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Aloe-emodin inhibits *Staphylococcus aureus* biofilms and extracellular protein production at the initial adhesion stage of biofilm development

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Abstract *Staphylococcus aureus* (*S. aureus*) biofilms are clinically serious and play a critical role in the persistence of chronic infections due to their ability to resist antibiotics. The inhibition of biofilm formation is viewed as a new strategy for the prevention of *S. aureus* infections. Here, we demonstrated that minimum inhibitory concentrations (MICs) of aloe-emodin exhibited no bactericidal activity against *S. aureus* but affected *S. aureus* biofilm development in a dose-dependent manner. Further studies indicated that aloe-emodin specifically inhibits the initial adhesion and proliferation stages of *S. aureus* biofilm development. Scanning electron microscopy (SEM) indicated that the *S. aureus* ATCC29213 biofilm extracellular matrix is mainly composed of protein. Laser scanning confocal microscope assays revealed that aloe-emodin treatment primarily inhibited extracellular protein production. Moreover, the Congo red assay showed that aloe-emodin also reduced the accumulation of polysaccharide intercellular adhesin (PIA) on the cell surface. These findings will provide new insights into the mode of action of aloe-emodin in the treatment of infections by *S. aureus* biofilms.

Keywords *Staphylococcus aureus* · Biofilm · Aloe-emodin · Inhibitor

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Introduction

Staphylococcus aureus (*S. aureus*) is the most common pathogen in cases of bacteraemia, endocarditis and sepsis. It is also involved in a variety of biofilm-associated infections such as those in heart implants and bloodline catheters, superficial skin infections and otitis media (Agarwal and Jain 2010; Thornton et al. 2013). Management of biofilm infections is extremely difficult due to the fact that bacterial cells embedded in biofilms exhibit inherent resistance to anti-microbial agents because they have a dormant phenotype; thus, biofilms can radically reduce cell metabolism and contribute to the bacterial persistence in chronic infections. In addition, biofilms represent a reservoir that can disseminate *S. aureus* infections to other sites in the host body, leading to recurrent infection and increased fatalities in cases of the most recalcitrant infections (Lewis 2010; Waters et al. 2016). Thus, it is important to identify new therapeutic strategies that interfere with biofilm development.

The development of bacterial biofilms can be divided into four stages: initial adhesion, proliferation, maturation and diffusion (Boles and Horswill 2011; Otto 2008b). In the initial stage, the planktonic *S. aureus* cells attach reversibly to a solid living or non-living substratum. The specific interaction between so-called MSCRAMMs (microbial surface components

recognizing adhesive matrix molecules) and human matrix proteins such as fibrinogen or fibronectin is most likely of much greater importance for the initiation of biofilm formation on biotic materials (Foster et al. 2014). In the absence of human matrix molecules, *S. aureus* may attach to abiotic surfaces through electrostatic and hydrophobic interactions (Kennedy and O'Gara 2004). The negatively charged teichoic acids and the major autolysin, AtlA, also have been shown to be involved in cell attachment to polystyrene and glass surfaces (Biswas et al. 2006; Houston et al. 2011; Gross et al. 2001).

After attaching to a surface, the adherent bacteria begin to divide, accumulate and form microcolonies (Stoodley et al. 2008). When a sufficient nutrient source is present and microcolonies are formed, the biofilm undergoes the maturation stage. This stage is characterized by intercellular aggregation and the establishment of a more complex mature biofilm with a more three-dimensional appearance and mushroom-like cell towers that surround fluid-filled channels, which are equipped to aid the flow of nutrients into the interior of the biofilm (Otto 2008a).

In the final stage of development, single cells or larger cell clusters can be dispersed from the biofilm via mechanical forces such as flow in a blood vessel and via detachment factors such as surfactants or enzymes that destroy the matrix. Detachment is crucial for dissemination of bacteria to new niches.

Targeting the different developmental stages of biofilm formation, three principal strategies have been developed: inhibition of attachment, disruption of biofilm architecture and signal transduction interference (Chung and Toh 2014). Several distinct inhibitor classes have been identified including synthesized small molecules or plant-derived natural compounds (Brackman and Coenye 2015; Mogosanu et al. 2015), silver ions and nanoparticles (Jia et al. 2017), matrix-targeting enzymes (Itoh et al. 2005), a cell wall-degrading enzyme from *S. aureus* bacteriophage (Kelly et al. 2012), synthetic anti-biofilm peptides (Pletzer and Hancock 2016) and anti-biofilm polysaccharides (Rendueles et al. 2013).

Currently, research work in our group mainly focuses on identifying new potential anti-infection small molecule compounds from medicinal herbs that target key bacteria virulence factors such as SrtA and α-haemolysin (Hla)in *S. aureus* and listeriolysin O in *Listeria monocytogenes* (Qiu et al. 2012; Wang et al. 2015a; Wang et al. 2015b). The capacity to form biofilms is now considered to be one of the important virulence traits in several *Staphylococcus* bacteria. By detecting the inhibition of biofilm formation, we have screened anti-biofilm molecules from TCM that have detoxification effects. Several compounds from the medicinal herb rhubarb (*Rheum officinale*) have been identified.

Chinese rhubarb (*R. officinale*) is one of the most well-known traditional Chinese herbal medicines and has been used for the treatment of various diseases including constipation, jaundice, gastrointestinal haemorrhage and ulcers for over 2000 years (Tang et al. 2012; ZHENG et al. 2013). In recent years, this rhubarb has also been shown to have anti-bacterial, anti-oxidant, anti-cancer, anti-angiogenesis and anti-inflammation effects (Hu et al. 2014).

Anthraquinone derivatives including aloe-emodin, chrysophanol, emodin and physcion are the primary active constituents of rhubarb (Komatsu et al. 2006). We had a strong interest in aloe-emodin because of its relatively high *S. aureus* biofilm inhibitory activities (Fig. 1a). Pharmacological data indicate that aloe-emodin has a broad spectrum of biological activities such as anti-bacterial, anti-viral, hepatoprotective and anti-cancer effects (Park et al. 2009); however, its effect on anti-biofilm activity is seldom reported. In the present study, we report the effect of aloe-emodin on biofilm formation by *S. aureus* on different materials and elucidate the primary mechanisms that are responsible for this kind of action.

Materials and methods

Bacterial strains, growth conditions and materials

S. aureus ATCC29213 (National Center for Medical Culture Collections) was used in this study, as it is proficient in biofilm formation (Abouelhassan et al. 2014). The strains were subcultured in brain-heart infusion (BHI) solution (Oxoid, Basingstoke, UK) that contained 3% NaCl and 0.5% glucose and were incubated for 12 h at 37 °C. Aloe-emodin and other compounds were obtained from the Changchun Baotaike Biotech Company (Changchun, China). Sytox green, fluorescein isothiocyanate (FITC), Syto 63 and Texas Red-concanavalin A (ConA) were obtained from Invitrogen-Molecular Probes (Invitrogen-Molecular Probes, Oregon).

Antibacterial activity assays

The minimum inhibitory concentration (MIC) of aloe-emodin was determined by a microtitre broth dilution method, as described in the CLSI guidelines (Kim et al. 2015). In brief, serial twofold dilutions of aloe-emodin in fresh BHI broth were undertaken, and *S. aureus* ATCC29213 was added at 1×10^5 CFU/ml to sterile 96-well polystyrene plates (Costar 3595; Corning Life Sciences). The assay plates were incubated at 37 °C for 24 h, and the bacterial growth inhibition assays were monitored by measuring the absorbance at 600 nm.

Crystal-violet screening assay for biofilm inhibitors

Biofilm inhibitors screening was based on a high-throughput assay described previously (Freeman et al. 1989). Overnight-cultured bacteria were diluted 1:100 into fresh BHI that was supplemented with 3% NaCl and 0.5% glucose. Then, 200 μl cultures were transferred into sterile 96-well polystyrene

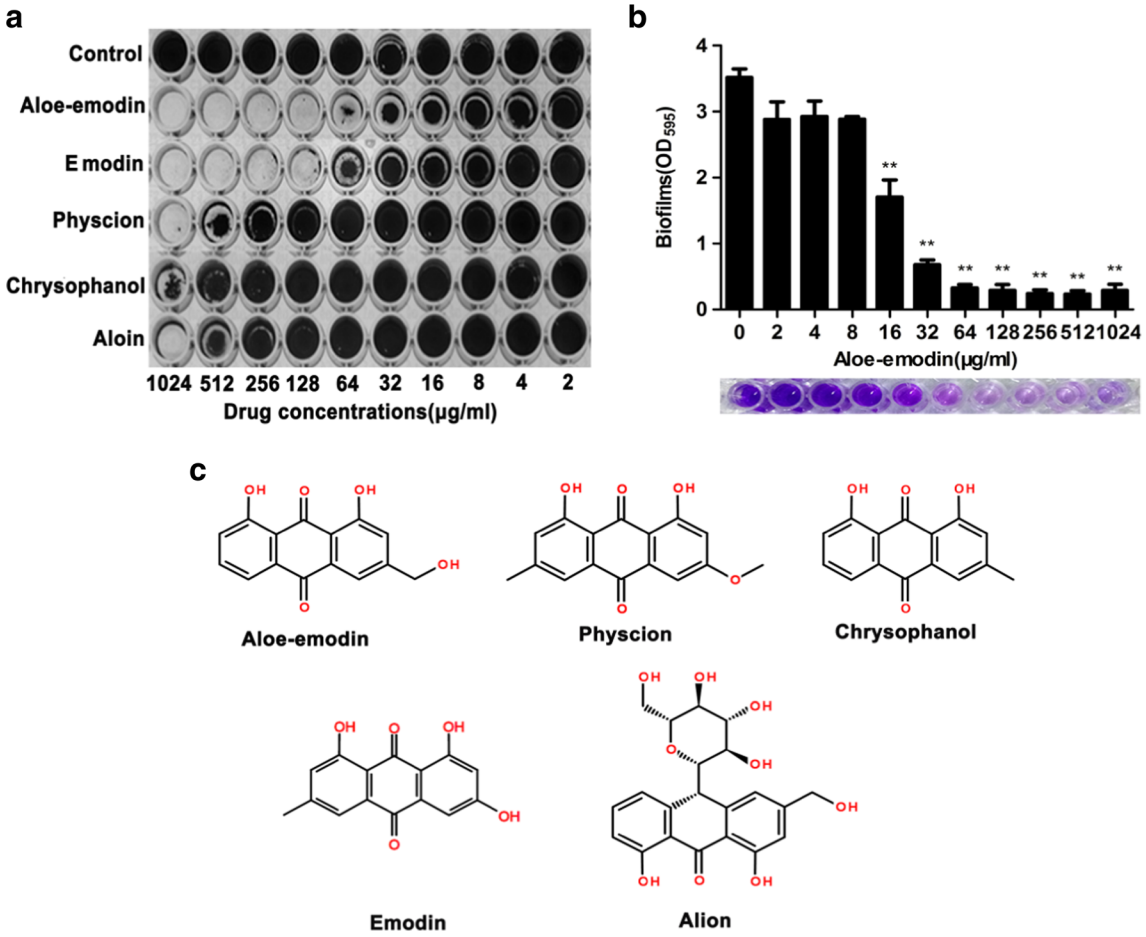

Fig. 1 **a** The anti-biofilm activities of anthraquinone compounds against *S. aureus* were determined by crystal violet staining. **b** The anti-biofilm activities (OD_{595}) of aloe-emodin against *S. aureus* were determined. The statistical significance was determined using one-way analysis of variance (ANOVA) (*$P < 0.05$; **$P < 0.001$). **c** Chemical structures of aloe-emodin and related compounds

plates and covered with 20% rabbit blood plasma at 4 °C overnight. Aloe-emodin and other compounds were added to the assay plates at different concentrations, and the plates were incubated for 12 h without shaking at 37 °C. Then, the supernatants were removed, and the wells were washed twice with PBS. The wells were stained with crystal violet for 15 min, and the plates were thoroughly washed with PBS solution and then dissolved in 200 μl of 100% ethanol for 30 min at room temperature. The biofilms were quantified using a microplate reader (Infinite®F500, Tecan, Shanghai, China) at OD_{595}. The biofilm formation of SrtA, Fnbps and SarA knockdown strains was based on methods described previously.

Confocal microscopy

S. aureus ATCC29213 biofilms were evaluated by confocal laser-scanning microscopy (Olympus, Shanghai, China). As described above, the biofilm was formed on glass with or without aloe-emodin at different concentrations (Hochbaum et al. 2011). The assay plates were incubated at 37 °C for 3, 6, 12 or 24 h. After culturing, the wells were washed twice with 2 ml of PBS. Sytox green (0.5 μM) was added to PBST (PBS supplemented with 0.5% Triton X-100), and the combination was added to assay plates that were then shaken for 30 min. FITC (0.001%) or ConA (50 μg/ml) and Syto 63 (100 μM) were added to PBS, which was added to the well without shaking. The supernatants were removed, and the wells were washed several times with PBS. Confocal microscopy images were observed using NIS-Elements C version 3.2 (Nikon eclipse).

Assay of anti-biofilm effects on different surfaces

The assay was based on a previously described method (Opperman et al. 2009). The biofilm adhered to glass, polystyrene, polyvinyl-chloride (PVC) and silicone. The ability of aloe-emodin to inhibit biofilm attachment to PVC and polystyrene was assayed using 96-well polyvinyl chloride (PVC) assay plates and 96-well polystyrene assay plates, respectively. Assays of aloe-emodin inhibiting biofilm attachment to glass and silicone were performed in six-well assay plates that contained glass and silicone disks, respectively. All assays

were conducted with or without aloe-emodin at different concentrations and were incubated for 12 h at 37 °C.

Effect of aloe-emodin on growth cycle of biofilm formation

S. aureus ATCC29213 biofilm formation was established as described above (Opperman et al. 2009). The assay plates were inoculated with or without 128 μg/ml aloe-emodin at various times. After inoculation, the biofilms were quantified as described previously.

Primary attachment assay

The static biofilm primary attachment assay was performed as described above (Cue et al. 2015). Bacteria were adhered for 1 h at 37 °C, washed with PBS and stained with crystal violet, and the OD_{595} of the samples was measured.

Identification of the main components of *S. aureus* biofilms

To determine the main components of the *S. aureus* ATCC29213 in BHI that was supplemented with 3% NaCl and 0.5% glucose (Mazmanian et al. 2002), the biofilm formation assay was carried out as previously described but without aloe-emodin (Opperman et al. 2009). After 12 h of incubation at 37 °C, the plates were washed with PBS, followed by the separate addition of 2 mg/ml DNase I (Sigma-Aldrich, Tokyo, Japan), 100 μg/ml proteinase K and 10 mM sodium metaperiodate; sterile BHI medium with sodium metaperiodate butter (50 mM sodium acetate buffer, pH 4.5) and saline was used as the control. After their incubation for 2 h at 37 °C without shaking, the plates were washed twice with PBS, and the biofilms were evaluated by crystal violet staining.

Scanning electron microscopy

S. aureus ATCC29213 biofilms were formed on six-well plates that contained glass coverslips and were examined using scanning electron microscopy (SEM) (Chu et al. 2016). After 12 h of incubation at 37 °C, the glass coverslips were washed with PBS and fixed with 2.5% glutaraldehyde at 4 °C for 12 h. Then, the wells were dehydrated with ethanol (60, 70, 80, 90, 95 and 100%) and coated with a 10-nm layer of gold/palladium. Scanning electron microscopy images were observed using a Hitachi S-5000 field emission SEM.

Congo red agar method

The Congo red agar method was used as previously reported (Chu et al. 2016). Briefly, the medium was composed of 37 g/l Tryptone Soy Broth (TSB), 50 g/l sucrose and 0.8 g/l Congo red. Congo red dye was autoclaved (121 °C for 15 min separately from other medium components) and was added when the agar had cooled to 55 °C. *S. aureus* ATCC29213 was incubated on Congo red agar plates with or without aloe-emodin (32, 64 and 128 μg/ml) for 24 h at 37 °C.

Autolysis assays

The assays were performed as described previously (Opperman et al. 2009). *S. aureus* ATCC29213 was incubated on medium with or without aloe-emodin (64 and 128 μg/ml) for 12 h at 37 °C. Then, the cultures were harvested by centrifugation, washed twice with ice-cold deionized water and suspended in 50 ml of 0.05% Triton X-100 solution (50 mM Tris-HCl [pH 7.5]). The cultures were then incubated at 30 °C with shaking. Samples were taken every 30 min, and their OD_{600} was measured.

Statistical analysis

The Student's *t* test was performed using SPSS 13.0 software, and the data were expressed as the mean ± standard deviation. Results with $P < 0.05$ and $P < 0.001$ were considered statistically significant.

Results

Aloe-emodin inhibited biofilm formation by *S. aureus* ATCC29213 without affecting planktonic cell growth

We have conducted screening of phytochemicals with anti-biofilm activity against *S. aureus* ATCC29213 in 96-well polystyrene plates that were covered with 20% rabbit freeze-dried plasma. Five anthraquinone compounds from *R. officinale* (aloe-emodin, emodin, physcoin, chrysophanol and aloin) inhibited *S. aureus* ATCC29213 biofilm formation at different concentrations (Fig. 1c). Among these compounds, aloe-emodin has the most potential (Fig. 1a). The results showed that aloe-emodin at 16 μg/ml markedly inhibited *S. aureus* ATCC29213 biofilm formation (Fig. 1a). Further evaluation showed that 32 μg/ml of aloe-emodin was required to inhibit biofilm formation by ≥85% (Fig. 1b).

Confocal laser microscopy was used to analyse changes in biofilm formation on glass and was in-line with biofilm data that were obtained using 24-well polystyrene plates. The fluorescent images indicated that in the control group, the biofilm gradually increased as time progressed from 3 to 24 h and aloe-emodin dose-dependently inhibited *S. aureus* ATCC29213 biofilm formation (Fig. 2a).

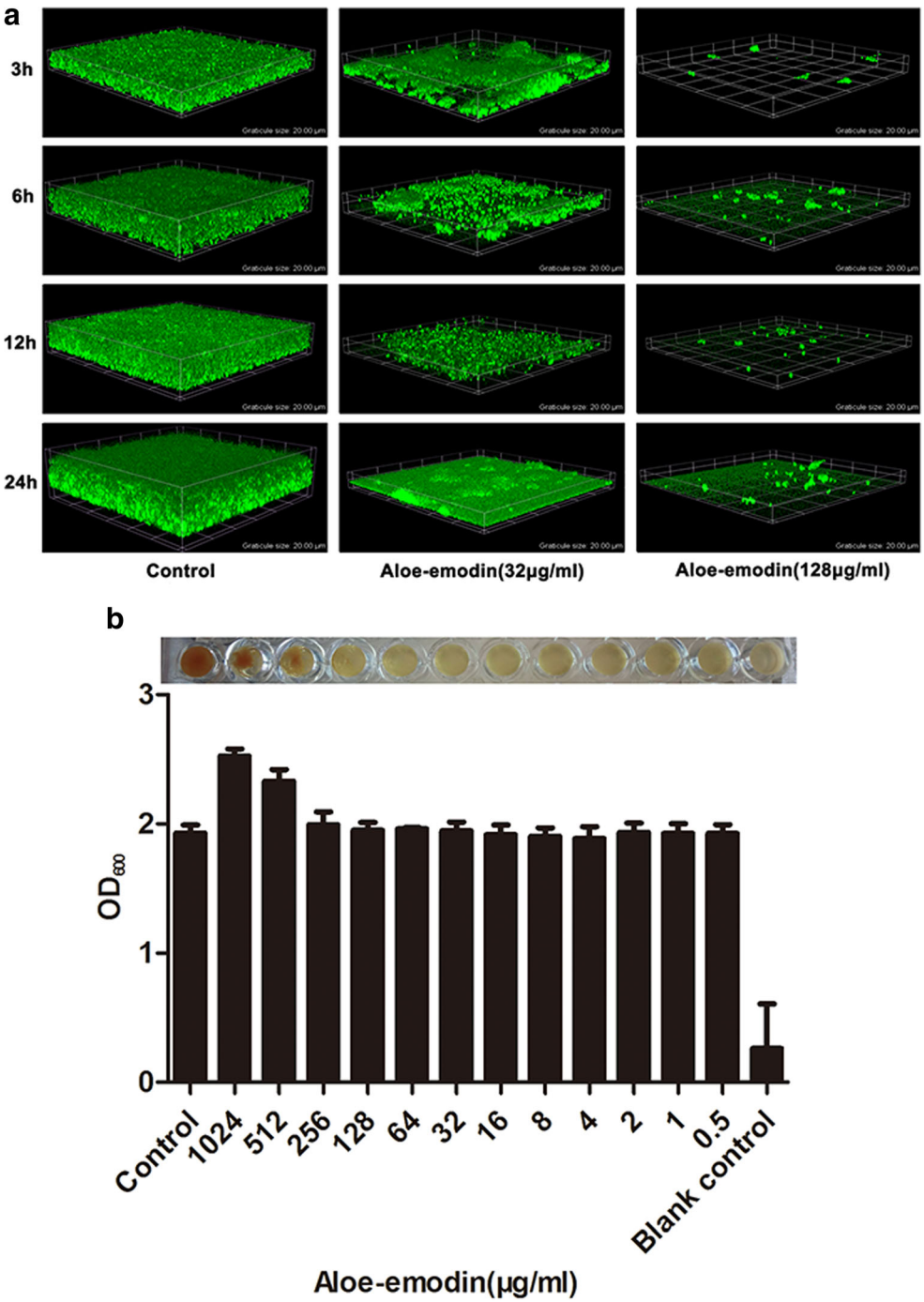

Fig. 2 **a** *S. aureus* ATCC29213 was incubated with different concentrations of aloe-emodin. Biofilm formation on glass was observed at 3, 6, 12 and 24h respectively by confocal laser microscopy. *Scale bar* represents 20 μm. **b** Minimum inhibitory concentration assay

To verify whether aloe-emodin inhibited *S. aureus* ATCC29213 biofilm formation by affecting bacterial growth, the anti-microbial activity of aloe-emodin was investigated by determining the MICs. As shown in Fig. 2b, the MICs of aloe-emodin against *S. aureus* ATCC29213 were >1024 μg/ml. These findings show that the reduced biofilm formation caused by aloe-emodin was due to its anti-biofilm activity and not to its anti-microbial activity.

Aloe-emodin inhibits biofilm formation on abiological surfaces

S. aureus can develop biofilms on the surfaces of different materials. To determine whether aloe-emodin inhibits biofilm formation on an abiological surface, the ability of aloe-emodin to inhibit biofilm formation of *S. aureus* ATCC 29213 on glass, polystyrene, polyvinyl chloride (PVC) and silicone

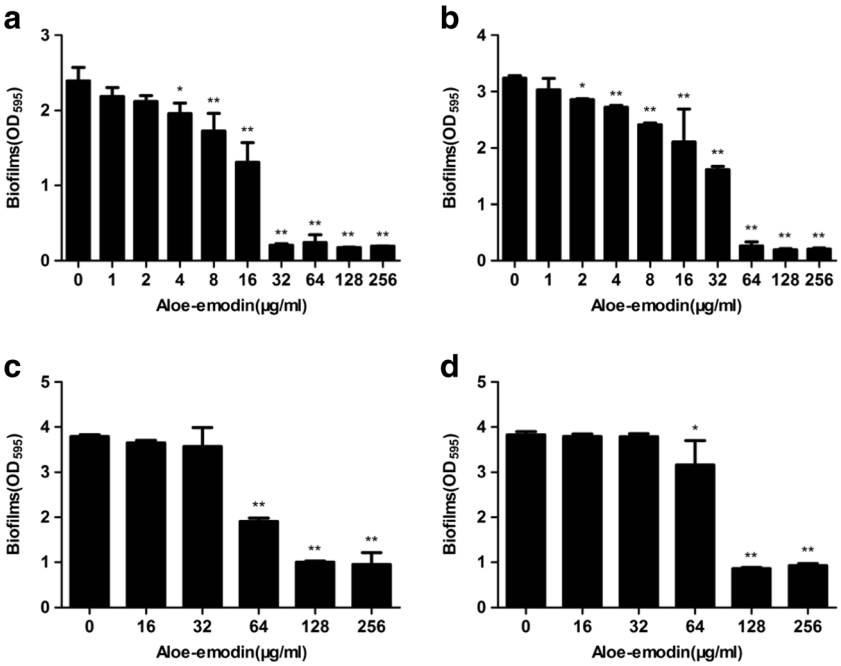

Fig. 3 Anti-biofilm activities of aloe-emodin against *S. aureus* ATCC 29213 on abiological surfaces. **a** PVC surface. **b** Polystyrene surface. **c** Glass surface. **d** Silicone surface. The statistical significance was calculated by one-way analysis of variance (ANOVA) (*$P < 0.05$; **$P < 0.001$)

was determined. Aloe-emodin exhibited significant anti-biofilm activity on polystyrene and PVC surfaces. At a concentration of 32 μg/ml, this compound almost completely inhibited biofilm formation on PVC surfaces. When the concentration of aloe-emodin was greater than 64 μg/ml, it inhibited biofilm formation on other abiological surfaces (Fig. 3). The results showed that aloe-emodin not only inhibits biological surface biofilm formation but also inhibits biofilm formation on abiological surfaces.

Aloe-emodin inhibited the initial adhesion and proliferation stages of biofilm development

To identify the biofilm development stages of *S. aureus* ATCC29213 under the above culture conditions (0.5% glucose, 3% NaCl), we performed tests based on the method previously described (Otto 2013). The results showed that the period from 0 to 1 h was the bacterial adhesion stage, 1–12 h was the bacterial proliferation stage, 12–72 h was the

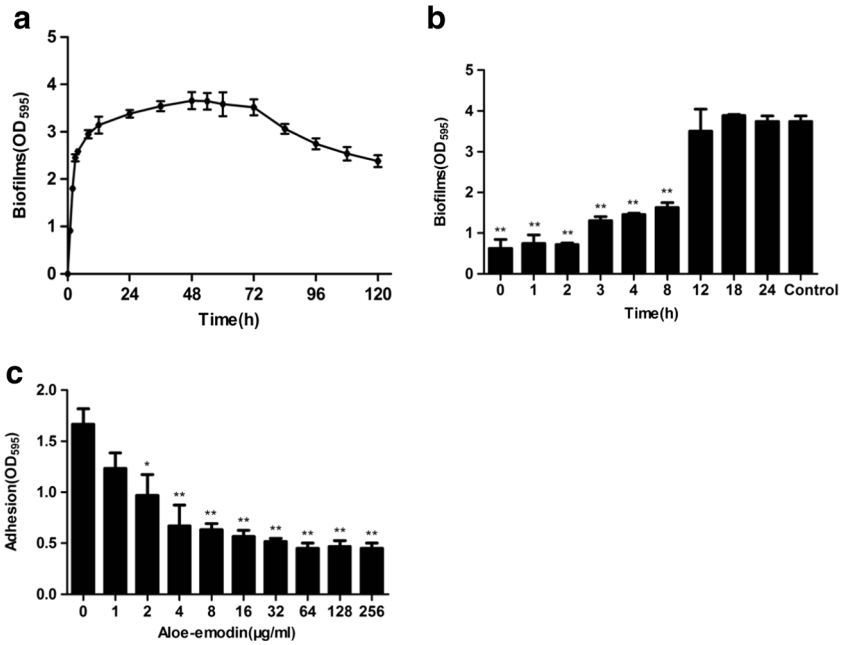

Fig. 4 **a** *S. aureus* ATCC29213 biofilm growth cycle. **b** Effect of aloe-emodin on the growth of biofilm. The time was the time that compound was added. **c** Effect of aloe-emodin on bacterial adhesion. The statistical significance determined by one-way analysis of variance (ANOVA) (*$P < 0.05$; **$P < 0.001$)

biofilm maturation stage and the biofilm diffusion stage occurred after 72 h (Fig. 4a).

To gain insight into the preliminary mode of action of aloe-emodin anti-biofilm activity, aloe-emodin (128 μg/ml) was added to the cultures of *S. aureus* ATCC29213 at different time points during the development of the biofilm. The influence on biofilm formation was measured after a 24-h incubation. Addition of aloe-emodin immediately after inoculation (0 h) resulted in 90% inhibition of biofilm formation (Fig. 4b). Aloe-emodin reduced the biofilm gradually when added at 1, 4 and 8 h into biofilm development. After 12 h of incubation, the biofilm culture was completely resistant to aloe-emodin (Fig. 4b). These results indicated that aloe-emodin would terminate the biofilm growth process when added during the initial adhesion and the proliferation stages.

To further test the effect of aloe-emodin on bacterial adhesion, the anti-adhesion activity of different concentrations of the compound was measured after 1 h of incubation. These results suggested that aloe-emodin reduces more than 50% of bacterial adhesion at concentrations of approximately 4 μg/ml with an inhibition rate of 65% (Fig. 4c). Collectively, these data showed that aloe-emodin significantly inhibited the attachment step in biofilm formation.

S. aureus ATCC29213 biofilm extracellular matrix is mainly composed of protein

The extracellular matrix composition of *S. aureus* biofilm is different under different culture conditions (Arce Miranda et al. 2011). To identify whether *S. aureus* biofilms are polysaccharide intercellular adhesin (PIA)-dependent or PIA-independent under these culture conditions, a biofilm detachment assay was carried out as previously described (Boles and Horswill 2008; Tetz et al. 2009). According to the results, the amount of residual biofilm after treatment with proteinase K, DNase I and sodium periodate was approximately 10, 35 and 50%, respectively (Fig. 5a). This result indicates that protein is the main component of the *S. aureus* ATCC29213 biofilm extracellular matrix.

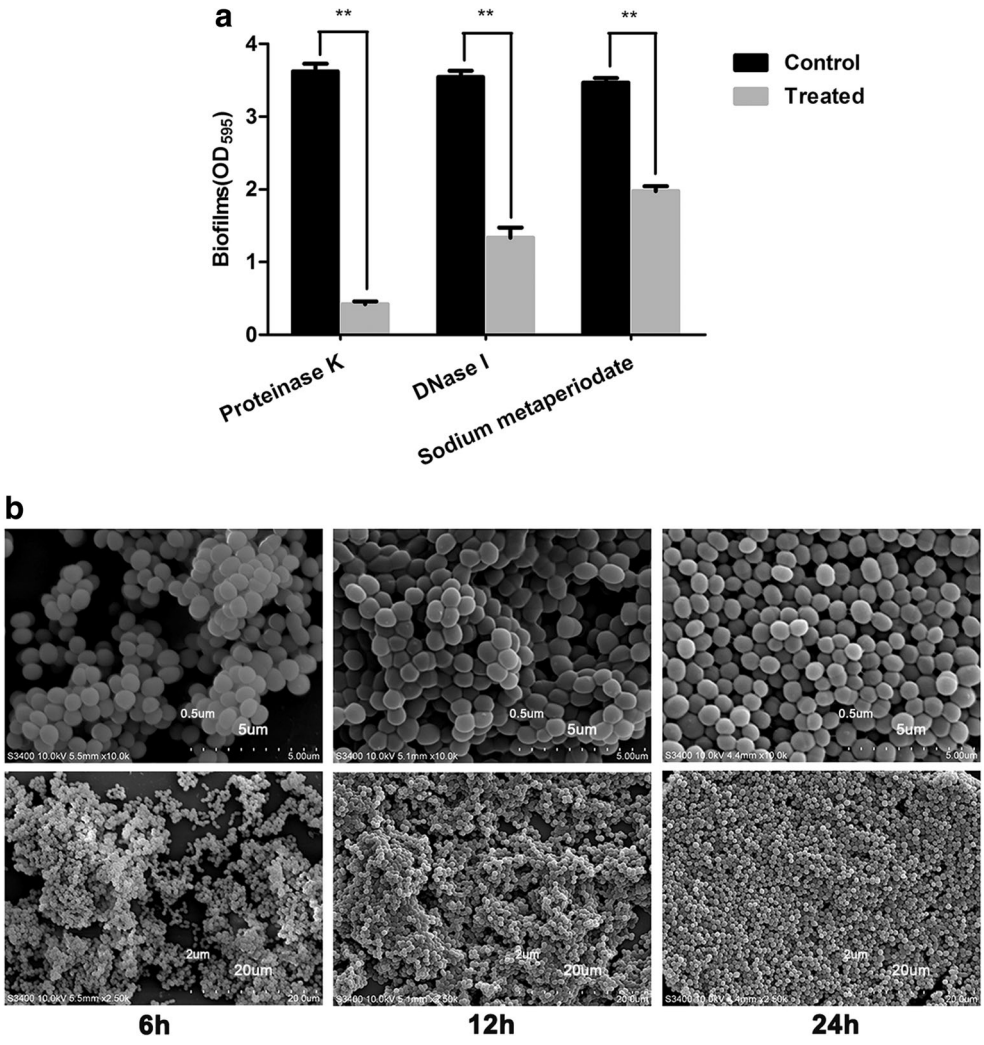

Fig. 5 **a** Enzymatic hydrolysis of biofilm. The amount of residual biofilm after treatment with or without proteinase K, DNase I and sodium periodate was measured at OD_{595}. The statistical significance was calculated using one-way analysis of variance (ANOVA) (*$P < 0.05$; **$P < 0.001$). **b** SEM analysis of *S. aureus* ATCC29213 biofilms. SEM images of biofilms formed by *S. aureus* ATCC29213 that had been incubated for 6, 12 and 24 h. *Scale bars* in the figures above represent 5 μm and scale bars in the figures below represent 20 μm and it was equally divided into ten units and each unit represents 0.5 or 2 μm, respectively

According to a previous study, *S. aureus* cells form highly dense aggregates without any detectable extracellular matrix when grown in a protein-mediated biofilm, and PIA/PNAG-dependent biofilm cells are embedded in an abundant extracellular material (Vergarairigaray et al. 2009). SEM of the biofilm carried out at different time points showed that the cells in the biofilm were smooth and there was not a visible filament that was attached to the bacterial surface (Fig. 5b). The detailed results further suggested that *S. aureus* ATCC29213 biofilm develops in a PIA-independent mode and that protein represents the main extracellular matrix under these culture conditions.

Aloe-emodin inhibits biofilm formation by reducing the production of extracellular proteins

The effect of aloe-emodin on extracellular biofilm proteins was studied by confocal microscopy. Syto63 dye stained the intracellular DNA red, and the FITC dye stained the extracellular proteins green. The control strain was surrounded by obvious green fluorescence, representing the extracellular matrix protein in the biofilm. After treatment with aloe-emodin (128 μg/ml), the surrounding green fluorescence decreased significantly (Fig. 6a).

Autolytic activity from cell subpopulations caused the release of extracellular DNA (eDNA), which promotes cell adhesion at the biofilm maturation stage. This eDNA contributes to the structure of the *S. aureus* biofilm matrix and to both cell-cell and cell-surface interactions (Mann et al. 2008; Rice et al. 2007). The effect of aloe-emodin on the autolysis of bacteria indirectly proved its effect on eDNA. The results showed that the treatment group and the control group were not significantly different (Fig. 6b). These results suggest that aloe-emodin has no effect on eDNA release from *S. aureus* ATCC29213 at 64 or 128 μg/ml concentrations.

Congo red can react with bacterial biofilm surface PIA, causing the colonies to become black. The present study showed that aloe-emodin treatment significantly reduced the number of black colonies in a dose-dependent manner (Fig. 6c). This result indicated that aloe-emodin inhibited the production of PIA from bacterial biofilms.

Discussion

S. aureus is a leading cause of nosocomial infections due in large part to rising rates of resistance to diverse antibiotics. The formation of a biofilm represents one of the mechanisms of drug resistance in *S. aureus*, because it is a physical barrier to antibiotics and results in differences in metabolism that further restrict antibiotic efficacy (Hochbaum et al. 2011). With the emergence of multidrug-resistant *S. aureus*, the need for more effective treatment of biofilm-associated infections has become imperative.

As natural products exhibit a large number of interesting structures, they have played an important role as a major source of new drugs for human therapy in persistent infections (Baker et al. 2007). Many categories of compounds from plants exhibit

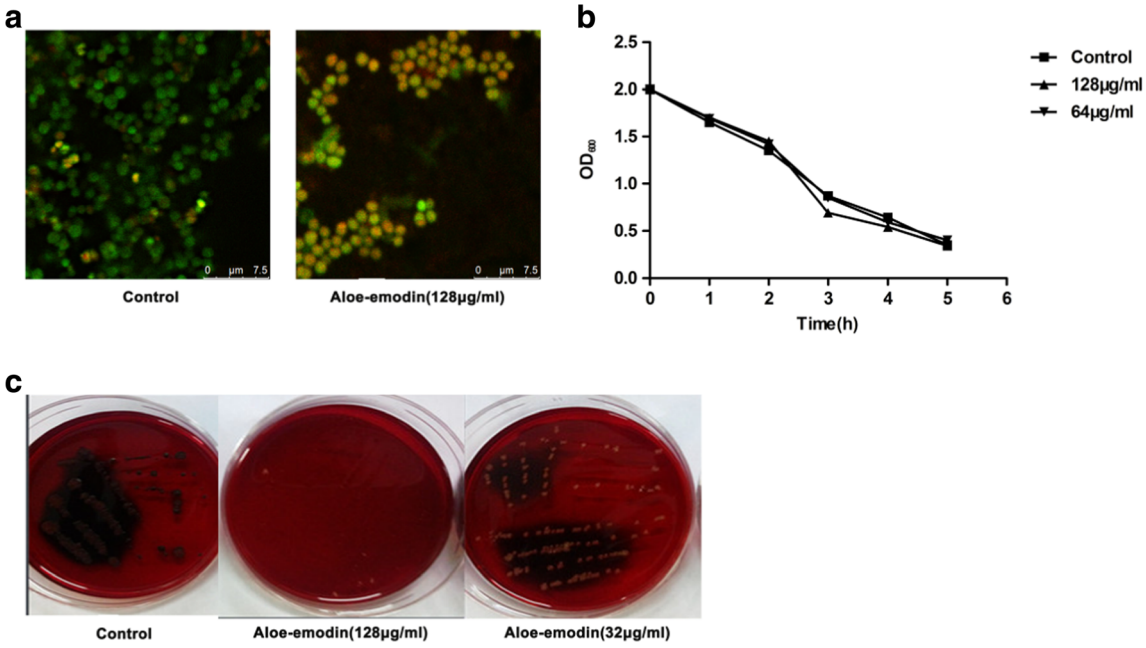

Fig. 6 **a** Confocal analysis of extracellular proteins. Syto 63 dye stained the intracellular DNA red, and the FITC dye stained the extracellular proteins green. **b** Autolysis assays of eDNA. Autolysis activity was monitored as the change in OD_{600} over time. **c** Congo red staining of PIA. *S. aureus* ATCC29213 was grown on Congo red medium and incubated with various concentrations of aloe-emodin

anti-biofilm activity against *S. aureus* in a variety of biofilm models while not causing significant bacterial growth inhibition. These compounds include, for example, oregano oil (Nostro et al. 2007), tea tree oil (Kwieciński et al. 2009), cinnamaldehyde (Jia et al. 2011), chelating pyrethroids (Payne et al. 2013), ginkgolic acids and *Ginkgo biloba* extract (Lee et al. 2014), gallic acid (Liu et al. 2016) and ellagic acid rhamnoside (Fontaine et al. 2017). Although these agents show great potential in the treatment of biofilm-associated infections, their mechanisms of action remain unclear.

The development of bacterial biofilms can be divided into four stages: initial adhesion, proliferation, maturation and diffusion. *S. aureus* biofilms are often encased in a matrix of self-produced extracellular polymeric substances (EPSs) that contain varying amounts of cell wall-associated proteins, PIA and eDNA (Arciola et al. 2012; Hochbaum et al. 2011). However, the composition of the matrix varies greatly under different growth conditions (Beenken et al. 2003).

We investigated the mode of action of aloe-emodin during the biofilm developmental stages and on matrix composition. We found that aloe-emodin terminated biofilm growth processes when it was added at the initial adhesion and proliferation stages (Fig. 6). We therefore determined the mechanism of aloe-emodin action against biofilms. The results indicated that aloe-emodin inhibits extracellular protein production and accumulation of PIA, leading to inhibition of biofilm formation by *S. aureus*.

In the initial adhesion stage, primary attachment to the biotic surface of the host tissues and to synthetic surfaces coated with plasma proteins is achieved by cell wall-anchored (CWA) proteins of *S. aureus*. Numerous surface proteins such as fibrinogen/fibronectin-binding proteins (FnBPA and FnBPB), clumping factors A and B (ClfA and ClfB), surface proteins C and G (SasC and SasG) and serine aspartate repeat protein (SdrC, SdrD and SdrE) are individually implicated in binding host matrix components to initiate cell adherence and biofilm matrix formation in *S. aureus* (Foster et al. 2014; Speziale et al. 2014).

After they attach to a surface, the adherent *S. aureus* cells will begin to grow, multiply and form microcolonies (Stoodley et al. 2008). Staphylococci produce several factors that could facilitate biofilm accumulation by stabilizing cell-to-cell interactions shortly after the initial attachment. Some of the CWA proteins such as the FnBPs, ClfB and SdrC proteins play dual roles in both attachment and accumulation (Speziale et al. 2014). In addition, protein A (Merino et al. 2009), SasC (Schroeder et al. 2009) and Bap (Cucarella et al. 2001) have all shown a propensity to aid in biofilm accumulation. Similarly, PIA has been implicated in early accumulation stage of *S. aureus* biofilm formation (Arciola et al. 2015).

Most of these surface proteins share a common cell wall-targeting motif (LPXTG) and are anchored to the cell wall envelope by sortase A (SrtA), which catalyses the covalent attachment of these proteins to the penta-glycine cross-linker component of the peptidoglycan (Mazmanian et al. 1999). Several categories of small molecule inhibitors of SrtA showed significant anti-biofilm activity by inhibiting the transpeptidase activity of SrtA and interfering with the functional display of surface proteins (Hochbaum et al. 2011). We have assessed the inhibitory activities of aloe-emodin against SrtA in vitro through a fluorescence resonance energy transfer (FRET) assay (Hochbaum et al. 2011). This compound did not show any potential transpeptidase inhibitory activity at concentrations up to 64 μg/ml (data not shown). This result indicated that aloe-emodin could not interfere with cell wall anchoring of surface proteins and that its biofilm development inhibition effects resulted from down-regulation of expression of certain surface proteins or direct blocking of adhesion of those proteins to other matrix components. Our future research will focus on identifying the precise molecular mechanism by which aloe-emodin inhibits *S. aureus* biofilm formation.

To conclude, aloe-emodin was effective at preventing *S. aureus* biofilm formation in a dose-dependent manner even at subinhibitory concentrations. Therefore, aloe-emodin can be used to prevent formation of biofilms on the surfaces of medical devices. We believe that this molecule is a potential novel treatment against *S. aureus* biofilm-related infections.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This article was conducted without the use of human or animal participants.

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