Candida albicans biofilm formation on peptide functionalized polydimethylsiloxane

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Abstract

In order to prevent biofilm formation by *Candida albicans*, several cationic peptides were covalently bound to polydimethylsiloxane. The salivary peptide Histatin 5 and two synthetic variants (Dhvar 4 and Dhvar 5) were used to prepare peptide functionalized PDMS using 4-azido-2,3,5,6-tetrafluoro-benzoic acid (AFB) as an interlinkage molecule. In addition, polylysine-, polyarginine- and polyhistidine-PDMS surfaces were prepared. Dhvar 4 functionalized PDMS yielded the highest reduction of the number of *C. albicans* biofilm cells in the Modified Robbins Device. Amino acid analysis demonstrated that the amount of peptide immobilized on the modified disks was in the nanomole range. Poly-D-lysine PDMS, in particular the homopeptides with low molecular weight (2,500 and 9,600) showed the highest activity against *C. albicans* biofilms, with reductions of 93 % and 91 %, respectively. The results indicate that the reductions are peptide dependent.

Keywords: biofilm, *Candida albicans*, peptide, grafting, Modified Robbins Device

Introduction

Biofilm formation on medical devices is frequently associated with infections and in many cases removal of the medical device is the only option to combat them (Khardori and Yassien 1995; Kadry et al 2009). Several approaches have already been evaluated to inhibit microbial biofilm formation on medical materials (Danese 2002; Von Eiff et al 2005). First, various materials have been passively coated e.g. alkanethiols on gold (Hou et al 2007), fish protein coatings on glass or vinyl plastic coverslips (Vejborg and Klemm 2008) and diamond-like carbon on stainless steel (Raulio et al 2008). These passive coatings are not antimicrobially active but alter the physico-chemical properties of surfaces, so that micro-organism/substrate interactions are weakened or even prevented (Hetrick and Schoenfish 2006). However, their effectiveness is limited, due to adsorption of conditioning films, masking the functional groups (Hetrick and Schoenfish 2006). Secondly, coatings releasing antimicrobial compounds including antibiotics (Price et al 1996; Kwok et al 1999a, 1999b; Stigter et al 2004), silver ions (Dowling et al 2003) or silver nanoparticles and antiseptics (for a review see Von Eiff et al 2005) have been designed for use in intravenous polyurethane catheters and urinary catheters (Wu and Grainger 2006). A third series of surface modifications includes polymers with positively charged moieties such as quaternary ammonium or phosphonium groups at their surface. These substrates can be synthesized via copolymerization (Kenawy and Mahmoud 2003; Kenawy et al 2006; Kenawy et al 2007) or using photo- or plasma-induced graft polymerization (Hsiue et al 1998; De Smet et al., unpublished data). Grafting results in the production of materials with altered surface properties without influencing the bulk properties (Pan et al 2003).

Covalent binding of cationic peptides may be a useful approach to prevent microbial biofilm formation. Histatins are a family of naturally occurring, histidine-rich, low molecular weight peptides in human saliva (Helmerhorst et al 1997) and tear fluid (Jumblatt et al 2006).

Of these, histatin (Hst) 1, 3 and 5 are the most abundant peptides in saliva (De Smet and Contreras 2005). Hst 5, which is a peptide fragment derived from Hst 3, possesses the highest activity against *Candida albicans* and salivary concentrations range from 15 to 50 µM (Edgerton et al 1998). In the oral cavity, salivary peptides are believed to inhibit *C. albicans* overgrowth, thus offering a natural protection from oropharyngeal candidiasis. In addition, homodimerization of histatin-derived peptides showed an improved *in vitro* bactericidal activity against *Staphylococcus aureus* (Welling et al 2007). Dhvar 4 and Dhvar 5 are synthetic variants of the active domain of Hst 5 (Den Hertog et al 2004). Exposure of micro-organisms to histatins results in leakage of intracellular components accompanied by a release of intracellular potassium and a decrease in the cellular ATP-level.

In certain circumstances, disease states can diminish the body's natural protective mechanisms against infection. For example, malignant laryngeal tumors of the vocal cords in humans are removed by a total laryngectomy. For voice rehabilitation of laryngectomized patients, a voice prosthesis (VP) is placed between the trachea and the oesophagus (Kasperbauer and Thomas 2004). This VP is highly susceptible to colonization by micro-organisms, particularly by *Candida* spp., growing in biofilms on the surface (Bauters et al 2002; Elving et al 2002). Laryngectomized patients show a decreased salivary secretion as a side effect of radiation therapy, thus reducing the concentration of natural protective peptides (Rodrigues et al 2006). The release of antimycotics from buccal, bio-adhesive tablets has not been successful in preventing biofilm formation on VPs because of this reduced saliva secretion (Ameye et al 2005). *In vitro* studies have already focussed on the removal of mixed species biofilms by Dhvar 4 and 5 (Oosterhof et al 2003). However, the antibiofilm effect against *C. albicans* by peptides (applied by covalent binding, unlike by adsorption or dip coating) completely immobilized on silicone rubber has not been determined so far.

In the present study, the antifungal effect of three histatin-like antimicrobial peptides (Hst 5, Dhvar 4 and Dhvar 5) and several polylysine, polyarginine and polyhistidine homopolymers covalently bound to silicone was assessed against planktonic and sessile *C. albicans* cells.

Materials and methods

Materials

Dhvar 4 and Dhvar 5 were synthesized at the Mousseron Institute (Montpellier, France). HEPES buffer, MES buffer, ethyl acetate, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), acrylic acid (AA) and Hst5 were purchased from Sigma (St. Louis, MO, USA). Ethanol was obtained from Chem Lab (Zedelgem, Belgium). 4-azido-2,3,5,6-tetrafluorobenzoic acid (AFB) was purchased from Invitrogen (Carlsbad, CA, USA) and RBS 35 solution was purchased from Chemical Products (Brussels, Belgium). Dichloromethane was obtained from Acros Fine Chemicals (Geel, Belgium). All homopolymers were purchased from Sigma. They included seven poly-D-lysine HBr salts (molecular weight distributions: 1,000-4,000; 4,000-15,000; 15,000-30,000; 30,000-70,000; 70,000-150,000; 150,000-300,000 and > 300,000); four poly-L-lysine HBr salts (molecular weight distributions: 500-2,000; 1,000-5,000; ≥ 15,000 and 15,000-30,000); two poly-DL-lysine HBr salts (molecular weight distributions: 1,000-4,000; 25,000-40,000); three poly-L-arginine HCl salts (molecular weight distributions: 5,000-15,000; 15,000-70,000 and > 70,000) and one poly-L-histidine HCl salt (molecular weight distribution: 5,000-25,000).

Polydimethylsiloxane (PDMS) production

Medical grade silicone rubber (Q7-4735, Dow Corning Corp., Midland, MI, USA) was prepared as described previously (Coenye et al 2008).

Synthesis of PDMS functionalized with peptides

To select the appropriate interlinkage molecule for covalent binding of peptides to PDMS, Dhvar 4 was bound onto the PDMS surface by grafting of AA or AFB. To initiate grafting of PAA onto PDMS, the surfaces were brought in contact with a benzophenone solution (5% [w/v] in ethanol). In a first approach, Dhvar 4 was coupled to the carboxylic groups of grafted AA using EDC as coupling reagent in combination with NHS. To this end, a stable activated ester on the PDMS surface was prepared by soaking the PAA-grafted PDMS surface for 4 h at room temperature in a solution of 1.76 mM EDC and 1.78 mM NHS, dissolved in MES-buffer (pH 5.5). The surfaces were washed in MES-buffer for 24 h at 37°C. Finally the terminal amino group of Dhvar 4 was coupled onto the surface in HEPES buffer pH 8.5, resulting in covalent binding of Dhvar 4 onto PDMS. After a contact time of 48 h the peptide containing disks were rinsed in sterile MilliQ water and treated aseptically until use.

The second approach to synthesize Dhvar 4-PDMS makes use of AFB. PDMS sheets were incubated in ethanol for 4 h, to remove the catalyst and subsequently cleaned in a 2 % (v/v) RBS solution. The sheets were incubated in a saturated AFB solution (5 mg/ml) prepared in dry ethyl acetate for 4 h, allowing AFB to adsorb onto the PDMS sheets. Afterwards the incubated sheets were dried under reduced pressure to remove ethyl acetate from the surface. The azido group of AFB readily reacts with methyl groups on the PDMS and both sides of the PDMS sheet were irradiated with UVC light (λ = 254 nm) for 5 min. Following polymerization, the surfaces were cleaned using dichloromethane to remove the non-reacted AFB. NHS esters were then synthesized by the reaction of the carboxylic group with EDC and NHS in MES-buffer pH 5.5 for 4 h at room temperature. The obtained surfaces

were cleaned in MES buffer for 24 h at 37°C. Finally, Dhvar 4 was coupled to the PDMS substrate as described above.

For covalent binding of all other (homo)peptides, the coupling procedure with AFB was used. Peptide concentrations of 5 or 20 mg/ml and 1 or 5 mg/ml were used for Dhvar 4 and Dhvar 5, respectively. The peptide concentration for covalent binding of Hst 5 was 0.5 mg/ml. All homopolymers were bound to PDMS using a concentration of 5 mg/ml of the homopeptide.

Characterization of modified PDMS surfaces

The peptide modified PDMS surfaces were characterized using Attenuated Total Reflection-Infrared spectroscopy (ATR-IR) and Attenuated Total Reflection-imaging (ATR-imaging)

Attenuated Total Reflection-Infrared spectroscopy (ATR-IR) measurements on the modified PDMS surfaces were performed at room temperature using a Biorad 575C spectrometer. All spectra were recorded between 4000 cm^{-1} and 720 cm^{-1} at 2 cm^{-1} resolution. A single beam reference spectrum of a freshly cleaned diamond crystal was recorded before the measurements and used as the background spectrum. All spectra were normalized by the C-H bending band of the PDMS backbone located at 1259 cm^{-1}.

For Attenuated Total Reflection-imaging (ATR-imaging) the Spotlight 300 instrument (Perkin Elmer Life and Analytical Sciences, Waltham, MA, USA), was used. Samples of 1.5 mm thickness were tightened between the sample stage and the Ge crystal. The Spotlight system was run with two scans per pixel and with a spectral resolution of 4 cm^{-1}. The data collection software collects rectangular image areas of 100 µm by 100 µm (De Smet et al., unpublished data).

Amino acid analyses were carried out to determine the amount of cationic peptide bound to PDMS. To this end, the peptide modified PDMS disks were cut in two pieces and introduced in a vial for hydrolysis. The vials were loaded in a vessel filled with 200 µl 6N HCl and subsequently with argon, closed and incubated at 106°C for 24 h. The hydrolysed amino acids were washed from the polymer with 200 µl of MilliQ water. From this mixture, 5 µl of sample was loaded on an automated Applied Biosystems amino acid analyzer using the phenylisothiocyanate chemistry. Each sample was measured in triplicate.

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Strain and culture conditions

Candida albicans SC5314 (ATCC MYA-2879) was used throughout. The strain was maintained at -80°C using the Microbank storage system (Pro-Lab Diagnostics, Richmond Hill, ON, Canada). After thawing at room temperature, one bead was transferred to 10 ml of a sterile Sabouraud broth (Becton Dickinson Co, Franklin Lakes, NJ, USA) and the inoculated broth was incubated at 37°C for 24 h. From this suspension, a pure culture was obtained on Sabouraud Dextrose agar (SDA, (Becton Dickinson). To prepare overnight cultures, one or two isolated colonies of the pure culture were transferred to Sabouraud broth.

Determination of the minimal inhibitory concentration

The minimal inhibitory concentrations (MICs) were determined using LYM broth (5 mM KCl, 5.6 mM Na_2HPO_4, 0.5 mM $MgSO_4$, 1.0 mM sodium citrate, 0.4 mg l^{-1} $ZnCl_2$, 2.0 mg l^{-1} $FeCl_3.6H_2O$, 0.1 mg l^{-1} $CuSO_4.5H_2O$, 0.1 mg l^{-1} $MnSO_4.H_2O$ and 0.1 mg l^{-1} $Na_2B_4O_7.10H_2O$) according to Rothstein et al (2001). Glucose, an amino acid mixture, and a vitamin mixture were added as well according to the manufacturer's instructions. Stock solutions of each peptide were diluted (1:2). Of each dilution, two ml were transferred to the wells of a flat-bottom 24-well microtiter plate (TPP, Trasadingen, Switzerland). A standardized cell

suspension was prepared as follows : overnight cultures were centrifuged and the cells of these freshly prepared overnight cultures were washed three times with and diluted in LYM broth to a concentration of 10^6 CFU ml^{-1}. One hundred µl of these standardized cell suspensions, containing appr. 10^5 *C. albicans* cells per ml LYM broth, were added to each well. Prior to use, the bacterial suspensions were vigorously mixed using a vortex mixer. Following 24 h of incubation at 37°C, the growth was measured by determining the absorbance at 530 nm using a Wallac Victor^2 (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) microtiter plate reader. The MICs for each peptide was defined as the lowest concentration resulting in a lack of visible growth. For the homopeptides, instead of LYM broth, Yeast Nitrogen Base (YNB) medium supplemented with 50 mM glucose was used. Each MIC test was carried out in triplicate. The following concentration ranges were tested : 125-0.12 µg/ml (Dhvar 4), 100-0.01 µg/ml (Dhvar 5), 850-0.001 µg/ml (Hst), 2500-78 µg/ml (poly-DL-lysine) and 2500-1 µg/ml (poly-L-lysine, poly-DL-lysine and poly-L-arginine).

Biofilm growth in the MRD

C. albicans biofilms were grown on PDMS disks in the Modified Robbins Device (MRD), as described previously (Coenye et al 2008). Prior to the determination of the antibiofilm effect, all the modified surfaces were rinsed in sterile saline (0.9% [w/v] sodium chloride solution). A flow system consisting of 6 custom made stainless steel Modified Robbins Devices (MRDs) was employed to allow *C. albicans* adhesion and subsequent biofilm formation. One such a device comprises six individual ports in a linear array along a channel of rectangular cross-section, having dimensions of 10.0 mm (width), 145.0 mm (length) and 3.5 mm (depth), respectively. Each port may hold a cylinder-shaped plug that serves as a substrate for biofilm development at the interior side of the MRD. These were placed in an aluminium heating

block to ensure a constant temperature of 37°C during biofilm growth. Prior to each run the tubing, the valves and the devices were cleaned with MilliQ water and finally autoclaved. The assembly of these different parts was carried out prior to sterilization. Two bottles containing the *C. albicans* inoculum suspension and the growth medium (YNB supplemented with 50 mM glucose) were connected to the silicone tubing under aseptic conditions. The inoculum suspension was prepared using saline (0.9 % sodium chloride solution). To this end *C. albicans* overnight cultures were centrifuged at 4000 rpm for 4 min and the pelleted yeasts were washed with saline. The cells were subjected to three wash cycles and finally resuspended in saline. One ml aliquots were added to 99 ml saline containing bottles resulting in the inoculum suspensions. The growth medium was prepared by adding 6.7 g of Yeast Nitrogen Base (Becton Dickinson) and 9 g glucose to 100 ml of MilliQ water. This solution was sterilized by vacuum filtration through a 0.45 µm cellulose acetate membrane filter (Corning Incorporated, Corning, NY, USA) and was aseptically diluted (1:10) in sterile MilliQ water, resulting in a YNB solution with final glucose concentration of 50 mM. The tubing was mounted on top of a pump head in a peristaltic pump (Watson-Marlow 505S, Bredel, Wilmington, MA, USA). The setup of each device includes a bypass to allow rinsing of the silicone tubing with either inoculum suspension or growth medium at the inlet side and removal of entrapped air bubbles. First the inoculum suspension was pumped towards the MRDs in order to fill the interior side of the MRD. Once filled, the pump was switched off and the MRDs were clamped off using a clamp at the inlet and one at the outlet side. Each device was flipped over to allow planktonic *C. albicans* cells adhering to the disks. After 1 h, the MRDs were flipped back and the clamps were loosened. The pump was started to permit a flow of continuous growth passing over the colonized disks. Flow rate was set at 0.5 rpm (corresponding with 7.9 ml/h) and *C. albicans* biofilms were allowed to develop at 37°C for 24 h.

After biofilm formation, the number of sessile *C. albicans* cells was determined by plating on SDA, as described previously (Coenye et al 2008). To this end, the sessile *C. albicans* cells were harvested following 1 h of adhesion and 1 h of adhesion followed by 24 h of biofilm formation. The disks were aseptically recovered from the plug (MRD) and added to Sab. The cells were harvested by 30 s of intensive mixing with a vortex (IKA® works, Inc., Wilmington, NC, USA) and 30 s of sonication (Bransonic® 3510, Soest, The Netherlands) three times. Finally the *C. albicans* cell suspensions were serially (1:10) diluted in Sab, one ml aliquots were transferred to petri dishes (Novolab, Geraardsbergen, België) and mixed with molten SDA (45°C). The plates were incubated at 37°C for 24 h, the number of colonies was counted and the number of colony forming units was calculated for all disks.

Statistical analysis

All cell counts were log transformed and data from the peptide modified disks were compared with those from the corresponding control disks. Analysis of variance with the Scheffe post hoc tests was performed using the SPSS 15.0 software package (SPSS Inc, Chicago, IL). Differences in biofilm cell counts between modified and control disks were considered significant when $p<0.05$. Correlation between molecular weight of peptides and MIC values was determined using Kendall's tau test ; correlations were considered statistically significant if $p < 0.05$.

Results and Discussion

Characterization of modified PDMS surfaces

The distribution of the cationic peptides bound to the PDMS surface, studied by ATR-Imaging was found to be homogeneous for both procedures, i.e. grafting of AA or AFB (see Figure 1 in Supplementary Data). However, after biofilm growth in the MRD visual inspection revealed a marked swelling (i.e. increase in surface area available for biofilm formation) of the AA grafted PDMS. In contrast, PDMS disks modified with AFB remained unchanged. This observation strongly indicates the need for the selection of the appropriate interlinkage molecules for covalent binding of antimicrobials on PDMS. For all further experiments, AFB was used as the interlinkage molecule.

Antifungal effect on planktonic C. albicans cells

The MIC of Dhvar 4, Dhvar 5 and Hst 5 for *C. albicans* SC5314 was 7 µM, 9 µM and 41 µM, respectively (Table 1). The MICs of poly-D-lysine, poly-L-lysine, poly-DL-lysine and poly-L-arginine where inversely correlated with the molecular weight of each peptide ($p<0.05$) (Table 2).

Antifungal effect of salivary peptides on sessile C. albicans cells

Table 1 lists the percent reduction in sessile *C. albicans* cell numbers on PDMS functionalized with Dhvar 4, using AA and AFB as interlinkage molecules. The reduction for each type of modification and for each replicate was calculated with reference to the cell count on the peptide-free control PDMS disks. The cell counts on the unmodified disks ranged from 10^4 to 10^5 after 1 h of adhesion and from 10^6 to 10^7 following 1 h of adhesion and 24 h of biofilm formation. The results clearly show that the highest reductions in the

numbers of sessile *C. albicans* cells, both after adhesion and biofim development were obtained for Dhvar 4 containing surfaces, modified using AFB. Dhvar 5 and Hst 5 yielded reductions of 64 % and 72 % ($p < 0.05$). Although a 10-fold higher concentration was used in the procedure for covalent binding of Dhvar 5 compared to Hst 5, similar reductions were observed. Maximum reduction (95 %) was obtained on Dhvar 4-PDMS surfaces.

Our results differ from those obtained by others, such as Oosterhof et al. (2003), who used an artificial throat (a biofilm model system similar to the MRD) to grow mixed species biofilms on intact Groningen button voice prostheses. They examined the biofilm eradication effect of the synthetic peptides, Dhvar 4 and Dhvar 5, applied by dip coating. After a 3-day growth period, mature biofilms were exposed 3 times a day to Dhvar 4 and Dhvar 5, dissolved in the salivary substitute Xialine in a final concentration of 4 mg per ml (well above the MIC). Dhvar 4 did not affect the number of bacteria and yeasts compared with the control prostheses. The highest reductions [78 % (bacteria) and 94 % (yeasts)] were obtained on VPs soaked in Dhvar 5 solutions, (Oosterhof et al 2003). This is unlike our own observations and may reflect the profound difference in activity between bound and free peptides.

Antifungal effect of homopeptides on sessile C. albicans cells

Histatins reportedly have intracellular targets (mitochondria). Their effects on cytoplasmatic membranes are limited and permeabilization of the microbial membrane is a secondary effect rather than the primary cause of their antimicrobial activity (Ruissen et al 2001, 2002; Den Hertog et al 2005, Welling et al 2007). The most potent anti-*Candida* member of this family is Hst 5 and analysis of its sequence showed the presence of lysine and arginine residues (Helmerhorst et al 1997). Covalent binding of peptides only consisting of lysine, arginine and histidine residues might increase the anti-*Candida* effect on biofilms, as these cationic amino acids are believed to be partly responsible for the antifungal effect.

Poly-D-lysine PDMS with molecular weights of 2,500 or 9,600 and poly-DL-lysine PDMS (molecular weight of 32,800) yielded a significantly lower *C. albicans* sessile cell count than the controls, with reductions of 93 %, 91 % and 79 %, respectively (Table 2). However, covalent binding of poly-D-lysine moieties containing more lysine residues drastically decreased the antibiofilm activity. *C. albicans* biofilm formation on other substrates was hardly affected (Table 2). In addition, neither poly-L-arginine PDMS nor poly-L-histidine PDMS affected *C. albicans* biofilm growth. These observations demonstrate that inhibition of *C. albicans* biofilm formation highly depends on the nature of the immobilized peptide, its molecular weight and its stereochemistry.

Conclusions

In the present study we have shown that salivary peptides and homopeptides can be bound to PDMS using preferably AFB as the interlinkage molecule. AA is less suitable as interlinkage molecule as it results in swelling of the PDMS after contact with aqueous media in the MRD. This highlights the importance of the interlinkage molecule to be bound to PDMS.

Although reductions of > 90 % (Dhvar 4 and low molecular weight poly-D-lysine) were obtained in an *in vitro* biofilm model system, it remains to be established if these would be sufficient for a prolonged, protective effect *in vivo*.

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Table 1. Reduction in *C. albicans* biofilm cells grown in the MRD recovered from peptide grafted PDMS.

Substrate	MIC (µM)	Interlinkage molecule	Time (h)	Concentration (mg ml^{-1})	Peptide amount (nmoles)	n	Sessile cells PDMS (control)		% reduction (mean ± SD)	*p*-value
							PDMS (control)	Modified		
Dhvar 4-PDMS	7	PAA	1	5	1	6	4.51 ± 0.26	4.51 ± 0.41	-	-
		AFB	1	5	1	6	4.51 ± 0.26	4.16 ± 0.18	55±12	NS
		PAA	24	5	1	6	6.57 ± 0.49	7.16 ± 0.39	-	-
		AFB	24	5	1	6	6.57 ± 0.49	5.17 ± 0.45	96±3	<0.001
Dhvar 5-PDMS	9	AFB	24	1	ND	12	6.67 ± 0.29	6.80 ± 0.18	-	-
		AFB	24	5	2	12	6.63 ± 0.24	6.16 ± 0.21	66±22	<0.05
Hst 5-PDMS	41	AFB	24	0.5	0.8	12	6.80 ± 0.22	6.21 ± 0.26	74±17	<0.05

n, number of disks tested; PAA, polyacrylic acid; AFB, azido tetrafluorobenzoate; PDMS, polydimethylsiloxane; ND : not determined; Concentration refers to the concentration in solution used for modification; the amount of peptide refers to total amount per disk; - : biofilm formation stimulated (i.e. cell counts on control disks lower than on modified disks) ; NS, not significant..

Table 2. Reduction in *C. albicans* biofilm cells grown in the MRD recovered from homopeptide grafted PDMS (using 5 mg ml^{-1} of petide and AFB as interlinkage molecule).

Substrate	Mol wt range	Mol wt	MIC (µM)	m	peptide amount (nmoles)	n	Sessile cells		% reduction (mean ± SD)	*p*-value
							PDMS (control)	Modified		
Poly-D-Lysine PDMS	1,000-4,000	2,500	500	17	31	12	6.76 ± 0.36	5.59 ± 0.38	93 ± 5	<0.01
	4,000-15,000	9,600	130	66	50	11	6.67 ± 0.32	5.58 ± 0.56	91 ± 7	<0.05
	15,000-30,000	27,200	46	186	34	12	6.67 ± 0.32	6.32 ± 0.54	55 ± 39	NS
	30,000-70,000	40,600	31	278	55	11	6.67 ± 0.32	6.49 ± 0.37	34 ± 34	NS
	70,000-150,000	150,000	8	1027	47	11	6.67 ± 0.32	6.41 ± 0.56	43 ± 40	NS
	150,000-300,000	262,300	ND	1797	49	12	6.67 ± 0.32	6.46 ± 0.39	37 ± 43	NS
	> 300,000	391,700	ND	2055	51	4	6.72 ± 0.37	6.70 ± 0.34	4 ± 66	NS
Poly-L-Lysine PDMS	500-2,000	584	67	4	ND	6	7.15 ± 0.24	6.78 ± 0.25	56 ±27	NS
	1,000-5,000	4,200	9	29	ND	5	7.15 ± 0.24	6.88 ± 0.38	45 ± 50	NS
	≥ 15,000	15,000	0.08	103	ND	6	7.15 ± 0.24	7.10 ± 0.27	12 ± 49	NS
	15,000-30,000	24,000	0.13	164	ND	6	7.15 ± 0.24	6.73 ± 0.44	61 ± 53	NS
Poly-DL-Lysine PDMS	1,000-4,000	4,000	10	27	ND	6	7.15 ± 0.24	6.97 ± 0.24	34 ± 29	NS
	25,000-40,000	32,800	0.06	255	ND	6	7.15 ± 0.24	6.46 ± 0.34	79 ± 13	<0.01
Poly-L-arginine PDMS	5,000-15,000	13,300	0.15	76	29	18	6.91 ± 0.33	6.84 ± 0.56	13 ± 72	NS
	15,000-70,000	49,500	0.10	284	33	12	6.92 ± 0.35	7.04 ± 0.35	-	-
	> 70,000	125,000	0.08	718	42	12	6.92 ± 0.35	7.09 ± 0.28	-	-
Poly-L-histidine PDMS	5,000-25,000	10,000	ND	65	ND	6	6.91 ± 0.36	7.45 ± 0.18	-	-

m, number of amino acids per peptide; n, number of disks tested; AFB, azido tetrafluorobenzoate; PDMS, polydimethylsiloxane; Mol wt, molecular weight of most abundant molecule in mixture ; ND, not determined; peptide amount refers to total amount per disk; - : biofilm formation stimulated (i.e. cell counts on control disks lower than on modified disks) ; NS, not significant.

Supplementary data - Figure 1. ATR image of unmodified PDMS (top) and PDMS modified with Dhvar-4 using AFB as interlinker.