cys0.5-nFeS or Cys0.5-nFeS + H2O2 (Fig. 5c). Moreover, the group treated with a combination regimen showed the best recovery from infected wounds, probably because the CAR effect enhances the antibacterial activity of nFeS. Our cell viability assay showed that nFeS (<100 μg mL⁻¹) was not toxic to mouse embryonic fibroblasts cells (3T3). In addition, the supernatant from nFeS solution (up to 500 μg mL⁻¹) showed no cytotoxicity, indicating that the released polysulfanes possess good biocompatibility (Fig. 5d, e). Importantly, the iron oxide (Fe3O4) nanoparticles in Cys0.5-nFeS regimen showed a long-term favorable effect in stimulating cell proliferation, which may facilitate wound recovery (Fig. 5f). In addition to Cyn-nFeS, DADS-nFeS also exhibited similar effects in the above wound healing tests, indicating all nFeS products have this potency in topical application. (Supplementary Fig. 28). Compared to DADS, DADS-nFeS not
only improved wound recovery, but also showed less cytotoxicity (Supplementary Fig. 29). Together, our experimental evaluations clearly demonstrate that nFeS regimen represent an effective therapeutic approach to accelerate wound healing by both reducing bacterial infection as well as stimulating host cell proliferation.

**Discussion**

Our work provided a facile strategy to convert natural organosulfur compounds into nFeS with enhanced antibacterial activity against pathogenic and resistant bacteria as well as antibiofilm efficacy. Strikingly, the conversion increased the antibacterial activity several 100-fold compared to the organosulfur precursors. As a potential antibacterial alternative, nFeS demonstrates the following features: (i) nFeS is a highly stable nanomaterial, making it suitable for long-term use. (ii) nFeS displays high dispersibility in aqueous solution, facilitating its reactivity under biological conditions. (iii) nFeS kills bacteria by releasing bactericidal polysulfanes. Such hydrogen polysulfanes have good diffusibility through biological barriers, including cell wall and...
biofilm matrix, which could facilitate killing of bacteria residing within a biofilm or invading tissues. The products of nFeS are odorless, which may merit their popularity in practical use. These features together provide potential advantages for practical applications of nFeS in biomedicine and consumer healthcare.

In our experiments, the conversion efficiency of nFeS showed strong correlation with the type of S-related chemical bond in the organosulfur compounds. The solvothermal condition (200 °C at high pressure) may induce pyrolysis of the S-related bonds to afford free sulfide for nFeS formation. It has previously been shown that thermal degradation occurs in cysteine thus releasing hydrogen sulfide when the temperature is increased to above 200 °C. Thus cysteine and GSH may undergo similar reaction in our solvothermal system. In addition, pyrolysis of the disulfide (DADS, Cystine) or trisulfide (DATS) forms are more favorable as the bond dissociation energies (BDEs) for C–S in these compounds are lower than those in AMS, DAS, and methionine. Therefore, accurate determination (experimentally or computationally) of the BDE of S-related bonds in organosulfur compounds is critical to better understand the conversion of nFeS. Here we chose a computational method to calculate BDE value of C–S and S–S in organosulfur compounds. However, the calculated values may differ from those experimentally obtained, because the reaction conditions are considered. For instance, the S–S and allylic C–S bond strengths in DADS were experimentally measured as 62 and 46 kcal mol−1, respectively. In comparison, our theoretical calculations resulted in lower values, namely 50.36 and 35.32 kcal mol−1, respectively. In addition, S–S bond in trisulfide was measured as 46 kcal mol−1, compared to a calculated value of 36.47 kcal mol−1 in DATS. These deviations may be ascribed to the different parameters and conditions used in experimental measurements and calculation models. Despite these deviations, the values obtained from both approaches are valuable for understanding the conversion process and mechanism of nFeS formation, and should thereby greatly assist in optimizing nFeS synthesis in solvothermal reaction system.

The antibacterial property of nFeS provides an alternative approach to understanding the bacterial killing mechanism of metal sulfides. Several metal sulfides have been previously reported to efficiently kill bacteria, such as MoS2 and CuS. However, previous research focused primarily on interpreting the antibacterial mechanisms of ROS32,37–40. Here we found that the release of polysulfanes plays a critical role in the antibacterial activity. Their release constitutes a coordinated process that involves nanostructure transformation, oxidation and sulfides release. Importantly, our data demonstrate that polysulfanes (H2S2 and H2S3) may be the primary bactericidal molecules to suppress bacterial viability and biofilm formation. We also ruled out the possibility of H2S in antibacterial action, which is an important point since H2S has been reported to enhance drug resistance of bacteria rather than kill bacteria. Together, our results provide insights into the biological functions of hydrogen polysulfanes, as the understanding for these molecules remains limited in biosystem42,43. Importantly, such mode of releasing polysulfanes may occur in any materials that contain iron sulfides. Many natural minerals are composed of iron sulfide, such as pyrite, marcasite, pyrrhotite and flint. In particular, flint has been found to be able to release hydrogen disulfanes and hydrogen trisulfanes, showing the potential as a donor of polysulfanes that affect microbial ecology. Besides our solvothermal system, nano-iron sulfides can be simply synthesized using a proper sulfur precursor and thus are expected to be able to release polysulfanes. Therefore, releasing polysulfanes may be a general property for inorganic metal sulfides, which will further expand biomedical applications of sulfur-containing nanomaterials.

Future studies will need to investigate the kinetics of polysulfane release, especially when nFeS enters biosystems for in vivo systemic medical applications. Our in vitro antibacterial and antibiofilm tests demonstrated that nFeS not only deformed the bacterial shape when attached on bacterial surface, but also directly inhibited the activity of enzyme excreted by bacteria. These observations suggest that nFeS readily interacts with cell surface and biomolecules at the nano-bio interface. The nanostructure transformation and release of polysulfanes may be affected once biomolecules (proteins, nucleic acids, or metabolites) bind to the surface of nFeS during transit (and thereby prolonged residence in biological fluids) to the targeted site within the body. We have found that protein-rich environment (simulating systemic use) affected the antibacterial effectiveness of nFeS (Supplementary Fig. 30). This effect is likely due to interactions of biomolecules (proteins) on the surface of nFeS thus forming a corona. However, the precise role and characteristics of nano-bio interfaces modulating antibacterial efficacy need to be investigated further, primarily to better understand how the biomolecular corona affects the polysulfanes release within biological fluids. These fundamental investigations will be helpful to specify which pathological status is suitable for nFeS treatment.

In summary, our findings offer a nano-conversion strategy to refine and potentiate the performance of natural products for biomedical application. Converting organosulfur compounds into inorganic nano-sulfides significantly improves antibacterial activity and antibiofilm efficacy. This enhanced antibacterial property benefits from nFeS releasing bactericidal polysulfanes. The effective inhibition on Pseudomonas aeruginosa and Staphylococcus aureus as well as drug-resistant strains, indicates that nFeS could be a potential antibacterial alternative to fight against these pathogens which are in the global priority list released by the WHO. Furthermore, the ability to apply nFeS topically to disrupt pathogenic biofilms and promote wound healing makes it possible for broader applications aimed at preventing or treating biofilm-related infections. Their applications can be extended to disinfect implant devices whose surfaces readily breed bacteria, such as ventilators and blood catheters, often resulting in acute or chronic infections. Therefore, nFeS represents a suitable future option in the fight against pathogenic bacteria with drug resistance or capable of forming intractable biofilms, thus contributing to maintaining high standards of human health presently available.

Methods

Materials. NaCl, NaOAc, glucose, glutaraldehyde were purchased from Sinoparm Chemical reagent (China), FeCl3·6H2O, ethylene glycol, dialyl sulfide (DAS), allyl methyl sulfide (AMS), dialyl disulfide (DADS), dialyl trisulfide (DATS), lysozyme, H2O2 (30%), 2′,7′-dichlorofluorescin diacetate (H2DCFDA), 3,3′,5′,5′-tetramethylbenzidine (TMB), phenylmethyl sulfonyl fluoride (PMSF), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Aldrich. Tryptone and yeast extract were purchased from Oxoid (UK). Hydroxyapatite discs were purchased from Clarkson Chromatography Products Inc. Alexa Fluor 647-dextran conjugate and SYTO 9 green-fluorescent nucleic acid stain were purchased from Life Technologies. Cysteine, cystine, glutathione (GSH), methionine, ethyldiamine, dimethylsulfoxigide (DMSO), and agar were purchased from Sangon Biotech (China). Agarose was purchased from Biowest (Spain). Trans2K Plus II DNA Marker was purchased from TransGen Biotech (China). Streptococcus mutans UA159 (ATCC 700610), Escherichia coli (E. coli, CMCC (B)44102), Pseudomonas aeruginosa (P. aeruginosa, ATCC 27853), Staphylococcus aureus (S. aureus, ATCC 29213), Salmonella enteritidis (S. enteritidis, SC4707) and Candida albicans (ATCC 10231) were purchased from the Institute of Microbiology of the Chinese Academy of Science. BALB/c mice were obtained from the Vital River Laboratories (Beijing). The human oral keratinocytes (HOK) were obtained from the Microbiology of the Chinese Academy of Science. Bacteria were obtained from the Vital River Laboratories (Beijing). The human oral keratinocytes (HOK) were obtained from the Microbiology of the Chinese Academy of Science. Bacteria were obtained from the Vital River Laboratories (Beijing). The human oral keratinocytes (HOK) were obtained from the Microbiology of the Chinese Academy of Science. Bacteria were obtained from the Vital River Laboratories (Beijing). The human oral keratinocytes (HOK) were obtained from the Microbiology of the Chinese Academy of Science.

In vitro antibacterial and antibiofilm activity. Bacterial strains were grown in Mueller-Hinton broth (MHB) at 37 °C for overnight and secreted into cell-free supernatant. The cell-free supernatant was collected by centrifugation and used as the bacterial growth powder for the following antibacterial activity. The bacteria were cultured for 18 h at 37 °C in MHB. The culture supernatant was used as a positive control.

Antibacterial activity tests. Bacterial cultures were grown in MHB at 37 °C for 18 h. A sterile field was prepared using a 96-well plate and 200 μL of each bacterial culture was added to each well. The bacteria were incubated at 37 °C for 18 h in MHB. The bacterial concentration was adjusted to OD600 = 0.05. The bacterial suspension was prepared in MHB. The bacterial suspension was added to the wells at a final concentration of 105 CFU/mL. The bacterial suspension was added to the wells at a final concentration of 106 CFU/mL. The bacterial suspension was added to the wells at a final concentration of 107 CFU/mL. Bacterial cultures were incubated for 18 h at 37 °C in 5% CO2 with 96-well plates. The antibacterial activity was measured by endpoint growth curves.

Antibacterial activity tests. Bacterial cultures were grown in MHB at 37 °C for 18 h. A sterile field was prepared using a 96-well plate and 200 μL of each bacterial culture was added to each well. The bacteria were incubated at 37 °C for 18 h in MHB. The bacterial concentration was adjusted to OD600 = 0.05. The bacterial suspension was prepared in MHB. The bacterial suspension was added to the wells at a final concentration of 105 CFU/mL. The bacterial suspension was added to the wells at a final concentration of 106 CFU/mL. The bacterial suspension was added to the wells at a final concentration of 107 CFU/mL. Bacterial cultures were incubated for 18 h at 37 °C in 5% CO2 with 96-well plates. The antibacterial activity was measured by endpoint growth curves.
**Determination of internal malondialdehyde.** Intracellular malondialdehyde (MDA) was quantified as an indicator of lipid peroxidation. After treatment with Cys-nFeS or 0.1 M NaOAc (control) for 30 min, S. mutans UA159 was lysed by lysozyme and proteinase K for MDA measurement. Bacterial lysates were collected by using a Takara MiniBEST Bacteria Genomic DNA Extraction Kit Ver. 3.0 (TaKaRa, Japan). In the latter experiments, DNA extracts were identified in agarose gel electrophoresis with ethidium bromide staining.

**Determination of DNA degradation.** After treatment with Cys-nFeS or 0.1 M NaOAc for 30 min, S. mutans UA159 was lysed by lysozyme and proteinase K for DNA extraction. Bacterial lysates were collected by using a Takara MiniBEST Bacteria Genomic DNA Extraction Kit Ver. 3.0 (TaKaRa, Japan). In the latter experiments, DNA extracts were identified in agarose gel electrophoresis with ethidium bromide staining.

**Bacteria characterization with SEM.** The morphology of S. mutans UA159 cells incubated with Cys-nFeS or 0.1 M NaOAc was examined by scanning electron microscope (SEM). First, bacteria suspensions washed with 0.89% (w/v) were resuspended in glutaraldehyde (2%, Sigma-Aldrich) for 4 h at 4 °C under dark conditions. Bacterial cells were then washed and treated with ethanol gradient dehydration (30%, 50%, 70%, 90%, and 100% twice), followed by drying with a critical point dryer and coating with platinum sputter. Finally, the bacterial cells were coated with platinum sputter and analyzed using a Hitachi S–4800 FE-SEM (Hitachi-S4800). Scanning electron microscope (SEM) images were obtained on a Hitachi S–4800 FE-SEM at a working voltage of 15.0 kV and a working current of 10 μA under magnification of 40k.

**Identification of polysulfanes release from nFeS.** For color observation at different times, 1 mL of nFeS (1.0 mg mL$^{-1}$) in distilled water was incubated in a 24-well plate (Corning Inc., NY). The color of the solution was recorded using a camera. The precipitates from the solution were collected by centrifugation at 3500 × g for 5 min. The collected precipitates were washed with ethanol three times and characterized with SEM for morphological and EDS analysis.

The free sulfide and acid-labile sulfide levels were measured by reverse-phase high-performance liquid chromatography (RP-HPLC) after derivatization with excess monobromobimane (MBB) as stable sulfdide-dibimide (SDB) products. Briefly, nanoparticle materials (1.0 mg mL$^{-1}$, dissolved in PBS) were centrifuged at 3500 × g for 5 min. The supernatants were derivatized using MBB. Thirty microliters of the sample was incubated with 100 μL of Tris-HCl reaction buffer (100 mM Tris, 0.1 mM DTPA, pH 9.5). The reaction was performed on a BEH C18 column (2.1 mm × 100 mm, 1.7 μm particle size) (Waters, Mississauga, ON, Canada). The mobile phases for LC were water (0.1% TFA, A) and acetonitrile (0.1% TFA, B), and the flow rate was set to 0.3 mL min$^{-1}$. The gradient was as follows: 0 min, 15% B; 0–10 min, 45% B (linear); 10–11 min, 95% B (linear) and hold for 1 min; 12–15 min, 15% B (linear) and hold for 2.0 min. Data were collected in MRM mode by screening parent and daughter ions simultaneously, the cone voltage was set depending upon each specific MRM for each metabolite, and the dwell time was automatically set by the MassLynxTM 4.1 software. The optimum collision energy was 30 V. Production ions for MBB derivatives of hydrogen sulfide (H2S), cysteine (cys-SH), disulfanes (HSSH, CSSSH), and trisulfanes (S-S-SH, Cy5-SSSH) levels were 192.1 m/z. Their levels were calculated by the peak area ratio of the signature productions (192.1 m/z) to the corresponding stable S34DB peak (internal standard).

**Enzyme-like activities of nFeS.** The peroxidase-like activity was determined by monitoring the absorbance change at 562 nm on a Microplate Reader (Tecan, Switzerland) in time-course mode at room temperature. The kinetic assays were carried out using 0.2 μg nFeS in 100 μL of reaction buffer (0.1 M NaOAc buffer, pH 4.5) in the presence of H2O2 and TMB. The kinetic analysis of nFeS with H2O2 as the substrate was performed by varying the concentrations of H2O2 with 0.8 mM TMB, and vice versa. The absorbance (562 nm) changes were calculated according to a double reciprocal concentration changes of TMB by using a molar absorption coefficient of 39,000 M$^{-1}$ cm$^{-1}$ for TMB-derivated oxidation products according to the Beer-Lambert law. All measurements were performed at least in triplicate, and the values were average. The results are given as the mean ± standard deviation (SD). The Michaelis-Menten constant was calculated using Lineweaver–Burk plots of the double reciprocal of the Michaelis-Menten equation (equation 2) by GraphPad Prism (GraphPad Software).

\[ v = \frac{V_{\text{max}} \times S}{K_m + S}, \]  

\[ \nu = \frac{V \times \text{fl}}{(E_A + E_B)}, \]  

where $E_A$, $E_B$, and $E_{\text{tot}}$ are the total energies of AR, A', and B, respectively. The larger $E_{\text{tot}}$ is the stronger bond A-B. The solvent effects of water were considered in all calculations using the SMD model. All calculations were performed using the GAUSSIAN 09 program.
where v is the initial velocity, Vmax is the maximal reaction velocity, [S] is the substrate concentration and Kₘ is the Michaelis-Menten constant.

For the second-order assay, all the reaction conditions were same, including the sample mass, H₂O₂ and TMB concentrations, pH and temperature. One unit (U) was defined as the amount of enzyme (sample, mg) required to generate 1 μM of TMB in 1 min at 37 °C in 0.1 M NaOAc (pH 4.5).

The catalase-like activity assay of nFeS was carried out at room temperature by measuring the generated oxygen using a specific oxygen electrode on a Multi-Parameter Analyzer (IPS-606L, Leici, China). The generated O₂ solubility (unit: mg L⁻¹) was measured at different reaction times and the effect of the H₂O₂ concentration on the generated O₂ was also detected by recording the O₂ solubility in NaOAc solution (pH 7.0). The Michaelis-Menten constant was calculated using the same method as mentioned above.

Biofilm formation. Biofilms were grown using saliva-coated hydroxyapatite (sHA) discs or dental films. Whole saliva (from one male volunteer, adult) was collected and mixed with equal volume of cold adsorption buffer (50 mM KCl, 1 mM potassium phosphate (0.35 mM K₃HPO₄ plus 0.65 mM KH₂PO₄), 1 mM CaCl₂, 0.1 mM MgCl₂, Adjust pH to 6.5). Next, cold 1 mM phenylmethyl sulfonyl fluoride (PMSF) was added to above saliva mixture containing oxygen electrode on a Multi-Parameter Analyzer (IPS-606L, Leici, China). The generated O₂ solubility (unit: mg L⁻¹) was measured at different reaction times and the effect of the H₂O₂ concentration on the generated O₂ was also detected by recording the O₂ solubility in NaOAc solution (pH 7.0). The Michaelis-Menten constant was calculated using the same method as mentioned above.

Cell viability assay of human oral keratinocytes (HOK). HOK cells (SC-2610, ScienCell, USA) were seeded into a sterile 96-well plate (Corning Inc., NY) at 10³ cells in the medium (DMEM with 10% FBS) and followed to attach for 24 h at 37 °C under 5% CO₂. The cells were treated with a series of Cys₅₋₅-nFeS solutions (31.25, 62.5, 125, 250, and 500 μM L⁻¹) for 10 min, and then, Cys₅₋₅-nFeS was washed away. Fresh medium was added, and the cells were cultured for 24 and 48 h. In addition, the cells were also treated with supernatant from the corresponding culture medium after co-incubation in Cys₅₋₅-nFeS for 10 min. Next, sHA discs or dental films were incubated in Cys₅₋₅-nFeS solutions (31.25, 62.5, 125, 250, and 500 μM L⁻¹) of Cys₅₋₅-nFeS. The wounds were photographed, and bandages were changed at 24 h intervals. After 6 days of therapy, the mice were killed, and the related wound tissue was collected and fixed by formalin. The wound tissues were then paraffin-sectioned, and analyzed via Hematoxylin-Eosin (HE) staining. The tissue sections were examined by a Nikon Eclipse Ci microscope in bright-field mode.

Mouse injury model. All animal studies were performed following a protocol approved by the Institutional Animal Care and Use Committee of the Institute of Biophysics, Chinese Academy of Sciences. Twenty-five 6–8-week-old male Balb/c mice were purchased from Vital River. The back of each mouse was cut and injected with 10⁷ CFU of P. aeruginosa bacteria to build the infected wound model according to a study reported by Cao et al.35. The mice were then divided into five groups (five mice per group). The mice with infected wounds in the different groups were treated with the gauze containing 10 μL of NaOAc buffer, 100 μM H₂O₂, 100 μg mL⁻¹ of Cys₅₋₅-nFeS, or 100 μM H₂O₂ + 100 μg mL⁻¹ of Cys₅₋₅-nFeS. The wounds were photographed, and bandages were changed at 24 h intervals. After 6 days of therapy, the mice were killed, and the related wound tissues were collected and fixed by formalin. The wound tissues were then paraffin-sectioned, and analyzed via Hematoxylin-Eosin (HE) staining. The tissue sections were examined by a Nikon Eclipse Ci microscope in bright-field mode.

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Dentin samples preparation. Twenty non-carious single-rooted teeth (adult) maintained in phosphate-buffered saline (PBS) were used. The crowns were sectioned at the level of the cementoenamel junction, and apical portions were ground to obtain root sections. The tooth specimens were then vertically sectioned along the mid-sagittal plane into 2 halves (mesial and distal). The root canal lumen was flattened using progressive 1000–4000 grit silicone carbide papers. Silicon carbide paper was used on the external root surface to ensure that the surfaces were parallel to the root canal wall in the section. The samples were then ultrasonically cleaned in deionized water for 30 min to obtain root canal dentin specimens without the presence of the smear layer. The samples were stored in double-distilled water until further use. The study was approved by the Sikhuan University Ethics Board (WCHSIRB-D-2016-140).

GtfB activity in the present of nFeS. The influence of the presence of Cys₅₋₅-nFeS on the synthesis of glucans by GtfB was determined in solution phase. The GtfB enzyme was prepared and purified using hydroxyapatite column chromatography. For glucan synthesis in the solution phase, GtfB (10 units) was mixed with Cys₅₋₅-nFeS (0.5 mg mL⁻¹), and incubated with (14% Clglocuculose)-succrose substrate (0.2 μM L⁻¹, 200 mM sucrose, 40 μM dextran 9000, 0.02% sodium azide in bi-distilled water at pH 6.5) to reach a final concentration of 100 mM sucrose (reaction volume of 200 μL), and incubated at 37 °C. After 4 h incubation, the total amount of glucans formed was measured by scintillation counting. The control contained the same reaction mixture with adsorption buffer but without Cys₅₋₅-nFeS. The solutions were incubated at 37 °C for 4 h to allow glucan synthesis. Subsequently, the glucans were precipitated with ice-cold ethanol (final concentration of 70%) for 18 h at 4 °C. The radiolabeled glucans were then determined by scintillation counting.

Mouse injury model. All animal studies were performed following a protocol approved by the Institutional Animal Care and Use Committee of the Institute of Biophysics, Chinese Academy of Sciences. Twenty-five 6–8-week-old male Balb/c mice were purchased from Vital River. The back of each mouse was cut and injected with 10⁷ CFU of P. aeruginosa bacteria to build the infected wound model according to a study reported by Cao et al.35. The mice were then divided into five groups (five mice per group). The mice with infected wounds in the different groups were treated with the gauze containing 10 μL of NaOAc buffer, 100 μM H₂O₂, 100 μg mL⁻¹ of Cys₅₋₅-nFeS, or 100 μM H₂O₂ + 100 μg mL⁻¹ of Cys₅₋₅-nFeS. The wounds were photographed, and bandages were changed at 24 h intervals. After 6 days of therapy, the mice were killed, and the related wound tissues were collected and fixed by formalin. The wound tissues were then paraffin-sectioned, and analyzed via Hematoxylin-Eosin (HE) staining. The tissue sections were examined by a Nikon Eclipse Ci microscope in bright-field mode.
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**Acknowledgements**

We thank the Testing and Analysis Centre at Yangzhou University for the nanomaterial characterizations. This work was supported by the National Natural Science Foundation of China (Grant No. 81671810), the Jiangsu Provincial Basic Research Program for the Interdisciplinary Science Foundation (Grant No. BK20161333), the Foundation of the Thousand Talents Plan for Young Professionals and Jiangsu Specially-appointed Professor, and in part by the National Institutes of Health/National Institute of Dental and Craniofacial Research (NIH/NIDCR) R01 DE025848.

**Author contributions**

L.G. conceived and designed the experiments. Q.L., Z.Q., D.L., Y.T. and J.Q. synthesized and characterized the materials. Z.X., Z.Q., D.L. and X.S. characterized sulfide release and the bacterial killing to suppress dental caries in vivo. Structural and functional characterization of the exopolysaccharide matrix and its interactions with the bacterial cell wall were performed. Molecular modeling and computational studies were conducted. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.
Identification. Q.L., Z.Q., and Y.G. contributed to the enzyme kinetics assay. Z.X. and Z.Q. performed the bacteria and biofilm assays and obtained the images. J.H. and J.X. provided the dentin sample. X.G. calculated the energy of the C–S bond in organosulfur compounds. Y.L. and H.K. contributed to the dental biofilm assay. Y.H., Z.X. and J.J. analyzed cell toxicity and K.F. performed wound healing experiment. L.G. and Z.X. analyzed the data and wrote the manuscript. H.K. and X.Y. helped revise the manuscript. All authors discussed the results and commented on the manuscript.

Additional information
Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-018-06164-7.

Competing interests: The authors declare no competing interests.

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