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Research Article

RESEARCH ARTICLE

Pentadecanal and pentadecanoic acid coatings reduce biofilm formation of *Staphylococcus epidermidis* on PDMS

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One sentence summary: The coating obtained by adsorption of pentadecanal and pentadecanoic acid on PDMS reduces the bacterial adhesion and biofilm formation of Staphylococcus epidermidis.

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ABSTRACT

Staphylococcus epidermidis is well known to be one of the major causes of infections related to medical devices, mostly due to its strong capacity to form device-associated biofilms. Nowadays, these infections represent a severe burden to the public health system and the necessity of novel antibacterial strategies for the treatment of these difficult-to-eradicate infections is urgent. The Antarctic marine bacterium *Pseudoalteromonas haloplanktis* TAC125 was found to be able to produce an anti-biofilm molecule, the pentadecanal, active against *S. epidermidis*. In this work, we modified one of the most widely used silicone-based polymers, polydimethylsiloxane (PDMS), by adsorption of pentadecanal and its most promising derivative, pentadecanoic acid, on the PDMS surface. The biofilm formation of *S. epidermidis* RP62A on both untreated and modified PDMS was performed in a parallel plate flow chamber system, demonstrating the capability of the proposed anti-biofilm coatings to strongly reduce the biofilm formation. Furthermore, drug-release capacity and long-term efficacy (21 days) were also proven for the pentadecanoic acid coating.

Keywords: anti-biofilm coatings; *Staphylococcus epidermidis*; medical devices; biofilm-related infections; *Pseudoalteromonas haloplanktis* TAC125; pentadecanal; pentadecanoic acid; PDMS; flow cell

INTRODUCTION

Healthcare-associated infections are among the most serious public health problems, globally and in Europe. The 'European Centre for Disease Prevention and Control' (ECDC) estimates that approximately 8.9 million healthcare-associated infections occur each year in European hospitals and long-term care facilities (Suetens *et al.* 2018). Infections that prolong hospital stays create long-term disability, increase resistance to antimicrobials, cause unnecessary deaths and represent a massive additional financial burden for health systems, accounting for

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approximately €7 billion per year in Europe, considering direct costs only (WHO 2011).

In a recent study, the five most frequently occurring bacteria have been identified among more than 3 million clinical isolates from hospitalized patients, and *Staphylococcus epidermidis* was one of the most common cause of nosocomial bacteraemia in North America (prevalence, 11.5%), Latin America (prevalence, 13.3%) and Europe (prevalence, 14.6%) (Biedenbach, Moet and Jones 2004).

S. epidermidis is currently the main pathogen in catheter-related bloodstream infections and is also a frequent cause of prosthetic joint infections, prosthetic valve endocarditis and other biomedical device-related infections (Widerström *et al.* 2012; Becker, Heilmann and Peters 2014). The main virulence factor possessed by *S. epidermidis* is its ability to rapidly attach to biotic and abiotic surfaces and form a biofilm (Otto 2012). Bacteria within biofilms embed themselves in a self-produced matrix and are more resistant to the classic antibiotic treatments than planktonic bacteria, resulting in recalcitrant biofilm-associated infections (Costerton *et al.* 2003). Considering the increasing impact of bacterial biofilms on human health, the discovery of new anti-infective strategies is becoming necessary. A viable approach should target the mechanisms involved in bacterial adhesion and biofilm formation or biofilm tolerance towards antibiotics, leading to novel therapies specifically designed to be combined with antibiotics against bacterial biofilm-associated infections (Beloin *et al.* 2014).

In the last years, several studies have been focused on the research of anti-biofilm molecules in extreme environments, like Antarctica, since it is already known that cold-adapted bacteria represent an unexploited source of biodiversity, able to produce a wide range of high added-value compounds, including anti-biofilm molecules (Papa *et al.* 2015). In a recent paper it has been demonstrated that the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 produces a long chain fatty aldehyde, the pentadecanal (Casillo *et al.* 2017), endowed with a strong anti-biofilm activity against *S. epidermidis* (Papa *et al.* 2013; Parrilli *et al.* 2015).

Moreover, with regard to pentadecanal mode of action, it was demonstrated to interfere with the AI-2/LuxS quorum sensing system, which has a crucial role in *S. epidermidis* biofilm formation process (Casillo *et al.* 2017).

Despite being very promising as anti-biofilm molecule, the pentadecanal is an aldehyde and a chemically reactive agent, which can easily undergo a variety of reactions that lead to many different products (Ricciardelli *et al.* 2018), losing therewith its anti-biofilm activity. Therefore, some pentadecanal derivatives were designed with the purpose to create new chemically more stable anti-biofilm molecules. By modifying the functional group, the corresponding acid, acetal and an ester of the pentadecanal were synthesized and the anti-biofilm activity of the designed derivatives was evaluated against *S. epidermidis* RP62A, a reference strain. Obtained results clearly indicated that pentadecanal and its derivatives show their anti-biofilm action against *S. epidermidis* when added in solution (Ricciardelli *et al.* 2018).

The aim of this study was to create a novel anti-biofilm coating for medical devices to prevent *S. epidermidis* infections, through the adsorption of the pentadecanal and pentadecanoic acid on a polydimethylsiloxane (PDMS) surface.

PDMS is a material frequently used for the manufacture of medical devices, such as phonatory and mammary prostheses, catheters and drug-delivery systems (Abbasi *et al.* 2011), due to its physiological inertness, high blood compatibility, low toxicity, good thermal and oxidative stability (Blanco 2018).

Though physico-chemical properties of PDMS makes it a good candidate for medical applications, in some cases surface or bulk modifications are necessary. For instance, bulk modification methods, like blending and copolymerization, or functionalization process are employed in controlled drug delivery systems for drug transport throughout the polymer (Abbasi *et al.* 2002).

However, because of its porosity and hydrophobicity, there is a concern that many drug-like compounds, especially small organic compounds, will adsorb to a large extent onto the PDMS surface (Jiao *et al.* 2016).

In this study, we investigated if pentadecanal and pentadecanoic acid can be used as a coating in order to reduce or avoid biofilm formation on PDMS.

In detail, two different anti-biofilm coatings were obtained through the adsorption of pentadecanal and pentadecanoic acid on PDMS. The influence of the anti-biofilm coatings on the surface properties of PDMS was evaluated in terms of hydrophobicity and surface roughness. Also, the anti-adhesive and biofilm-inhibiting effects of the coatings were studied *in vitro* using a parallel plate flow chamber with *in situ* observation and image analysis systems (Busscher and van der Mei 2006). Finally, taking into account its highest stability and strong biofilm-inhibiting effect, the pentadecanoic acid coating was also analysed for its drug-release capacity and long-term efficacy using convertible flow cells.

MATERIALS AND METHODS

Preparation of the anti-biofilm PDMS coatings

PDMS surfaces were prepared using SYLGARD®184 silicone elastomer kit (Dow Corning Corporation, Midland, MI). PDMS substrates were fabricated using a ratio base to curing agent 10:1 w/w. The base and curing agent were mixed and then degassed under vacuum until all air was removed (ca. 30 min).

For the use of PDMS in the parallel plate flow chamber, the polymer mixture was poured into a specific polymethylmethacrylate (PMMA) mold, whereas for the use in the convertible flow cells, the polymer mixture was poured into the bottom of the flow cell. In both cases, the polymer mixture was cured at 65°C for at least 5 h.

PDMS surfaces were then sterilized by soaking in 70% ethanol for 30 min, washed with sterile distilled water and dried at room temperature under sterile conditions.

Pentadecanal, the anti-biofilm molecule produced by the Antarctic bacterium *P. haloplanktis* TAC125 (Casillo *et al.* 2017), and pentadecanoic acid, a synthetic derivative of pentadecanal (Ricciardelli *et al.* 2018), were used to coat PDMS by drop-casting. In this work ,the commercial pentadecanal (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) and pentadecanoic acid (Sigma-Aldrich, Inc., St. Louis, MO # 2018 Merck KGaA, Darmstadt, Germany) were used. Briefly, solutions of pentadecanal and pentadecanoic acid in acetone (5 mg/ml) were deposited dropwise onto the PDMS surface and dried under ambient sterile conditions until complete solvent evaporation. Then the modified surfaces were washed with sterile distilled water, dried at room temperature under sterile conditions and stored until use.

Contact angle measurements

Advancing type contact angles with ultrapure water on PDMS with and without the anti-biofilm coatings were measured with a locally manufactured contour monitor using the sessile drop technique (Van Oss, Good and Chaudhury 1988). On each

sample, at least five droplets were placed at different positions and the images of the droplets were taken directly after 40 seconds from deposition. Results of three separately prepared surfaces with and without anti-biofilm coatings were averaged.

Atomic force microscopy

Atomic force microscopy (AFM) analysis of the PDMS with and without the anti-biofilm coating were performed using a Nanoscope IV (Digital Instruments, Veeco). Contact Mode AFM was used to measure topography by sliding the tip on the cantilever over the PDMS with and without the coating. During measurements, the tip was in direct contact with the sample, therefore variations in topography caused deflections in the cantilever which were converted into height information.

Bacterial strains and growth conditions

S. epidermidis RP62A was grown at 37°C in brain heart infusion broth (BHI, OXOID, Basingstoke, UK). Overnight pre-cultures were transferred to fresh medium for main cultures inoculation. Then, *S. epidermidis* cells from overnight main cultures were harvested by centrifugation at 5000 *g* for 10 min at 4°C, washed twice with fresh phosphate-buffered saline (PBS, 10 mM potassium phosphate and 150 mM NaCl, pH 7) and then resuspended in fresh PBS to the desired bacterial concentration (3×10^8/ml).

Parallel-plate flow chamber and image analysis

A parallel-plate flow chamber (Sjollema, Busscher and Weerkamp 1989) was used to study the adhesion and biofilm formation of *S. epidermidis* RP62A on PDMS with and without the anti-biofilm coatings. The parallel-plate flow chamber consists of a PMMA bottom plate with dimensions 76 × 26 × 2 mm, containing an inset for a PDMS piece with dimensions 15 × 10 × 1 mm and a top plate of glass with dimensions 76 × 26 × 1 mm. After PDMS sample polymerization, the top and bottom slides were cleaned by sonicating for 5 min in a commercially available surfactant cleaning solution (2% RBS35 in water, Societé des Traitements Chimiques de Surface, Lambersat, France), rinsed thoroughly with tap water and then rinsed with demineralized water. Subsequently, the PDMS was coated with the anti-biofilm agent, when necessary, and then top and bottom slides were mounted in the housing of the flow chamber, separated by 0.075 cm Teflon spacers. Images were taken from the PDMS with or without the anti-biofilm coating on the bottom slide. Bacterial adhesion and biofilm formation on different surfaces were observed with a CCD-MXRi camera (High Technology, Eindhoven, The Netherlands) mounted on a phase-contrast microscope (Olympus BH-2) equipped with a 40 × ultra-long working distance objective (Olympus ULWD-CD Plan 40 PL). The camera was coupled to an image analyzer (TEA, Difa, Breda, The Netherlands). Each live image (512 × 512 pixels with 8-bit resolution) was obtained after summation of 15 consecutive images (time interval 1 s) in order to enhance the signal to noise ratio and to eliminate moving microorganisms from the analysis.

Prior to each experiment, all tubes and the flow chamber were filled with PBS, and care was taken to remove air bubbles from the system. Flasks, containing microbial suspension and buffer, were positioned at the same height with respect to the chamber to ensure that, immediately after the flow was started, all fluids would circulate by hydrostatic pressure through the chamber at the desired flow of 0.4 ml/min.

To analyse the bacterial adhesion to the surfaces, the microbial suspension was circulated through the system for 4 h and images were obtained from PDMS with or without the anti-biofilm coatings. The initial increase in the number of adhering microorganisms was expressed as deposition as a function of time. All values presented in this work are the averages of at least two measurements on PDMS surfaces with or without the anti-biofilm coatings, and were carried out with separately grown microorganisms.

Fluorescence microscopy analysis of adhering bacteria to the PDMS with and without an anti-biofilm coating

The analysis of the bactericidal effect of the anti-biofilm coatings were performed by fluorescence microscopy. The bacterial suspension was flowed through the flow chamber for 2 h, then PBS buffer was flowed through the flow chamber for 30 min to remove non-adhered bacteria. The adhering bacteria were stained with live/dead stain. Live/dead stain of SYTO®9 nucleic acid (green fluorescent) and propidium iodide (red fluorescent), respectively with a volume ratio of 1:1, was used (Bac*Light*, Invitrogen, Breda, The Netherlands) with 3 μl of stain diluted in 1 ml of demineralized water. After allowing the stain to react with the sample for 15 min in the dark, the samples were imaged using a fluorescence microscope (Leica DM4000B, Leica Microsystems GmbH, Heidelberg, Germany) with a 40 × water lens and GFP and N21 filters. Three fluorescent images were taken at different spots on each sample.

Optical Coherent Tomography analysis of the biofilms on PDMS with and without an anti-biofilm coating

To grow a biofilm in the flow chamber, first all components of the flow chamber system and the solutions were sterilized by autoclaving. For biofilm formation, bacterial cells were suspended in a solution of BHI 2% (v/v) in PBS. The microbial suspension was circulated through the system for 1 h and the non-adhering bacteria were washed away with sterile PBS for 15 min. Then, fresh medium (BHI 50% (v/v) in PBS) was circulated overnight through the system with a flow rate of 0.1 ml/min to let the biofilm form. After 20 h incubation, fresh PBS buffer was flowed through the flow chamber for 30 min to remove non-adhering bacteria and then the biofilms were analysed by Optical Coherent Tomography (OCT) (Ganymede; Thorlabs Inc., Munich, Germany). Biofilms were visualized by OCT B-scan images (cross-sectional tomography, 5000 × 373 pixels). The biofilm growth experiments were performed at 37°C.

Drug-release from the coatings on PDMS

To evaluate the stability of the pentadecanoic acid coating, PDMS discs coated with the anti-biofilm molecules were immersed in PBS buffer for 8 days to investigate the release from the coating on PDMS. The release of the anti-biofilm molecule was monitored over time. In detail, PDMS substrates were fabricated as previously described and small PDMS discs with a diameter of 9 mm were cut by using a surgical punch. The pentadecanoic acid coating was prepared by the drop-casting method, as described above. Coated PDMS discs were placed in the middle of a well of a 6-well flat-bottomed polystyrene plate and a volume of 6 ml PBS was added into each well (time T_0). The plates were incubated at room temperature under agitation (80 rpm). After designated time intervals of 1, 4 and 8 days (T_1, T_4, T_8) the total

Table 1. Surface parameters values of PDMS with and without the adsorbed anti-biofilm molecules obtained from atomic force microscope: Height data type, Rq (root mean square average of height deviations taken from the mean data plane), Ra (arithmetic average of the absolute values of the surface height deviations measured from the mean plane), Rmax (maximum vertical distance between the highest and lowest data points in the image).

	Height	Rq (nm)	Ra (nm)	Rmax (nm)
Uncoated PDMS	28.9 ± 18.1 nm	3.8 ± 1.9	2.5 ± 1.8	57 ± 20
Pentadecanal coating	6.8 ± 0.3 μm	1072 ± 224	880 ± 230	7964 ± 1445
Pentadecanoic acid coating	6.4 ± 0.4 μm	915 ± 52	736 ± 48	5234 ± 450

volume (6 ml) of PBS buffer was collected, or, in parallel, 3 ml aliquots were withdrawn and then replaced with the same volume of PBS, to avoid a possible saturation of the solution. Subsequently, the presence of pentadecanoic acid was evaluated in the collected PBS samples by Gas Chromatography-Mass Spectrometry (GC-MS) analyses.

GC-MS analysis

The collected PBS samples were extracted three times with ethyl acetate (*v*/*v* 1:1). The organic layers were collected, concentrated and analysed on an Agilent Technologies gas chromatograph 7820A equipped with a mass selective detector 5977B and a HP-5 capillary column (Agilent, 30 m × 0.25 mm i.d., flow rate 1 ml/min, He as carrier gas), by using the following temperature program: 150°C for 3 min, from 150 to 300°C at 15°C/min, at 300°C for 5 min. The pentadecanoic acid in the samples was revealed by comparison of both GC retention time column and EI-mass spectrum with those of an authentic standard (Sigma-Aldrich, Italy).

Long-term efficacy analysis in convertible flow cells

An alternative flow system was set-up for long-term efficacy analysis of the pentadecanoic acid coating (Fig. S1). Convertible flow cells (Stovall Life Science, Inc., Greensboro, NC, USA) were assembled as per the manufacturer's instructions. The single chamber (7.7 cm^3, 24 mm × 40 mm × 8 mm) has a detachable, re-attachable top, which allowed the polymerization of the PDMS directly into the flow chamber and then coated with pentadecanoic acid. PBS was flowed through the cell at a controlled flow rate of 160 μl/min using a Ismatec IPC 4 Peristaltic Pump (Cole-Parmer GmbH, Germany). The flow system was kept free of air bubbles using a bubble trap, which created a low positive pressure with PBS flow, thus mitigating undesirable peristaltic pulsation in liquid delivery to the flow cell. After a predetermined time interval of 21 days, the efficacy of the anti-biofilm coating was evaluated by the biofilm formation assay of *S. epidermidis* RP62A. In detail, a bacterial suspension of *S. epidermidis* RP62A in a solution of BHI 2% (v/v) in PBS (3×10^8/ml) was flowed into three convertible flow cells: the cell with the anti-biofilm coating exposed for 21 days to PBS, under constant flow (C21), a cell with a fresh anti-biofilm coating (C1) and a cell with the uncoated PDMS (NC). Bacterial suspension was circulated through the system for 2 h to allow bacterial adhesion, then non-adherent cells were washed away with sterile PBS for 30 min. Finally, fresh medium (BHI 50% (v/v) in PBS) was circulated for 24 h through the system to allow the biofilm formation on the coated and uncoated PDMS surfaces. Biofilms were analysed by confocal laser scanning microscopy.

Confocal laser scanning microscopy analysis

For confocal laser scanning microscopy (CLSM) analysis, the bacteria viability in the biofilms was determined by live/dead stain, which was injected with a syringe into the convertible flow cells, without removing them from the flow system, and incubated for 20–30 min at room temperature, protected from light. Then, fresh PBS was flowed to remove the excess stain. All microscopic observations and image acquisitions were performed with a confocal laser scanning microscope (CLSM; LSM700-Zeiss, Jena, Germany) equipped with an Ar laser (488 nm), and a He-Ne laser (555 nm). Images were obtained using a 20x/0.8 objective. The excitation/emission maxima for these dyes are 480/500 nm for SYTO®9 stain and 490/635 nm for propidium iodide. Z-stacks were obtained by driving the microscope to a point just out of focus on both the top and bottom of the biofilms. Images were recorded as a series of .tif files with a file-depth of 16 bits.

RESULTS

Characterization of the anti-biofilm PDMS coatings

Water contact angle (WCA) measurements were performed to assess the hydrophobicity of the PDMS surface with and without the anti-biofilm coatings (Fig. S2). Contact angle of unmodified PDMS was 110 ± 1 degrees, whereas after the pentadecanal adsorption the contact angle increased (WCA 134 ± 2 degrees) and decreased in case of pentadecanoic acid (WCA 91 ± 1 degrees).

The surface morphologies were analysed by atomic force microscopy (AFM) (Fig. S3). In Table 1, the height values and the surface roughness parameters values (Rq, Ra, Rmax), calculated from the AFM images, are summarized. The uncoated PDMS is a perfectly flat surface. After adsorption of the anti-biofilm molecules the surface roughness increased for both coatings, with pentadecanal slightly rougher than pentadecanoic acid. Although the chemical structures of the anti-biofilm molecules are very similar to each other, it is interesting to note how dissimilar the coated surfaces are in terms of hydrophobicity.

Anti-adhesive properties evaluation of the anti-biofilm PDMS coatings

The adhesion of *S. epidermidis* RP62A to PDMS with and without the anti-biofilm coatings was evaluated in a parallel plate flow chamber. Fig. 1A presents the deposition kinetics, indicating the initial increase in the number of adhering microorganisms as a function of time. A reduction in the adhesion of bacteria on the coated PDMS with both anti-biofilm coatings, compared to the uncoated PDMS surface was observed. The number of adhering microorganisms after 4 h (n_{4h}) (Fig. 1B), showed a

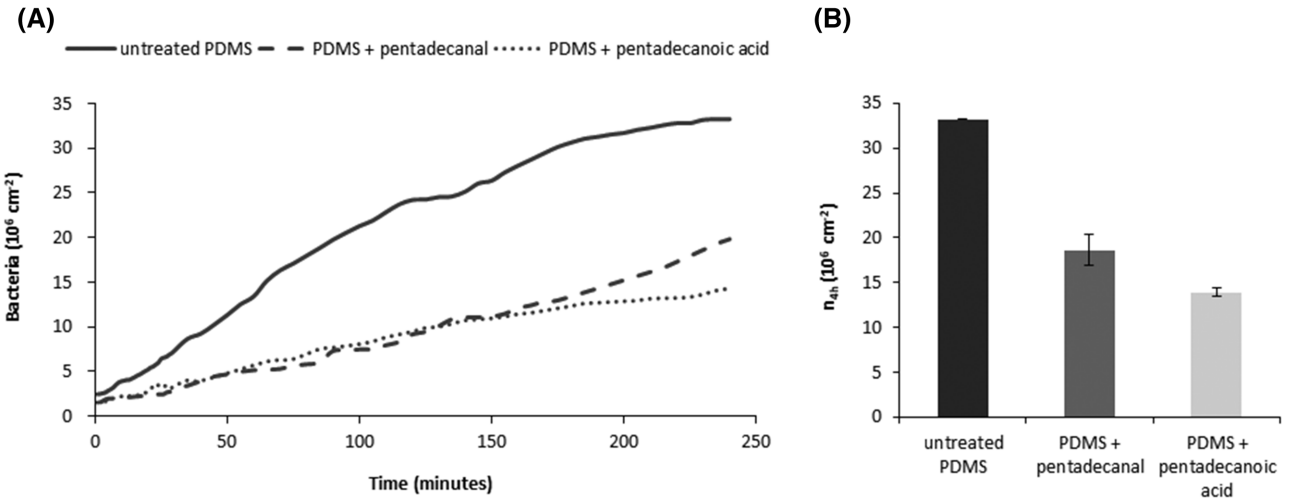

Figure 1. A) Deposition kinetics as a function of time in a parallel plate flow chamber for *S. epidermidis* RP62A on PDMS with and without the adsorbed anti-biofilm molecules, in PBS, pH 7.0, at a concentration of 3×10^8 bacteria per ml. B) Number of microorganisms adhering after 4 h (n_{4h}) on silicone rubber with and without the adsorbed anti-biofilm molecules. According to the Student t-test, for both the samples, the difference between the observed means is significant for $P < 0.01$.

2-fold reduction for both coatings compared to PDMS. Furthermore, to exclude any bactericidal effect of the coatings, *S. epidermidis* RP62A adherent cells were stained with the live/dead stain and analysed by fluorescence microscopy. Results revealed that bacteria adhering to the PDMS with the anti-biofilm coatings were not killed by the coatings (Fig. S4).

Evaluation of biofilm formation on the anti-biofilm PDMS coatings

The capability of PDMS coatings of inhibiting the biofilm formation of *S. epidermidis* RP62A was also performed in a parallel plate flow chamber and evaluated using OCT. Fig. 2 shows the staphylococcal biofilms formed on the PDMS with and without the coatings. The OCT images demonstrated a strong biofilm-inhibiting activity of the coatings, showing a decrease in biofilm thickness for both coatings when compared to the biofilm on PDMS. The PDMS biofilm was much more compact, shown by the much whiter biofilm, than those present on the two anti-biofilm coatings, which show a more porous structure.

Evaluation of drug-release capability of the anti-biofilm coatings

Considering the strong biofilm-inhibiting effect of the proposed coatings against *S. epidermidis* RP62A, their capability to release the anti-biofilm molecules in solution was evaluated over time. After designated time intervals, different aliquots of immersion buffer were collected and analysed by GC-MS. The presence of pentadecanoic acid has been detected in all the tested samples (Fig. S5), by comparison with an authentic standard, demonstrating an effective release of the anti-biofilm molecule in solution over a period of 8 days.

Long-term efficacy of the anti-biofilm coating on biofilm formation in a flow cell system

Biofilm growth in the flow cells was tested on PDMS (NC), pentadecanoic acid coating (C1) and a pentadecanoic acid coating which was previously exposed for 21 days to PBS flow (C21) in the flow cell. Staphylococcal biofilms were grown for 24 h and the biofilm structures and viability of the bacteria were analysed by confocal laser scanning microscopy (CLSM), as shown in Fig. 3. Bi-dimensional (SNAP) and three-dimensional (Z-STACK) biofilm structures were obtained using the live/dead staining, indicating viable bacteria by green fluorescence and red for dead (cell membrane damaged) bacteria. CLSM analysis demonstrated the strong capability of the coating to reduce the biofilm formation of *S. epidermidis* RP62A, and even after 21 days of exposure to PBS the coating is still active, proving its long-term efficacy. In fact, biofilms formed on the uncoated PDMS surface (NC) are thick and have a more compact structure, compared to those formed on PDMS surfaces coated with pentadecanoic acid, which appear less homogeneous and thinner, as also shown in the OCT images. Moreover, it is interesting to note a large number of dead cells on the freshly coated surface, compared to the one exposed for 21 days to PBS.

DISCUSSION

S. epidermidis has emerged as leading cause of biofilm-related nosocomial infections, particularly in case of implanted medical devices (Otto 2009; Widerström *et al.* 2016). Nowadays, the treatment of patients with *S. epidermidis* biofilm-related infections typically involves removal of the offending device and subsequent replacement. Current biofilm-targeting approaches aimed at biofilm prevention are based on surface-coating or eluting coatings, which ensure high localized antimicrobial concentrations for long periods by *in situ* antimicrobial delivery (Wi and Patel 2018).

In this study, we modified the PDMS surface by adsorption of two anti-biofilm molecules, pentadecanal and pentadecanoic acid, to prevent biofilm formation of *S. epidermidis*. PDMS was selected as ideal candidate for this purpose due to its capability to adsorb small hydrophobic molecules, in addition to its biocompatibility, optical transparency, high flexibility, ease of fabrication and molding properties (Mata, Fleischman and Roy 2005; Gokaltun *et al.* 2017; Valentin *et al.* 2019). Therefore, easy-to-fabricate PDMS anti-biofilm coatings were developed and characterized, and their biofilm-inhibiting properties were analysed using flow systems.

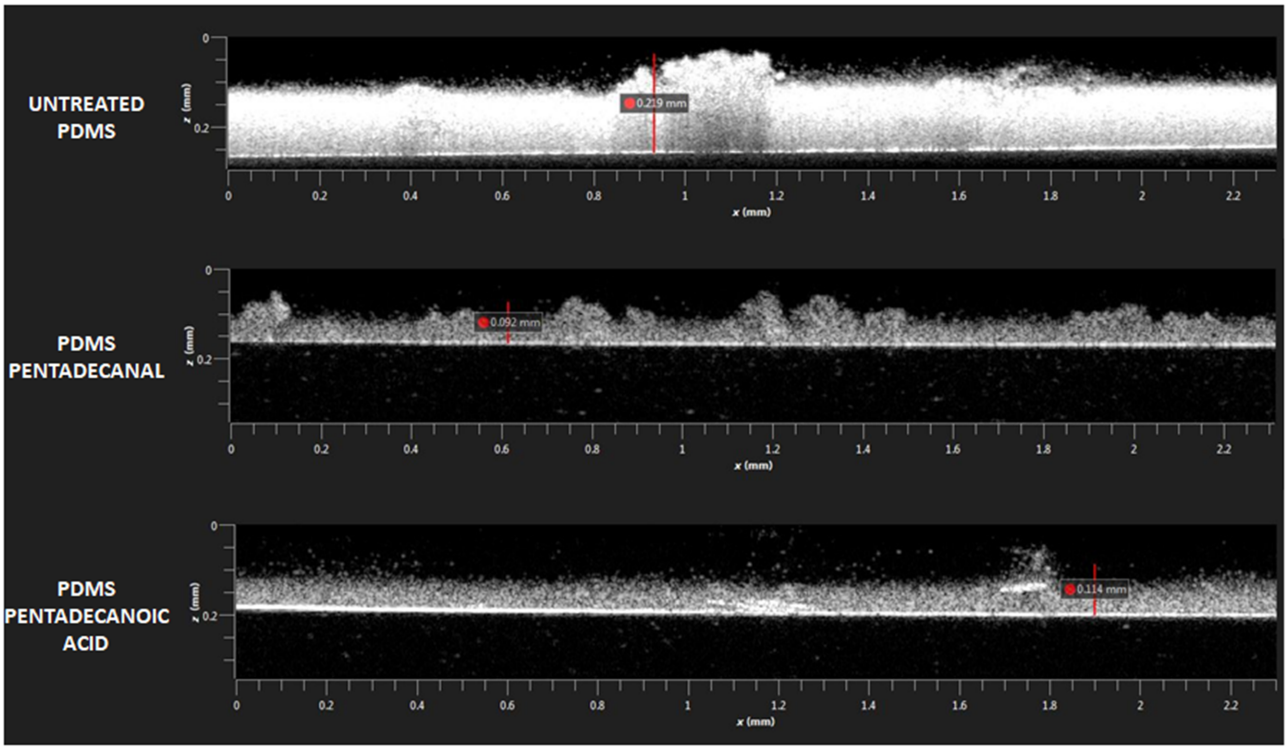

Figure 2. OCT images of *S. epidermidis* RP62A biofilms grown in a parallel plate flow chamber on PDMS with and without the adsorbed anti-biofilm molecules.

In a previous work, the pentadecanal and pentadecanoic acid molecules have been proven to be useful agents for the development of innovative anti-biofilm approaches, aimed at the prevention of biofilm formation (Ricciardelli *et al.* 2018). It has been reported that pentadecanal and pentadecanoic acid were able to reduce the biofilm formation of *S. epidermidis* RP62A at very low concentrations (12.5 μl/ml), probably acting as quorum-sensing modulators. Moreover, the effect of pentadecanal and pentadecanoic acid has also been investigated on immortalized human cell lines, demonstrating their biocompatibility at concentrations up to 50 μl/ml (Ricciardelli *et al.* 2018).

Taking into account the physicochemical properties of the anti-biofilm molecules and their capability to act against *S. epidermidis* when dissolved in the growth medium (Ricciardelli *et al.* 2018), a suitable coating strategy for medical devices should involve week intermolecular interactions between pentadecanal or pentadecanoic acid and the surface of the medical device, with a subsequent slow release of the active molecule.

To obtain a physic adsorption, the anti-biofilm molecules were drop-casted onto the PDMS surface and then the surface properties were evaluated by atomic force microscopy (AFM) and water contact angle (WCA) analysis. The topographical surface characterization, obtained by AFM analysis, showed that both the coatings strongly increased the surface roughness, probably due to the selected deposition technique. In fact, although drop-casting is a widespread, simple and low cost deposition technique, it is not easy to obtain coatings with controlled and uniform thickness and morphology.

The relationship between roughness and wettability was defined in 1936 by Wenzel, who stated that adding surface roughness would enhance the hydrophobicity or hydrophilicity caused by the chemistry of the surface (Wenzel 1936). For example, if the surface is chemically hydrophobic, it will become even more hydrophobic when surface roughness is added. Therefore, the high surface roughness, resulting from the adsorption of the pentadecanal, strongly affects the wettability of the surface, thus influencing the water contact angle measurements. On the contrary, the chemical nature of the pentadecanoic acid, and in particular the presence of the carboxylic group, makes the PDMS surface more hydrophilic, thus justifying the lower contact angle.

Although the anti-biofilm coatings were turned out to be different to each other in terms of hydrophobicity, their interactions with staphylococcal cells resulted quite similar. In fact, the analysis of the initial attachment to the surface in the parallel plate flow chamber showed that both the coatings induced a 2-fold reduction in the adhesion of *S. epidermidis* RP62A, compared to the unmodified PDMS surface (Fig. 1). This reduction in the bacterial adhesion is probably due to the irregularity of the surfaces. It is indeed known that surface topography of materials is one of the key factors affecting bacterial adhesion. The most interesting result was the clear and strong biofilm-inhibiting effect that both the coatings showed against *S. epidermidis* RP62A.This evident anti-biofilm effect is not related to anti-adhesive properties of the coatings, since the extent of the reduction in cell adhesion was not so obvious as to cause such a strong inhibition of biofilm formation. This clear biofilm-inhibiting effect is probably due to a release of the molecules from the coating, probably acting as quorum sensing modulators (Casillo *et al.* 2017; Ricciardelli *et al.* 2018).

Therefore, the proposed coating strategy has proven to be successful in obtaining weak interactions between active compounds and surfaces, leading thus to a release of the anti-biofilm molecules over time, which was confirmed by GC-MS measurements.

The poor water solubility of the anti-biofilm molecules (0.000097 g/l for pentadecanal and 0.00033 g/l for pentadecanoic acid) (VCCLAB 2005) is the key factor in obtaining a slow release

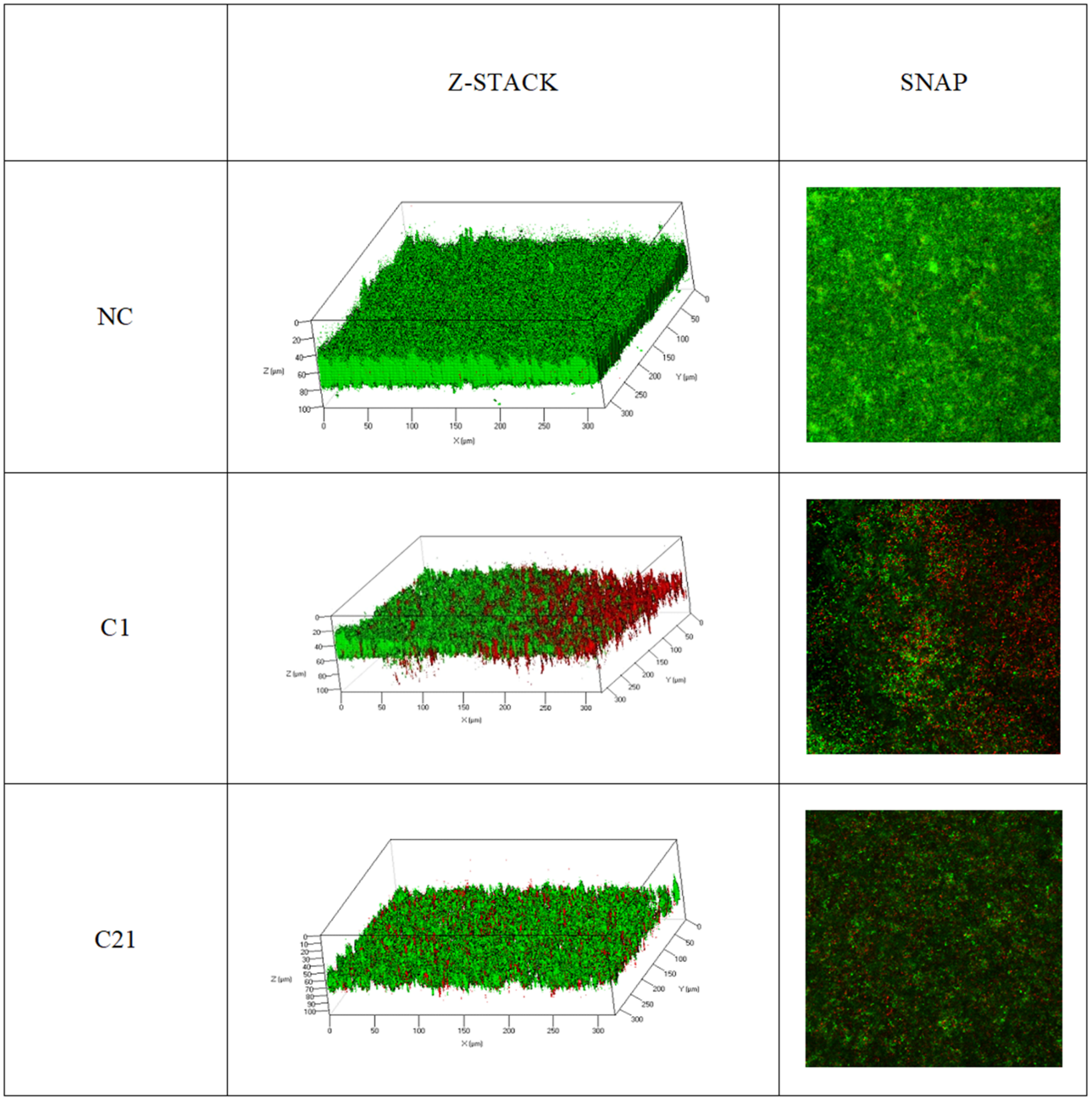

Figure 3. CLSM analysis of *S. epidermidis* RP62A biofilms formed in convertible flow cells on uncoated PDMS (NC), PDMS freshly coated with pentadecanoic acid (C1) and PDMS coated with pentadecanoic acid after 21 days incubation in presence of a constant PBS buffer flow (C21). Bi-dimensional (SNAP) and threedimensional (Z-STACK) biofilm structures were obtained using the LIVE/DEAD® Biofilm Viability Kit.

of the compounds in a water solution, which nevertheless allows a strong reduction in *S. epidermidis* biofilm formation. Moreover, even when the coating has been exposed to PBS, under a constant flow, for 21 days, there is still a clear anti-biofilm effect (see Fig. 3).

The freshly coated PDMS surface, besides showing a clear reduction of the staphylococcal biofilm, also induced a toxic effect on the bacterial cells, clearly indicated by the presence of dead cells zones. This effect could be probably due to an uneven and inhomogeneous distribution of the pentadecanoic acid on the surface after drop-casting. This effect could be avoided by optimizing the coating technique. In fact, future perspectives will aim to the evaluation of different casting methods, like spray-coating or spin coating, in order to obtain a more uniform adsorption of the anti-biofilm molecules onto the PDMS surface. An improved deposition could also lead to a reduction in the amount of compound used per unit area and probably prolong the coating effectiveness over time. Furthermore, a release kinetic assay will be performed to study the release profile of the anti-biofilm molecules from the coatings.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSPD* online.

Conflicts of interest. None declared.

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