Adhesion force sensing and activation of a membrane-bound sensor to activate nisin efflux pumps in Staphylococcus aureus under mechanical and chemical stresses

Carniello, Vera; Harapanahalli, Akshay K.; Busscher, Henk J.; van der Mei, Henny C.

Published in:
Journal of Colloid and Interface Science

DOI:
10.1016/j.jcis.2017.10.024

Publication date:
2018

Journal of Colloid and Interface Science 512 (2018) 14–20

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

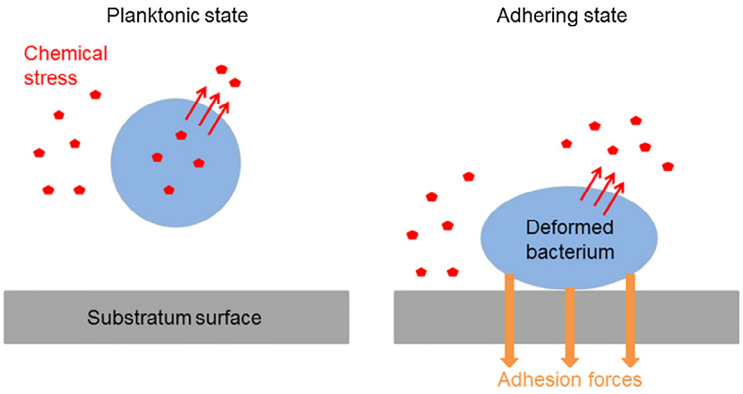
Regular Article

Adhesion force sensing and activation of a membrane-bound sensor to activate nisin efflux pumps in *Staphylococcus aureus* under mechanical and chemical stresses

Vera Carniello [1], Akshay K. Harapanahalli [1], Henk J. Busscher, Henny C. van der Mei *

University of Groningen and University Medical Center Groningen, Department of Biomedical Engineering, Groningen, The Netherlands

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 August 2017
Revised 5 October 2017
Accepted 6 October 2017
Available online 7 October 2017

Keywords:
Nisin
NsaRS response-regulator
Antibiotic susceptibility
Biofilms
Cell wall deformation
Surface sensing
Adhesion force
Atomic force microscopy
Gene expression

ABSTRACT

Nisin-associated-sensitivity-response-regulator (NsaRS) in *Staphylococcus aureus* is important for its adhesion to surfaces and resistance against antibiotics, like nisin. NsaRS consists of an intra-membrane-located sensor NsaS and a cytoplasmatically-located response-regulator NsaR, which becomes activated upon receiving phosphate groups from the intra-membrane-located sensor.

Hypothesis: The intra-membrane location of the NsaS sensor leads us to hypothesize that the two-component NsaRS system not only senses "chemical" (nisin) but also "mechanical" (adhesion) stresses to modulate efflux of antibiotics from the cytoplasm.

Experiments: NsaS sensor and NsaAB efflux pump transcript levels in *S. aureus* SH1000 adhering to surfaces exerting different adhesion forces were compared, in presence and absence of nisin. Adhesion forces were measured using single-bacterial contact probe atomic force microscopy.

Findings: Gene expression became largest when staphylococci experienced strong adhesion forces combined with nisin-presence and the two-component NsaRS response to antibiotics was enhanced at a stronger adhesion force. This confirms that the intra-membrane-located sensor NsaS senses both chemical and mechanical stresses to modulate antibiotic clearance through the NsaAB efflux pump. This finding creates better understanding of the antibiotic resistance of bacteria adhering to surfaces and, in the

Abbreviations: AFM, atomic force microscopy; CFU, colony forming unit; EPS, extracellular polymeric substances; MIC, minimal inhibitory concentration; NsaRS, nisin-associated-sensitivity-response-regulator; PE, polyethylene; SS, stainless steel.

* Corresponding author at: Department of Biomedical Engineering (FB-40), University Medical Center Groningen, P.O. Box 196, 9700 AD Groningen, The Netherlands.
E-mail addresses: v.carniello@umcg.nl (V. Carniello), akshay77@gmail.com (A.K. Harapanahalli), h.j.busscher@umcg.nl (H.J. Busscher), h.c.van.der.mei@umcg.nl (H.C. van der Mei).

[1] Both author contributed similarly.

https://doi.org/10.1016/j.jcis.2017.10.024

fight against antibiotic-resistant pathogens, may aid development of advanced biomaterials on which bacterial efflux pumps are not activated.

1. Introduction

Staphylococci possess an extensive infection capacity in a plethora of ecological niches within the host, including the surfaces of biomaterial implants and devices. An infection related to the presence of a biomaterial implant surface starts with the reversible adhesion of bacteria to the implant surface, after which adhering bacteria embed themselves in a matrix of extracellular polymeric substances (EPS) to yield a transition to irreversible adhesion and biofilm growth commences. The EPS matrix protects biofilm inhabitants against biological, mechanical and chemical stresses, such as the host immune response, fluid shear and antibiotic treatment [1]. Different biomaterials used in the clinical practice have different tendencies to become colonized and cause infection [2]. Moreover, antibiotic resistance of *Staphylococcus epidermidis* biofilms is related to the biomaterial used [3]. This indicates that adhering bacteria can sense properties of the biomaterial they adhere to.

Staphylococcus aureus interacts with its surrounding through a wide range of environmental sensors, often intra-membrane located, to regulate gene expression in response to environmental stimuli. Bacteria, including *S. aureus*, can either use one- or two-component systems to process these stimuli [4–6]. Nisin associated sensitivity response regulator (NsaRS) is a recently discovered two-component system in *S. aureus*, consisting of an intramembrane bound histidine kinase NsaS and a cytoplasmatically located response regulator NsaR that becomes activated upon receiving phosphate groups from the NsaS sensor [7]. Intramembrane located NsaS can sense environmental stimuli, such as chemical stress due to antibiotics or antimicrobial peptides targeting the cell wall, like nisin [8], and reprograms bacterial gene expression to reduce stress (see Fig. 1). The cytoplasmatically located response regulator NsaR then activates or represses the target gene expression [9]. One of the target genes is *nsaA*, which upregulates the NsaAB efflux pump (Fig. 1) to detoxify the cell and promote survival in the presence of a chemical stress, such as antibiotics [8].

Bacterial interactions with their environment are not only stimulated by chemical stresses, but mechanical stresses exerted by adhesion forces between a substratum surface and adhering bacteria, constitute another mechanism of environmental sensing by bacteria [10]. In *Escherichia coli* for instance, initial surface adhesion causes mechanical stress to the cell envelope and activates

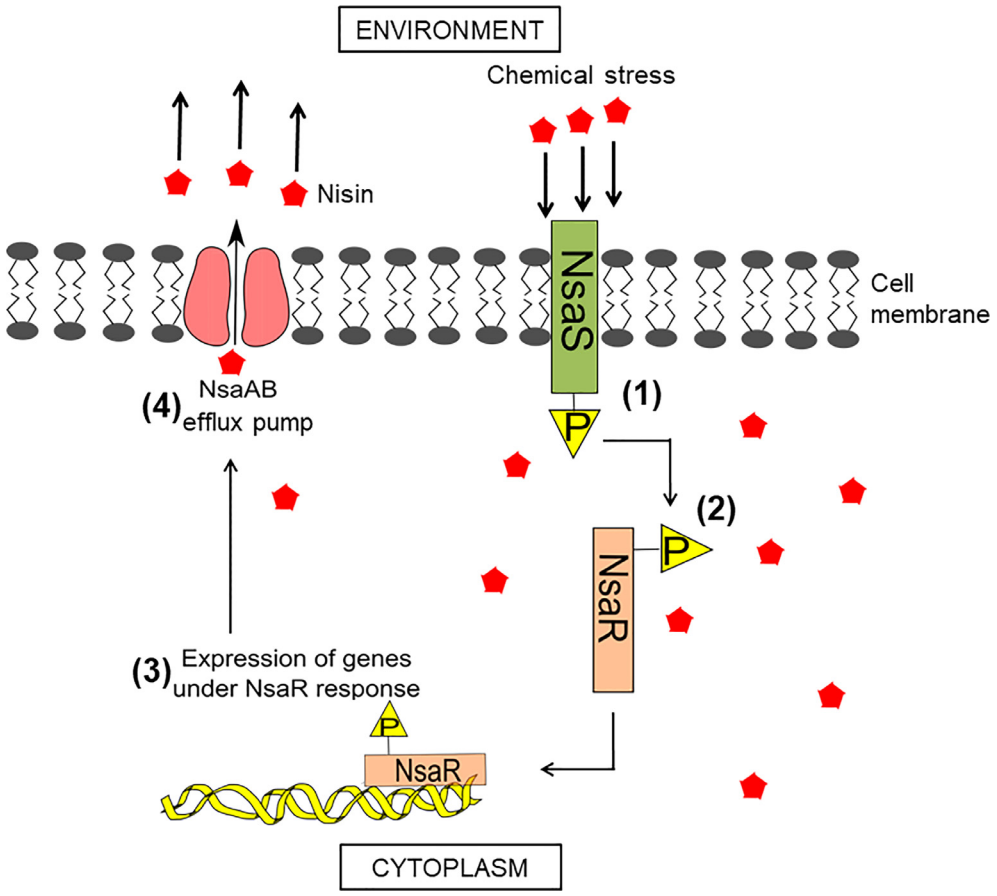


Fig. 1. Schematic presentation of the NsaRS two-component system regulating the downstream transporter NsaAB to remove possible antibiotics such as nisin, from the cytoplasm. The NsaS intra-membrane bound histidine kinase sensor rearranges itself upon sensing an environmental, chemical stress (1) and subsequently autophosphorylates to transfer phosphate to the NsaR response regulator (2). The NsaR activates or represses target gene expression (3), such as *nsaA*, which leads to upregulation of the NsaAB efflux pump (4). The NsaAB efflux pump modulates antibiotic resistance by pumping chemicals out of the bacterial cells [7,8].

the Cpx pathway for biofilm formation [6]. Similarly, *Vibrio parahaemoliticus* uses the ScrABC pathway [11] for surface sensing and biofilm formation. Adhesion forces arising from substratum surfaces exerted on adhering staphylococci have been shown to impact cell wall deformation [12], *ica* expression and associated production of the extracellular matrix component poly-*N*-acetylglycosamine [13]. Although in *S. aureus*, NsaRS activation is clearly associated with chemical stress [7,8], the intra-membrane location of the NsaS sensor also suggests its possible activation by membrane deformation as a result of mechanical stress, i.e. the adhesion forces experienced. Therefore, we here hypothesize that the NsaRS system can sense both chemical and mechanical stresses and therewith plays an important role in biomaterial induced modulation of antibiotic resistance via the NsaAB efflux pump.

Here, we report on the verification of the above hypothesis by investigating differences in transcript levels of the NsaS sensor and NsaAB efflux pump during early *S. aureus* biofilm formation in the presence and absence of nisin on two common biomaterials (stainless steel (SS) and polyethylene (PE)) exerting different adhesion forces on *S. aureus*. Bacterial adhesion forces are measured using single-bacterial contact probe atomic force microscopy (AFM) as an indicator of mechanical stress, while the presence of nisin constitutes a chemical stress.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The bacterial strain *S. aureus* SH1000 was used in this study and kindly provided by Dr. Lindsey N. Shaw, Department of Cell Biology, Microbiology and Molecular Biology, University of South Florida, Tampa, FL, USA. The strain was cultured aerobically at 37 °C on blood agar. One colony was inoculated in 10 ml Tryptone Soya Broth (TSB, OXOID, Basingstoke, UK) and grown for 24 h at 37 °C. The pre-culture was used to inoculate a main culture, by suspending staphylococci in 10 ml TSB (1:100) and culturing for 16 h. Subsequent processing of the main culture was done as described under the different experimental sections.

2.2. Materials and preparation

SS and PE were prepared to possess a comparable surface roughness in the micron-range, i.e. 1.8 ± 0.2 μm and 1.0 ± 0.2 μm for SS and PE, respectively [13]. Briefly, SS was polished using 1200 grit SiC paper followed by MetaDi 3 μm diamond suspension (Buehler, Lake bluff, IL, USA) on a polishing mat for 15 min, using a mechanical polisher Phoenix Beta, fitted with Vector™ power head (Buehler, Dusseldorf, Germany). PE was used as received from the manufacturer (Goodfellow Cambridge Ltd, Huntingdon, England). Coupons were made to fit into a 6-wells plate with a total surface area of 7.5 cm^2, sterilized with ethanol (96%), washed with sterile PBS and stored in sterile demineralized water until use.

2.3. Water contact angles measurements

Water contact angles were measured on SS and PE surfaces at 25 °C using the sessile drop technique with a home-made contour monitor. Droplets of 1.5–2 μl ultra-pure water were put on clean surfaces and contours measured between 5 and 10 s after placing a droplet, from which contact angles were subsequently calculated after grey-value thresholding. Contact angles were measured in triplicate on each of three different coupons for each material.

2.4. Adhesion force measurements

In order to measure adhesion forces between the *S. aureus* SH1000 and SS and PE surfaces, staphylococci from a main culture were suspended to a low (pivotal to create single-bacterial contact AFM probes) concentration of 3×10^6 bacteria ml^{-1} in 10 mM potassium phosphate buffer, pH 7.0, and immobilized on a tipless cantilever (NPO, Bruker AFM Probes, Camarillo, CA, USA) for AFM via electrostatic interaction with poly-l-lysine (PLL; Sigma-Aldrich, USA) using a micromanipulator (Narishige Groups, Tokyo, Japan). The far end of the cantilever was dipped in a droplet of PLL for 1 min and dried in air (2 min), followed by 1 min immersion in a droplet of bacterial suspension to let one bacterium adhere. To prove that we had a single-bacterial contact probe (see Fig. 2), a calibration grid (HS-20MG BudgetSensors, Innovative Solutions Bulgaria Ltd., Sofia, Bulgaria) was imaged with a bacterial probe, a tipless cantilever and a regular AFM tip (NP; Bruker AFM Probes, Camarillo, CA, USA; length and width at the top 3 and 1 μm respectively, with a half angle at the tip end of 18 degrees), as described in detail before [14].

Freshly prepared bacterial probes were directly used for adhesion force measurements. Adhesion force measurements were performed at room temperature in phosphate buffered saline (PBS; 10 mM potassium phosphate, 0.15 M NaCl, pH 7.0) using a Dimension 3100 system (Nanoscope V, Digital Instruments, Woodbury, NY, USA). For each bacterial probe, force-distance curves were measured without surface delay at a 2 nN trigger threshold. Bacterial probes were prepared out of three different cultures. Three bacterial probes, prepared from three separately grown bacterial cultures, were used. With each probe, three different locations were measured on one coupon of each material, recording 10 force curves on each location. The spring constant of each cantilever was determined using the thermal method [15]. The integrity of a bacterial probe was monitored before and after the onset of each ten adhesion force measurements by comparing adhesion forces measured on a clean glass surface. Whenever this adhesion force had a difference >0.5 nN, data obtained last with that probe were discarded and a new bacterial probe was made.

2.5. nsaS and nsaA gene expression

The main culture was diluted (1:5) and 3 ml was used to grow 3 h biofilms on coupons made of SS and PE in a 6 wells plate at 37 °C aerobic under static conditions in the presence and absence of 2 μg ml^{-1} nisin, a sub-minimal inhibitory concentration (MIC for *S. aureus* SH1000 is 4 μg ml^{-1} [7]). Biofilms were harvested by transferring the coupons with biofilms to a new 6 wells plate, washing the coupons twice with PBS and resuspending the staphylococci in 1 ml PBS by repeated pipetting. The suspended biofilm was centrifuged at 4000*g* for 10 min, the supernatant was removed and the pellets were stored at −20 °C until RNA isolation.

Total RNA was isolated using RiboPure™-Bacteria Kit (Ambion, Invitrogen, Foster City, CA) according to the manufacturer's instructions. Traces of genomic DNA were removed using DNA-*free*™ kit (Ambion, Applied biosystems, Foster City, CA) and the absence of genomic DNA contamination was verified by real-time PCR prior to cDNA synthesis. The amount and quality of extracted RNA was based on the 260/280 nm ratio measured by NanoDrop ND-1000 (NanoDrop technologies LLC, ThermoFisher Scientific, Wilmington, DE). A ratio of around 2.0 (± 10%) was accepted as "pure" for RNA. 200 ng of RNA was used for cDNA synthesis, 4 μl 5× iScript Reaction Mix, 1 μl iScript Reverse Transcriptase, in a total volume of 20 μl (Iscript, Biorad, Hercules, CA) according to manufacturer's instructions. Real time RT-qPCR was performed in a 384-well plate HSP-3905 (Bio-RAD, Laboratories, Foster city, CA, USA) with the primer sets for *16S rRNA*, *nsaS* and *nsaA* (Table 1).

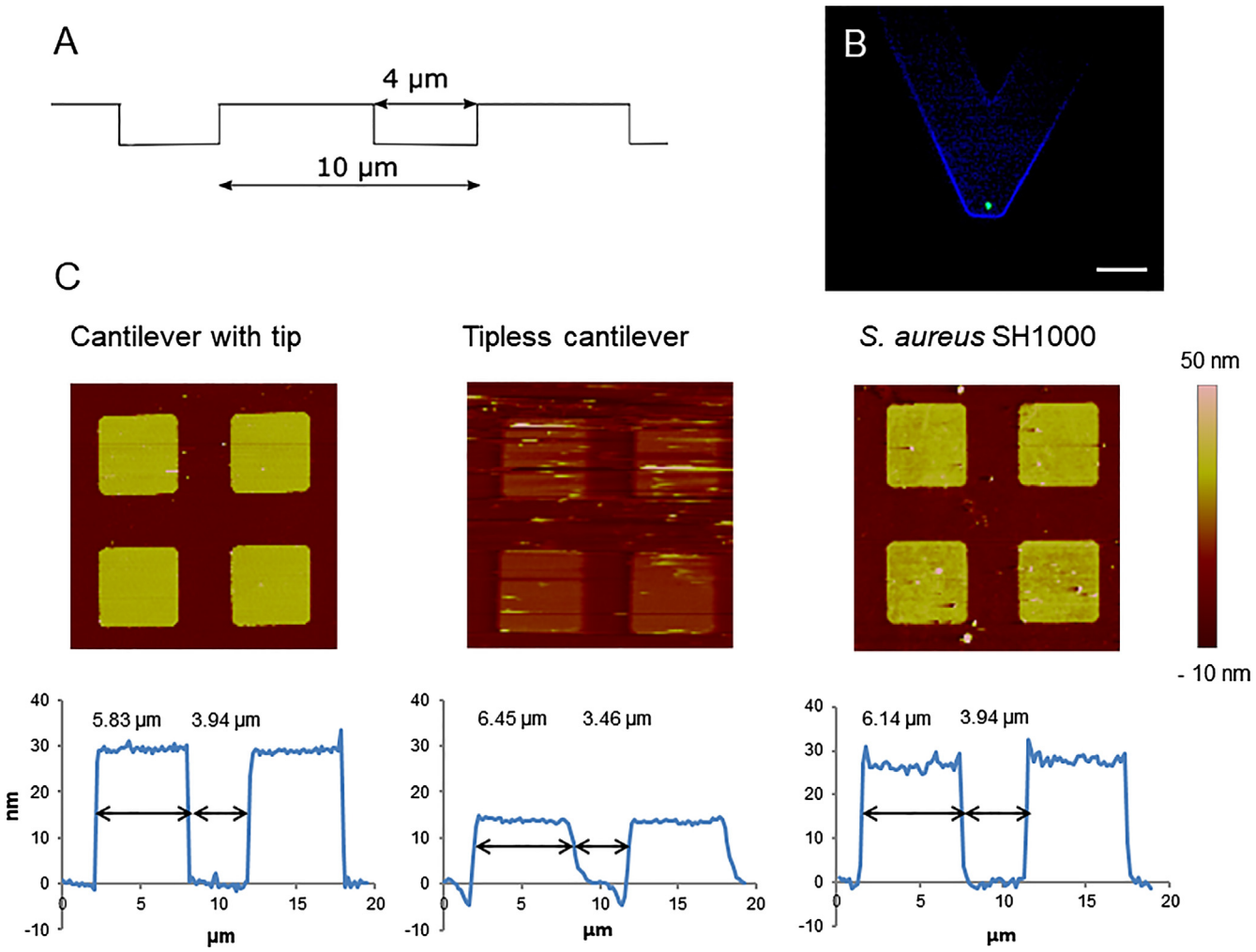


Fig. 2. Use of a calibration grid to demonstrate single-bacterial contact probe AFM. (A) Schematic presentation of the dimensions of the AFM calibration grid used to check whether bacterial AFM probes yield single-bacterial contact. Multiple-bacterial contact of the AFM probe would not correctly reflect the dimensions of the grid and yield double contour lines in imaging. (B) Fluorescence image of LIVE/DEAD stained *S. aureus* SH1000 (Baclight viability stain; Molecular Probes Europe BV, Leiden, The Netherlands) on a tipless AFM cantilever. Viable bacteria are green fluorescent, scale bar represents 20 µm. (C) Height images (20 µm × 20 µm) of the topography of the calibration grid (see panel A) and corresponding height profiles obtained with – a regular AFM tip, – a tipless, PLL-coated cantilever without bacteria and – a cantilever with *S. aureus* SH1000. Note that double contour lines are equally absent when using the bacterial-probe as when using a cantilever with a regular AFM tip.

Table 1
Primer sequences for RT-qPCR used in this study.

Primer	Sequence (5′–3′)
nsaS-forward	GCAACATGGCATGCACCTC
nsaS-reverse	AGGTTATAATGGCCAGCGCC
nsaA-forward	TGCATGCCATGTTGCT
nsaA-reverse	TTCACCAGCTTCAACT
16S-forward	TACGGGAGGCAGCAG
16S-reverse	ATTACCGCGGCTGCTGG

The following thermal conditions were used for all qPCR reactions: 95 °C for 15 min and 40 cycles of 95 °C for 15 s and 59 °C for 20 s. The mRNA levels were quantified in relation to endogenous control gene *16 s rRNA*. The *nsaS* and *nsaA* expression levels in the biofilms were normalized to *S. aureus* SH1000 planktonic culture. Gene expression was assessed in triplicate experiments with separately grown staphylococcal cultures.

2.6. Nisin susceptibility of S. aureus SH1000 biofilms

In order to determine the nisin susceptibility of *S. aureus* SH1000 biofilms, the efficacy of the NsaS sensor and NsaAB efflux pump system was evaluated in early *S. aureus* biofilms. First, biofilms were grown on PE and SS coupons as described before in the presence and absence of increasing concentrations of nisin above the nisin-MIC of *S. aureus* SH1000. Bacteria were then collected by repeated pipetting, serially diluted, plated on TSB agar and incubated overnight at 37 °C for colony forming units (CFU) counting. Experiments were performed in triplicate with separately grown staphylococcal cultures.

2.7. Statistical analysis

GraphPad Prism, version 5 (San Diego, CA) was employed for statistical analysis. Statistical significance was analyzed with a one-way analysis of variance (ANOVA), followed by Tukey HSD post-hoc test considering a confidence interval of 95% ($p < 0.05$).

3. Results and discussion

3.1. Staphylococcal adhesion forces to SS and PE surfaces

Adhesion forces for *S. aureus* SH1000 were measured using single-bacterial contact probe AFM [16]. Single-bacterial contact

was verified in separate experiments as demonstrated in Fig. 2, in which images of a calibration grid (Fig. 2A) are compared for a single-bacterial contact probe prepared on a tipless cantilever, a tipless cantilever without bacteria and a silicon nitride AFM tip. The bacterial probe measured the width of the AFM calibration grid equally well as the AFM tip, while the cantilever itself without a bacterium yielded a greatly distorted image of the grid with wrong grid dimensions (Fig. 2C). Note that the bacterium should be located at the end of the cantilever in order to ensure contact between the bacterium and the substratum surface only (Fig. 2B), as only this position yields an undistorted image of the grid. Thus prepared probes were used to obtained force-distance curves (Fig. 3A) between *S. aureus* SH1000 and SS and PE coupon surfaces, yielding adhesion forces between 3.5 ± 0.2 nN and 6.2 ± 0.2 nN, respectively (Fig. 3B). These force values are in the same order of magnitude [17] or smaller [18] than staphylococcal adhesion forces obtained using other methods to prepare single-contact bacterial AFM probes, confirming our adhesion forces represent single-bacterial contact.

Bacterial adhesion forces were significantly stronger ($p < 0.005$) on PE (6.2 ± 0.2 nN) than on SS (3.5 ± 0.2 nN) surfaces. Such differences in adhesion forces can arise from several factors, including the physico-chemical properties of the SS and PE. Bacteria experience in general stronger adhesion forces on hydrophobic surfaces than on hydrophilic surfaces. Thewes et al. [19] have shown that *Staphylococcus carnosus* adhered two times stronger to hydrophobic silicon wafers than to hydrophilic ones, suggesting that short-range, non-specific hydrophobic interactions present between bacterial cell wall proteins and substratum surfaces enhance the adhesion forces. Our results show a similar trend with significantly stronger adhesion forces to hydrophobic PE (water contact angle 85 ± 2 degrees) compared to hydrophilic SS (water contact angle 35 ± 3 degrees) for *S. aureus* SH1000.

3.2. nsaS and nsaA gene expression under mechanical stress

Gene expression of *nsaS* and *nsaA* of *S. aureus* SH1000 upon mechanical stress, i.e. adhesion to SS and PE surfaces, was measured by isolating total RNA from biofilms grown on SS and PE surfaces (see Supplementary material Fig. S1 for RT-qPCR amplification plots and melting curves of gene transcripts). The normalized level of *nsaS* and *nsaA* gene expression did not vary significantly on the two surfaces, despite exerting different adhesion forces ($p > 0.05$, Fig. 3C and D). However, all normalized gene expression levels were above unity, representing the gene expression level measured for planktonic bacteria, although this was not statistically significant.

Surface sensing under mechanical stress occurs through deformation of the bacterial cell wall, and adhesion forces in the order of magnitude of 0.5–1 nN have been shown to yield substantial cell wall deformation in staphylococci, as demonstrated using surface fluorescence enhancement [13]. Whereas in *S. aureus* ATCC 12600 expression of *icaA* and poly-N-acetylglucosamine upon adhesion to different biomaterial surfaces increased with increasing adhesion forces [13], the levels of *nsaS* and *nsaA* gene expressions of *S. aureus* SH1000 do not respond to increasing adhesion

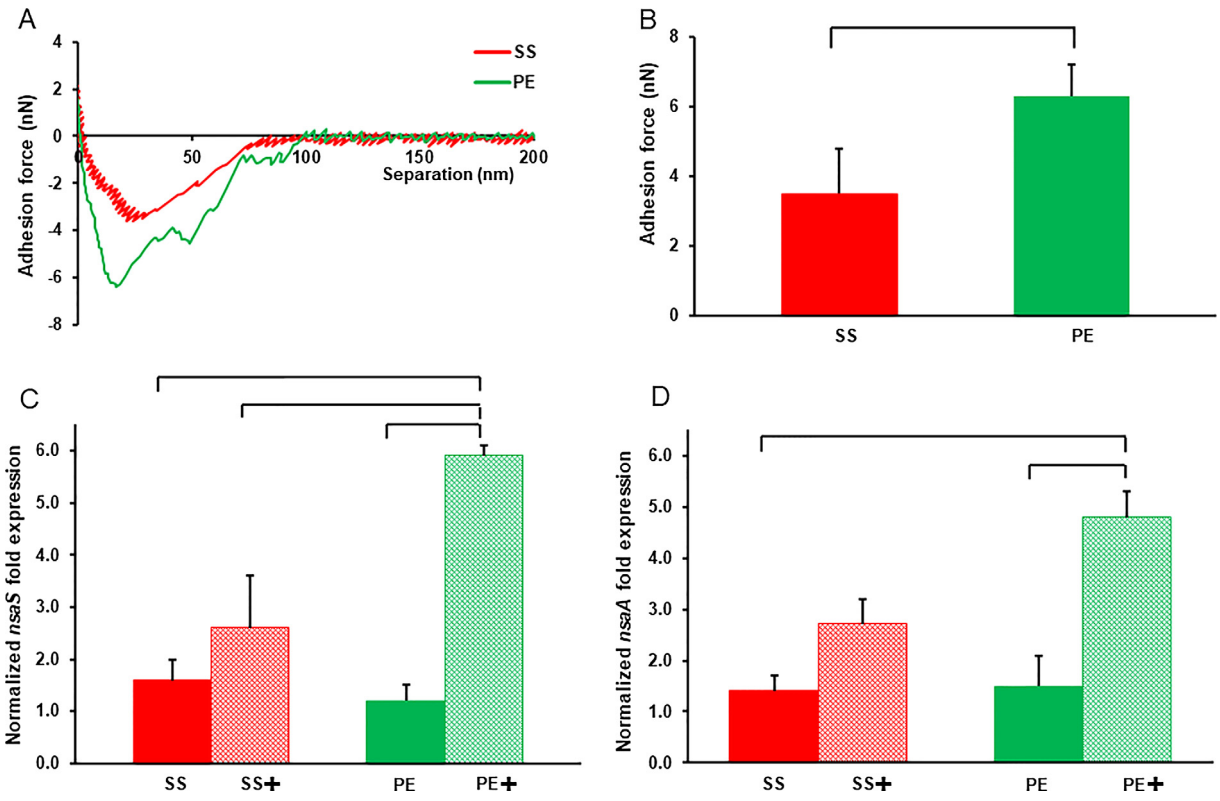


Fig. 3. (A) Force-distance retraction curves for *S. aureus* SH1000 on SS (red line) and PE (green line). (B) Adhesion forces of *S. aureus* SH1000 on SS (red column) and PE (green column). Each column represents an average ± standard deviation over 90 AFM force distance curves, obtained using three bacterial probes, prepared from three separately grown bacterial cultures and using each probe to measure adhesion forces on three different locations on one coupon of each material, recording 10 force curves on each location. (C) *nsaS* expression normalized to *16s rRNA* in *S. aureus* SH1000 adhering to stainless steel (SS) and polyethylene (PE) under mechanical and chemical (2 μg ml^{-1} nisin) stress. For control, gene expression in a planktonic state was taken as a reference and set to unity. SS^+ and PE^+ (red and green shaded, respectively) indicate the presence of nisin for the respective conditions. Each column represents an average ± standard error of the mean over three individual experiments with separately grown staphylococcal cultures. (D) Same as (C), now for *nsaA* expression. Connecting lines between bars show statistical significance ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

forces over the range of force values observed here, but seem to respond to the absence (planktonic state) or presence of adhesion forces (adhering state).

3.3. nsaS and nsaA gene expression under chemical and mechanical stresses

In order to study the combination of a chemical and mechanical stress on *nsaS* and *nsaA* gene expression, expression was also measured in the presence of nisin for *S. aureus* SH1000 biofilms grown on SS and PE. Normalized expression levels of both *nsaS* and *nsaA* increased in the presence of nisin for biofilms on both surfaces, compared to normalized expression levels in biofilms grown in the absence of nisin. However, gene expression levels only increased significantly ($p < 0.05$) on PE (Fig. 3C and D), exerting the strongest adhesion force (6.2 nN) on adhering *S. aureus* SH1000. On less hydrophobic SS exerting a smaller adhesion force (3.5 nN), no increased gene expression levels were found (Fig. 3C and D).

Thus it can be concluded that the influence of mechanical stress, i.e. adhesion forces arising from a substratum surface, in *S. aureus* SH1000 only become manifest in combination with the presence of a chemical stress, such as provided here by nisin. Since increased gene expression levels were only observed upon combination of a chemical stress and a high mechanical stress, this suggests that a threshold mechanical stress exists above which it is likely that the intra-membrane located NsaS sensor triggers additional expression of *nsaS* and *nsaA*.

3.4. Nisin susceptibility of S. aureus SH1000 biofilms on SS and PE

In order to demonstrate that a stronger adhesion force and increased expression of intra-membrane bound *nsaS* and *nsaA* also lead to increased phosphorylation of the cytoplasmatically located response regulator NsaR, required for a more effective two-component efflux pump system and nisin clearance from the cytoplasm, viability of *S. aureus* SH1000 biofilms grown on SS or PE during exposure to nisin were determined. In absence of nisin, less staphylococci were found adhering in biofilms on PE than on SS, (Fig. 4) which matches the slightly lower levels of *nsaS* expression

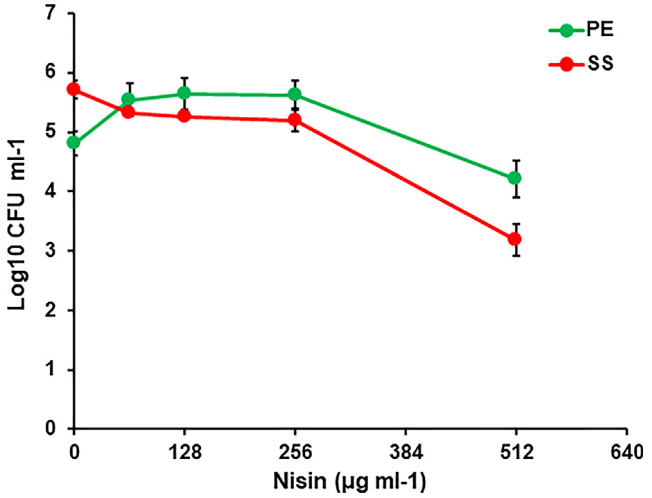


Fig. 4. The number of CFU retrieved from *S. aureus* SH1000 biofilms grown on PE (green) or SS (red) as a function of the nisin concentration in the growth medium. MIC for nisin is 4 µg ml^{-1} [7] and the minimum biofilm inhibitory and eradication concentrations are 64 µg ml^{-1} [20] and > 256 µg ml^{-1} [20], respectively. Each data point represents an average ± standard error of the mean over six coupons of each material prepared from three separately grown bacterial cultures. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

on PE compared with SS in the absence of nisin (Fig. 3C). As soon as nisin was introduced in a concentration above the nisin MIC, staphylococci adhering strongly to PE survived in higher numbers than those more weakly adhering on SS. Although this trend was not significant at lower nisin concentrations, the difference in survival between bacteria adhering to PE and SS coupons increased with increasing nisin concentrations, reaching a statistically significant 10-fold difference at a nisin concentration of 512 µg ml^{-1}, a value that approaches the nisin minimum biofilm eradication concentration [20]. Similarly, also biofilms grown on silicone rubber, exerting strong adhesion forces on bacteria, exhibited lower susceptibility to antibiotics compared to biofilms grown on polymer-brush coated silicone rubber, generating extremely low adhesion forces on adhering bacteria [21].

Since it has been demonstrated in general that stronger adhesion forces are associated with larger nanoscale cell wall deformation, including deformation of the cytoplasmic membrane, activation of membrane located sensors may constitute the onset of increased survival of bacteria strongly adhering to a substratum surface. Bacterial cell wall deformation changes the pressure profile across the lipid membrane [22] with a possible impact on the membrane located proteins constituting the NsaS sensor.

4. Conclusions

In conclusion, while activation of NsaRS efflux pumps in *S. aureus* has been associated with chemical stress in planktonic staphylococci [7,8], we here demonstrate that adhesion to substratum surfaces constitutes a mechanical stress that plays an important role too in antibiotic clearance from the cytoplasm in *S. aureus* through membrane located efflux pumps. The intra-membrane located NsaS sensor was more sensitive to nisin when staphylococci adhered more strongly to a biomaterials surface. The enhanced expression of the *nsaS* and *nsaA* genes due to this increased sensitivity directly leads to increased phosphorylation of cytoplasmatically located NsaR sensor and therewith to stimulation of the two-component NsaRS efflux pump system, as evidenced by increased survival of staphylococci adhering more strongly to PE as compared to SS. These findings contribute to a better understanding of the influence of adhesion forces on antibiotic resistance in bacteria adhering to surfaces, and may lead to the development of advanced biomaterials on which bacterial efflux pump systems are not stimulated. Such developments are direly needed in the fight against increasing numbers of antibiotic-resistant bacterial pathogens.

Acknowledgements

This study was entirely funded by UMCG, Groningen, The Netherlands.

H.J.B is also director of a consulting company SASA BV. The authors declare no potential conflicts of interest with respect to authorship and/or publication of this article. Opinions and assertions contained herein are those of the authors and are not construed as necessarily representing views of the funding organization or their respective employer(s).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jcis.2017.10.024.

References

[1] H.-C. Flemming, J. Wingender, The biofilm matrix, Nat. Rev. Microbiol. 8 (2010) 623–633, https://doi.org/10.1038/nrmicro2415.

[2] Y.H. An, R.J. Friedman, Concise review of mechanisms of bacterial adhesion to biomaterial surfaces, J. Biomed. Mater. Res. 43 (1998) 338–348, https://doi.org/10.1002/(SICI)1097-4636(199823)43:3<338::AID-JBM16>3.0.CO;2-B.
[3] T. Nuryastuti, B.P. Krom, A.T. Aman, H.J. Busscher, H.C. van der Mei, *Ica*-expression and gentamicin susceptibility of *Staphylococcus epidermidis* biofilm on orthopedic implant biomaterials, J. Biomed. Mater. Res. A. 96 (2011) 365–371, https://doi.org/10.1002/jbm.a.32984.
[4] L.E. Ulrich, E.V. Koonin, I.B. Zhulin, One-component systems dominate signal transduction in prokaryotes, Trends Microbiol. 13 (2005) 52–56, https://doi.org/10.1016/j.tim.2004.12.006.
[5] M. Kawada-Matsuo, Y. Yoshida, N. Nakamura, H. Komatsuzawa, Role of two-component systems in the resistance of *Staphylococcus aureus* to antibacterial agents, Virulence 2 (2011) 427–430, https://doi.org/10.4161/viru.2.5.17711.
[6] K. Otto, T.J. Silhavy, Surface sensing and adhesion of *Escherichia coli* controlled by the Cpx-signaling pathway, Proc. Natl. Acad. Sci. USA 99 (2002) 2287–2292, https://doi.org/10.1073/pnas.042521699.
[7] K.L. Blake, C.P. Randall, A.J.O.' Neill, In vitro studies indicate a high resistance potential for the lantibiotic nisin in *Staphylococcus aureus* and define a genetic basis for nisin resistance, Antimicrob. Agents Chemother. 55 (2011) 2362–2368, https://doi.org/10.1128/AAC.01077-10.
[8] S.L. Kolar, V. Nagarajan, A. Oszmiana, F.E. Rivera, H.K. Miller, J.E. Davenport, J.T. Riordan, J. Potempa, D.S. Barber, J. Koziel, M.O. Elasri, L.N. Shaw, NsaRS is a cell-envelope-stress-sensing two- component system of *Staphylococcus aureus*, Microbiology 157 (2011) 2206–2219, https://doi.org/10.1099/mic.0.049692-0.
[9] P. Lejeune, Contamination of abiotic surfaces: what a colonizing bacterium sees and how to blur it, Trends Microbiol. 11 (2003) 179–184, https://doi.org/10.1016/S0966-842X(03)00047-7.
[10] A.K. Harapanahalli, J.A. Younes, E. Allan, H.C. van der Mei, H.J. Busscher, Chemical signals and mechanosensing in bacterial responses to their environment, PLoS Pathog. 11 (2015) e1005057, https://doi.org/10.1371/journal.ppat.1005057.
[11] R.B.R. Ferreira, L.C.M. Antunes, E.P. Greenberg, L.L. McCarter, *Vibrio parahaemolyticus* ScrC modulates cyclic dimeric GMP regulation of gene expression relevant to growth on surfaces, J. Bacteriol. 190 (2008) 851–860, https://doi.org/10.1128/JB.01462-07.
[12] J. Li, H.J. Busscher, J.J.T.M. Swartjes, Y. Chen, A.K. Harapanahalli, W. Norde, H.C. van der Mei, J. Sjollema, Residence-time dependent cell wall deformation of different *Staphylococcus aureus* strains on gold measured using surface-enhanced-fluorescence, Soft Matter 10 (2014) 7638–7646, https://doi.org/10.1039/c4sm00584h.
[13] A.K. Harapanahalli, Y. Chen, J. Li, H.J. Busscher, H.C. van der Mei, Influence of adhesion force on *icaA* and *cidA* gene expression and production of matrix components in *Staphylococcus aureus* biofilms, Appl. Environ. Microbiol. 81 (2015) 3369–3378, https://doi.org/10.1128/AEM.04178-14.
[14] J. Sjollema, H.C. van der Mei, C.L. Hall, B.W. Peterson, J. de Vries, L. Song, E.D. de Jong, H.J. Busscher, J.J.T.M. Swartjes, Detachment and successive re-attachment of multiple, reversibly- binding tethers result in irreversible bacterial adhesion to surfaces, Sci. Rep. 7 (2017) 4369, https://doi.org/10.1038/s41598-017-04703-8.
[15] N.A. Burnham, X. Chen, C.S. Hodges, G.A. Matei, E.J. Thoreson, C.J. Roberts, M.C. Davies, S.J.B. Tendler, Comparison of calibration methods for atomic-force microscopy cantilevers, Nanotechnology 14 (2003) 1–6, https://doi.org/10.1088/0957-4484/14/1/301.
[16] Y. Liu, J. Strauss, T.A. Camesano, Adhesion forces between *Staphylococcus epidermidis* and surfaces bearing self-assembled monolayers in the presence of model proteins, Biomaterials 29 (2008) 4374–4382, https://doi.org/10.1016/j.biomaterials.2008.07.044.
[17] G. Zeng, T. Müller, R.L. Meyer, Single-cell force spectroscopy of bacteria enabled by naturally derived proteins, Langmuir 30 (2014) 4019–4025, https://doi.org/10.1021/la404673q.
[18] N. Thewes, A. Thewes, P. Loskill, H. Peisker, M. Bischoff, M. Herrmann, L. Santen, K. Jacobs, Stochastic binding of *Staphylococcus aureus* to hydrophobic surfaces, Soft Matter 11 (2015) 8913–8919, https://doi.org/10.1039/C5SM00963D.
[19] N. Thewes, P. Loskill, P. Jung, H. Peisker, M. Bischoff, M. Herrmann, K. Jacobs, Hydrophobic interaction governs unspecific adhesion of staphylococci: a single cell force spectroscopy study, Beilstein J. Nanotechnol. 5 (2014) 1501–1512, https://doi.org/10.3762/bjnano.5.163.
[20] N. Ooi, K. Miller, C. Randall, W. Rhys-Williams, W. Love, I. Chopra, XF-70 and XF-73, novel antibacterial agents active against slow-growing and non-dividing cultures of *Staphylococcus aureus* including biofilms, J. Antimicrob. Chemother. 65 (2009) 72–78, https://doi.org/10.1093/jac/dkp409.
[21] A.K. Muszanska, M.R. Nejadnik, Y. Chen, E.R. van den Heuvel, H.J. Busscher, H.C. van der Mei, W. Norde, Bacterial adhesion forces with substratum surfaces and the susceptibility of biofilms to antibiotics, Antimicrob. Agents Chemother. 56 (2012) 4961–4964, https://doi.org/10.1128/AAC.00431-12.
[22] E. Perozo, A. Kloda, D.M. Cortes, B. Martinac, Physical principles underlying the transduction of bilayer deformation forces during mechanosensitive channel gating, Nat. Struct. Biol. 9 (2002) 696–703, https://doi.org/10.1038/nsb827.