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Strain-Dependent Adsorption of *Pseudomonas aeruginosa*-Derived Adhesin-Like Peptides at Abiotic Surfaces

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Abstract: Implant-associated infections are an increasingly severe burden on healthcare systems worldwide and many research activities currently focus on inhibiting microbial colonization of biomedically relevant surfaces. To obtain molecular-level understanding of the involved processes and interactions, we investigate the adsorption of synthetic adhesin-like peptide sequences derived from the type IV pili of the *Pseudomonas aeruginosa* strains PAK and PAO at abiotic model surfaces, i.e., Au, SiO₂, and oxidized Ti. These peptides correspond to the sequences of the receptor-binding domain 128–144 of the major pilin protein, which is known to facilitate *P. aeruginosa* adhesion at biotic and abiotic surfaces. Using quartz crystal microbalance with dissipation monitoring (QCM-D), we find that peptide adsorption is material- as well as strain-dependent. At the Au surface, PAO(128–144) shows drastically stronger adsorption than PAK(128–144), whereas adsorption of both peptides is markedly reduced at the oxide surfaces with less drastic differences between the two sequences. These observations suggest that peptide adsorption is influenced by not only the peptide sequence, but also peptide conformation. Our results furthermore highlight the importance of molecular-level investigations to understand and ultimately control microbial colonization of surfaces.

Keywords: adsorption; peptide; microbial adhesion; biofilm; quartz crystal microbalance

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1. Introduction

The past century has seen a continuous increase in the number of surgical procedures aimed at the restoration of body functions via the implantation of artificial biomaterials. While tremendous advances in the fields of reconstructive medicine and biomaterials science and engineering have resulted in highly efficient implants with low probability of rejection by the body, implant-associated infections have developed into a serious problem over the last two decades [1]. All implants from heart valves and prosthetic joints to vascular prostheses and dental implants attract microorganisms and thus exhibit a certain risk of deep infection [2]. Due to the rapid spreading of antimicrobial-resistant pathogens [3–5], implant-associated infections represent an increasingly severe burden on healthcare systems worldwide. For the US alone, it was estimated that the total number of infected total hip and total knee arthroplasties will increase from 8326 in 2010 to 37,059 in 2030, corresponding to additional costs resulting only from these two procedures of up to US\$ 5 billion [6]. Suppressing implant-associated infections via the rational engineering of implant surfaces to render them resistant to microbial colonization has thus become a very important goal in the field of biomedical materials research [7]. For such engineering strategies to succeed, however, a detailed understanding of the molecular mechanisms that govern microbial colonization of abiotic surfaces is required.

One of the most common nosocomial pathogens encountered in healthcare settings all over the world is the Gram-negative bacterium *Pseudomonas aeruginosa*. *P. aeruginosa* infections are particularly hard to treat and associated with exceptionally high mortality

rates [8]. This is because of the intrinsic high resistance of *P. aeruginosa* to many antimicrobials and its remarkable ability to acquire additional mechanisms of resistance to multiple groups of antimicrobials [9]. Pandrug-resistant strains that do not respond to any available antimicrobial are an increasing problem worldwide [8,9]. Consequently, in 2017, carbapenem-resistant *P. aeruginosa* was ranked second on the WHO priority list of antimicrobial-resistant bacteria [10].

When colonizing surfaces, *P. aeruginosa* relies predominantly on dynamic protein filaments called type IV pili (see Figure 1a) that facilitate initial adhesion as well as migration and motility of the adhering bacteria [11–13]. Each of these filaments is anchored in the inner membrane and consists of several thousand copies of a single protein subunit called pilin (see Figure 1b). These type IV pilins are structurally highly conserved in different *P. aeruginosa* strains and also other bacteria [12–15]. Of particular medical importance is the C-terminal D-region, which contains a loop structure defined by a disulfide bridge (see Figure 1b) and facilitates adherence to host-cell epithelia [12,13]. However, Giltner et al. demonstrated that this receptor-binding domain between residues 128 and 144 is also involved in *P. aeruginosa* adhesion to abiotic surfaces and in particular stainless steel, polystyrene, and polyvinylchloride [14]. Therefore, in this work, we investigate the adsorption of synthetic peptide sequences corresponding to the receptor-binding domains of *P. aeruginosa* pilins at abiotic model surfaces, i.e., Au, SiO₂, and oxidized Ti (TiO_x/Ti). While Au is used as a well-defined, inert reference surface, SiO₂ and TiO_x/Ti films serve as model surfaces for implants based on bioactive glasses [16] and titanium alloys [17], respectively. Since the receptor-binding domain shows considerable sequence variability between different *P. aeruginosa* strains, we are comparing the adsorption of two peptides derived from two different strains, i.e., PAK and PAO (see Figure 1c). Using quartz crystal microbalance with dissipation monitoring (QCM-D), we find that peptide adsorption is not only surface-dependent, but also strain-dependent. In particular, PAO(128–144) shows stronger adsorption at Au surfaces than PAK(128–144), whereas at the oxide surfaces, adsorption of both peptides is markedly reduced and almost negligible. Our results suggest that peptide adsorption at these surfaces is influenced not only by the peptide sequence, but also the peptide loop conformation and stability.

Figure 1. (a) Schematic representation of *P. aeruginosa* with cell surface-associated type IV pili. (b) Structure of the major PAK pilin protein taken from the RCSB PDB (rcsb.org), PDB ID 1OQW [15]. The C-terminal receptor-binding domain PAK(128–144) is highlighted in red. The image was created using Mol* Viewer [18]. (c) Sequences, isoelectric points (pI, calculated using Pep-Calc.com [19]), and grand average of hydropathicity (GRAVY, calculated using the ExPASy ProtParam tool [20]) of the pilin-derived peptides used in this work. Positively charged amino acids are indicated in red, negatively charged ones in blue, and hydrophobic ones in green. Cysteines are colored orange with disulfide bridges indicated.

2. Materials and Methods

2.1. Peptide Sample Preparation

PAK(128–144) and PAO(128–144) were purchased from the Biomolecular Synthesis Facility of the Center For Molecular And Cellular Bioengineering, TU Dresden, Germany, and used without further purification. The peptides were dissolved in phosphate buffered saline (PBS, Sigma-Aldrich, Steinheim, Germany) containing 10 mM Na_2HPO_4 , 1.8 mM KH_2PO_4 , 138 mM NaCl, and 2.7 mM KCl with a pH value of 7.4. To avoid bubble formation during the QCM-D measurements, the buffer solution was degassed with Ar for 1 h. The final peptide concentration was 100 μM in each case. For all solutions, HiPerSolv Chromanorm water for HPLC (VWR International, Fontenay-sous-Bois, France) was used.

2.2. Surface Preparation

Gold-coated QCM-D crystals with a fundamental resonance frequency of 4.95 MHz were purchased from Fil-Tech Inc., Boston, MA, USA. Before use, the sensors were cleaned with freshly prepare RCA1 solution (1:1:5 $\text{NH}_4\text{OH}:\text{H}_2\text{O}_2:\text{H}_2\text{O}$) at 75 °C for 30 s. Afterwards, the sensors were rinsed with water and ethanol and dried in a stream of ultrapure air. The cleaned surface of the Au electrode was either used as is in the subsequent adsorption experiments or coated with SiO_2 and Ti as described below.

A thin film of Ti was deposited on the cleaned Au surface on the QCM-D sensor as previously described [21]. In brief, RF-magnetron sputter deposition (Ion'X-2" UHV from Thin Film Consulting, Grafenberg, Germany, with a BDS-HF 300 AFP generator from BDISCOM SRL, Vellezzo Bellini, Italy) was performed under normal incidence at room temperature in a vacuum chamber with a base pressure of $\sim 10^{-7}$ mbar. A high-purity Ti target (purity 99.995%, EVOCHEM, Offenbach am Main, Germany) was used. During deposition, an Ar flow rate of 30 sccm was applied, resulting in a Ti deposition rate of 0.07 Å/s. The total applied film thickness was 100 nm.

SiO_2 was deposited using reactive RF-magnetron sputter deposition (Nexdep physical vapor deposition system, Angstrom Engineering Inc., Kitchener, ON, Canada, base pressure $\sim 5 \times 10^{-7}$ mbar). First, 10 nm of Ti (purity 99.995%, Angstrom Engineering Inc., Kitchener, ON, Canada) were deposited as an adhesion layer at an Ar flow rate of 20 sccm and a deposition rate of 0.10 Å/s. Then, 100 nm of SiO_2 were deposited by sputtering a Si target (undoped, purity 99.999%, Angstrom Engineering Inc., Kitchener, ON, Canada) using a gas mixture of 20 sccm Ar and 1 sccm O_2 . The resulting deposition rate was 0.16 Å/s.

2.3. X-ray Photoelectron Spectroscopy (XPS)

The surface chemistry of the SiO_2 and TiO_x/Ti films was characterized by means of X-ray photoelectron spectroscopy (XPS). All measurements were done with an Omicron ESCA+ system (Omicron NanoTechnology, Taunusstein, Germany). The pressure was below 3×10^{-10} mbar and monochromatic Al $K\alpha$ radiation (1486.7 eV) was used. The measurements were carried out at a take-off angle of 30° with respect to the surface plane. The survey and core level spectra were detected with a pass energy of 100 eV and 20 eV, respectively. During the measurements, neutralization with a beam energy of 5.0 eV and an emission current of 10.0 μA was performed. The evaluation of the spectra was done with CasaXPS, V 2.3 (Casa Software Ltd., Teignmouth, UK). The spectra were calibrated to the aliphatic C1s peak at 284.6 eV. The background of the spectra was subtracted with a Shirley type function and for the peak fitting Gaussian and Lorentzian peak shapes with 30% of Lorentzian contribution were used.

2.4. Atomic Force Microscopy (AFM)

The topographies of all surfaces were analyzed by atomic force microscopy (AFM) using a JPK NanoWizard 3 (JPK GmbH, Berlin, Germany) and HQ:NSC15/AlBS cantilevers (MikroMasch, Wetzlar, Germany) in intermittent contact mode in air. The images had a size of $5 \times 5 \mu\text{m}^2$ and a resolution of 1024×1024 px. All AFM images were processed using Gwyddion (version 2.51) [22] and the RMS surface roughness S_q was obtained for

each image. The S_q values were averaged over four images and the standard deviations are given as errors.

2.5. QCM-D

QCM-D experiments were performed using a QSense E4 from Biolin Scientific, Gothenburg, Sweden, at a temperature of 20 °C. Peptide adsorption at the different surfaces was measured in flow mode at a constant flow rate of 10 $\mu\text{L}/\text{min}$ provided by a peristaltic pump (IPC4, Ismatec, Wertheim, Germany). Due to the sometimes very small frequency shifts (ΔF), which were of the order of the drift of the QCM-D sensor, the recorded ΔF curves were drift-corrected in OriginPro, version 2020 (OriginLab Corporation, Northampton, MA, USA) by subtracting a linear function. In the following, only data for the 5th overtone are shown, while overtones 3 to 9 are shown in Figures S7–S12.

3. Results and Discussion

3.1. Surface Characterization

The topographies of the prepared Au, SiO_2 and TiO_x/Ti surfaces have been analyzed by means of AFM. The AFM image of the as-received Au surface presented in Figure 2a shows the individual grains of the polycrystalline electrode, which dominate the surface morphology. Deposition of the SiO_2 and Ti thin films results in notable modifications of the overall surface topography. While larger grains are obtained after depositing the amorphous SiO_2 film (Figure 2b), the oxidized Ti film exhibits a smoother surface (Figure 2c). This is also reflected in the calculated root-mean-square (RMS) roughness (S_q) values. Whereas $S_q = 3.09 \pm 0.14$ nm for the uncoated Au electrode surface, SiO_2 deposition results in a higher surface roughness of $S_q = 4.63 \pm 0.08$ nm. For the TiO_x/Ti surface, however, a lower value of $S_q = 2.40 \pm 0.02$ nm was determined. These differences can be attributed to different growth modes of these amorphous (SiO_2) and polycrystalline (Ti) films and the different deposition rates (0.07 vs. 0.16 $\text{\AA}/\text{s}$). Higher deposition rates typically result in rougher film topographies [23], which is in line with the present observations. Despite these notable differences in surface topography, we expect peptide adsorption to be predominantly affected by surface chemistry and not surface topography. This is mostly because the peptides studied in this work are very small in relation to the surface topographic features. Significant effects of nanoscale surface topography at this scale on biomolecular adsorption are typically observed only for larger proteins [21,24] or in the presence of strong peptide–peptide interactions which result in nanometer-sized aggregates [25]. However, because of the large S_q values of the surfaces, which are about one order of magnitude larger than those of silicon wafers [24], the adsorbed peptides with their small sizes and molecular weights below 2 kDa cannot be identified by AFM [26,27].

Figure 2. Representative AFM images of the employed (a) Au, (b) SiO_2 , and (c) TiO_x/Ti surfaces. Height scales are 40 nm (a), 30 nm (b), and 20 nm (c), respectively.

The chemical composition of the deposited films was assessed by XPS. The surface of the SiO₂ film was found to be composed predominantly of silicon and oxygen with some contributions from adventitious carbon species (see Figure S1 and Table S1). The high-resolution Si2p spectrum in Figure S2 additionally reveals only a single peak corresponding to SiO₂ without any contributions from suboxides [28].

For the native surface oxide of the Ti thin film, only oxygen, titanium, carbon, and nitrogen are observed (see Figure S3 and Table S1), the latter two resulting from air exposure. More importantly, deconvolution of the Ti2p spectrum revealed that the surface oxide is mainly composed of TiO₂ with only minor contributions from suboxides (see Figure S4 and Table S2). The O1s spectrum of the oxide-covered Ti film (see Figure S5) further indicates a significant hydroxylation of the ultra-thin surface oxide. In particular, deconvolution of the O1s spectrum reveals an OH[−]/O^{2−} ratio of 0.49 ± 0.06 (after subtraction of the COOC and COOH contributions; see Figure S6). Based on the energy difference between the oxide and the hydroxyl O1s contribution of +1.5 eV, we conclude that these surface OH groups are acidic [29]. The surface hydroxylation is thus indicative of a significant negative surface charge formation above the isoelectric point caused by the deprotonation of surface OH groups.

3.2. Peptide Adsorption

The results of the QCM-D measurements (frequency shift, ΔF , and change in dissipation, ΔD) are shown in Figure 3. For PAK(128–144) adsorption at the Au surface (Figure 3a, left panel), a sharp decrease of ΔF can be observed upon peptide injection, equivalent to an increase in sensor mass due to peptide adsorption at the electrode surface. This adsorption is accompanied by a rapid increase in the dissipation ΔD . About 20 min after peptide injection, the decrease in ΔF is slowing down until a constant value of about −10 Hz is eventually reached. At the same time, ΔD is decreasing again, resulting in a spike-like curve. Since ΔD is a measure of the viscoelasticity of the adsorbate film [30], such a behavior is indicative of structural rearrangements of the adsorbed peptides, resulting in a more compact film that traps fewer water molecules. This rearrangement appears to be a comparatively slow process that proceeds for more than 90 min without reaching a steady state. The compaction is partially reversed upon flushing the surface with peptide-free buffer solution, which results in a second, yet smaller, increase of ΔD . After another 15 min, ΔD saturates at an intermediate value. ΔF , on the other hand, is barely affected by flushing, indicating that the majority of peptides are irreversibly adsorbed. The observed structural rearrangement in the adsorbed peptide film can be attributed to the high complexity of the amino acid sequence of the PAK(128–144) peptide, which features several different amino acid residues with very different properties (see Figure 1c). It is well known that the adsorption energies of different amino acids at Au surfaces can differ by a factor of two, with cysteine playing a special role due to its ability to form covalent bonds with the Au surface via its thiol group [31]. Therefore, it appears reasonable that the PAK(128–144) peptide, which contains two cysteines, undergoes significant conformational adjustments after adsorption that in turn affect the packing of the neighboring peptides and the availability of free adsorption sites.

This interpretation of residue-driven conformational changes is further supported by the fact that a rather similar behavior is also observed for the adsorption of PAO(128–144) at the Au surface (see Figure 3b, left panel). In this case, however, a larger frequency shift and a larger change in dissipation are observed. While it is rather tempting to attribute the considerably strong adsorption of both peptides at the Au surface to the formation of S–Au bonds between the two cysteines and the Au surface, these differences in adsorbed mass underscore the relevance of the other residues. As shown in Figure 1c, the amino acid sequences of both peptides differ in eight residues. Using adsorption energies for isolated but capped amino acids at Au [31], we calculated the adsorption energies $E_{\text{ads}}(\text{PAK})$ and $E_{\text{ads}}(\text{PAO})$ for both peptides. We found that the calculated adsorption energy of PAK(128–144) is slightly lower than that of PAO(128–144), i.e., $E_{\text{ads}}(\text{PAK}) = -1488.0 \text{ kJ/mol}$

and $E_{\text{ads}}(\text{PAO}) = -1457.7 \text{ kJ/mol}$, which is at variance with the above experiments that showed stronger adsorption of PAO. This discrepancy can be attributed to two factors that are not considered in this simple calculation. First, the published adsorption energies of the amino acids are for adsorption at a flat Au(111) surface in vacuum [31], while our experiments were performed at a polycrystalline Au surface in physiological buffer. The presence of water, ions, and additional crystal facets may alter the adsorption energies of individual amino acids in a species-dependent manner. Therefore, we next calculated the grand average of hydrophobicity (GRAVY) [32] for both peptides (see Figure 1c). As it turns out, PAO(128–144) is slightly more hydrophobic than PAK(128–144). Since non-oxidized Au surfaces in general have a somewhat hydrophobic character [33], this slight increase in peptide hydrophobicity may indeed result in stronger adsorption, even though both peptides are still rather hydrophilic in nature.

Figure 3. Frequency shift ΔF (left axes) and change in dissipation ΔD (right axes) for the adsorption of (a) PAK(128–144) and (b) PAO(128–144) at the Au (left), SiO₂ (center), and TiO_x/Ti surface (right). Vertical lines indicate the time points of peptide injection and flushing with peptide-free buffer.

Another important factor that has been neglected in the above considerations is the conformation of the two peptides. In particular, both peptides used in the present study feature two cysteines close to their termini, which are expected to form disulfide bridges and thus facilitate the formation of a peptide loop similar to that observed in the full pilin protein (see Figure 1b). Loop formation will most likely restrict the mobility of the amino acid sidechains and thereby affect their interaction with the Au surface. Furthermore, changes in amino acid sequence will also affect the conformation and stability of the loop itself [34]. This in particular concerns the presence of prolines [35], of which PAO(128–144) features an additional one between the two cysteines (see Figure 1c). Indeed, structural investigations of the complete PAK and PAO pilin proteins revealed small but significant differences in loop conformation [14].

For the SiO₂ surface, adsorption of both peptides is strongly reduced. At this surface, peptide adsorption obviously does not involve covalent bond formation. Furthermore, peptide adsorption also requires the displacement of water molecules from the surface-

bound hydration layer [36]. Both of these effects may contribute to weakening peptide adsorption. However, there are also notable differences between the two peptides. For PAK(128–144), similar trends as for the Au surface are observed for ΔF and ΔD , albeit with much smaller absolute values (Figure 3a, central panel). Interestingly, a slight overshooting in the adsorbed mass is observed, with ΔF dropping to about -3 Hz within the first 10 min of adsorption, after which it slowly recovers and finally saturates at about -2 Hz. This overshooting is again indicative of structural rearrangements occurring in the adsorbate film, which now appear to be substantially different from those at the Au surface where no overshooting was observed (Figure 3a, left panel). Furthermore, the overall adsorbed mass at equilibrium is significantly smaller for the SiO_2 than for the Au surface.

For PAO(128–144), on the other hand, barely any adsorption at the SiO_2 surface can be detected (Figure 3b, central panel). This observation is rather surprising since protein and peptide adsorption at SiO_2 surfaces, which are negatively charged under physiological conditions [37], is mostly driven by electrostatic interactions [24,25,38,39]. The studied peptides, on the other hand, both have essentially the same isoelectric points and should thus have a negative net charge of about -1 at pH 7.4 (see Figure 1c). Nevertheless the peptide sequences shown in Figure 1c reveal that PAK(128–144) features three positively and three negatively charged amino acid residues, whereas PAO(128–144) has only two of each. Furthermore, the negatively charged residues in PAK(128–144) are all clustered within a short loop region (aa 132 to 134), while the positively charged ones are located close to the termini and thus end up in the stem region (see Figure 4a). Considering the dimensions of the peptide loop and the short Debye length of PBS of $\lambda_D \sim 0.7$ nm [40], we assume that this peptide initially adsorbs in an upright configuration, in which two to three of the positively charged residues are in close contact with the negatively charged SiO_2 surface (see Figure 4a). In this geometry, the negatively charged residues are located more than 0.7 nm from the like-charged surface, so that electrostatic repulsion is effectively screened by the electrolyte. Later, however, short-range van der Waals interactions may take over and result in the peptide loop assuming a flat orientation (see Figure 4b), which may be the structural rearrangements observed in the corresponding ΔD curve. In contrast, the charged residues in the PAO(128–144) sequence are more evenly distributed (see Figure 1c), so that this mechanism will be less effective, resulting in weaker adsorption as observed experimentally (see Figure 3).

Figure 4. Proposed adsorption geometry for PAK(128–144) at the SiO_2 surface. (a) Initial adsorption occurs in an upright conformation with the positively (red) and negatively charged (blue) amino acid residues being located within and beyond the Debye length λ_D , respectively. (b) Subsequently, short-range van der Waals interactions may result in the peptide loop assuming a flat geometry. Structure of the PAK(128–144) peptide loop taken from the RCSB PDB (rcsb.org), PDB ID 1OQW [15]. The images were created using Mol* Viewer [18].

At the TiO_x/Ti surface, PAK(128–144) shows a similar adsorption behavior as at the SiO_2 surface, including the overshooting in adsorbed mass (Figure 3a, right panel). However, the absolute frequency shifts ΔF are even smaller than for the SiO_2 surface, with values of about -1.4 Hz and -0.5 Hz at the peak and in equilibrium, respectively. Nevertheless, the spike in the ΔD curve is higher and sharper than in the previous case, which indicates that structural rearrangements are faster and more pronounced. This is in line with the proposed model of PAK(128–144) adsorption in Figure 4 because TiO_2 has a significantly larger Hamaker constant than SiO_2 [41] so that van der Waals interactions should be more dominant at the TiO_x/Ti surfaces and thus result in the peptide assuming the flat configuration in shorter time. In contrast, adsorption of PAO(128–144) at the TiO_x/Ti surface shows a frequency shift in equilibrium that is comparable to that of PAK(128–144) with no indication of an overshooting (Figure 3b, right panel). In addition, the ΔD curve shows only a very small change upon adsorption without any visible spike. This indicates that this particular peptide immediately assumes a flat conformation at the TiO_x/Ti surface upon adsorption without any subsequent rearrangements. This rather pronounced difference to PAO(128–144) adsorption at the SiO_2 surface where barely any adsorption was observed may be a result of the stronger van der Waals interactions in combination with slightly reduced electrostatic repulsion. Although the isoelectric points of both SiO_2 and TiO_2 can vary quite drastically between different material formulations, the one of TiO_2 should, on average, be higher [42] and may thus result in a less negatively charged surface at pH 7.4. The reduced electrostatic repulsion may enable the PAO(128–144) peptide, which has a more neutral character than PAK(128–144) because of its more evenly distributed charges (see Figure 1c), to get closer to the surface. Then, the attractive van der Waals interactions will take over and result in the adsorption of the peptide in a flat conformation.

Finally, we quantified the irreversibly adsorbed peptide mass after flushing. In the stationary regime after flushing, the obtained ΔD values in all cases are rather low with $\Delta D \leq 1.1 \times 10^{-6}$, while the spread between individual overtones is very small as well (see Figures S7–S12). Therefore, we calculated the irreversibly adsorbed mass using the Sauerbrey relation [30,43]. This was done by first individually averaging the ΔF values recorded for overtones 3 to 9 in the time window from 20 to 30 min after flushing. Figure 5a presents the averages of the four different overtones for each peptide-surface combination with the standard deviations as errors. As can be seen, the trends of the irreversibly adsorbed mass reflect the overall trends seen in the ΔF data reported in Figure 3 rather well, despite minor deviations and the sometimes rather large error bars, which result from measurement artefacts and, in particular, variations between individual overtones (see Figures S7–S12).

Based on the determined Sauerbrey masses shown in Figure 5a, we further attempted to estimate the equilibrium surface coverage for the different peptide-surface combinations. For this, the footprint of each adsorbed peptide must be calculated. This was done by estimating their hydrodynamic radii r_H using the empiric formula developed by Nöbbmann [44]. For both peptides, this results in $r_H \sim 0.8$ nm, which is in fair agreement with the dimensions of the peptide loop in the complete pilin protein (see Figure 4). Using this value for surface coverage estimation thus corresponds to the assumption of the intact peptide loops lying flat on the surface. As can be seen in Figure 5b, this rather crude estimation reveals a surface coverage of about one monolayer for PAK(128–144) at the Au surface. In line with the increased Sauerbrey mass, PAO(128–144) shows about twice this coverage. While such a double-layer coverage is certainly possible, it may just as well be a result of PAO(128–144) adsorbing in a more upright instead of a flat configuration. In contrast, for the oxide surfaces, surface coverage is well below one monolayer, i.e., 0.04 to 0.15 ML. At such a low coverage, a flat adsorption geometry as suggested in Figure 4 appears certainly possible.

Figure 5. (a) Irreversibly adsorbed peptide mass m determined for the different peptide-surface combinations using the Sauerbrey equation. Values represent averages of overtones 3 to 9 with the standard deviations as error bars. (b) Corresponding surface coverage in monolayers (ML) calculated from the molecular weights and the estimated hydrodynamic radii r_H of the peptides.

4. Conclusions

In this work, we investigated the adsorption of two *P. aeruginosa*-derived pilin peptides with sequences corresponding to the receptor-binding domains of the two strains PAK and PAO at three abiotic surfaces using QCM-D. Strongest adsorption of both peptides is observed at the Au surface, which is accompanied by strong structural rearrangements of the adsorbed peptide films. While adsorption at this surface probably involves covalent S-Au bond formation between the peptides' cysteins and the surface, the large differences in adsorbed peptide mass (see Figure 5) suggest that the other amino acid residues and the exact conformation of the peptide loop play important roles in adsorption as well. At oxide surfaces, adsorption of both peptides is drastically reduced and most likely driven by electrostatic and van der Waals interactions. However, also in this case, the peptide-specific and material-specific differences in adsorption hint at the importance of the exact peptide conformation and possibly the clustering of charged amino acid residues on opposing sides of the loop in the case of PAK(128–144). Our work thus suggests that colonization of abiotic surfaces by *P. aeruginosa* and most likely other pathogenic microbes is highly dependent on the surface material and the microbial strain, with comparatively small sequence variations potentially having a strong effect on initial adhesion. Finally, our results also highlight the importance of molecular-level investigations to understand and ultimately control microbial colonization of the surfaces of medical implants as well as everyday materials and devices.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/micro1010010/s1>, Figure S1: Ex-situ XPS survey of the SiO₂ film, Figure S2: Ex-situ XPS Si2p high-resolution spectrum of the SiO₂ film, Figure S3: Ex-situ XPS survey of the TiO_x/Ti film, Table S1. XPS quantification results for the SiO₂ and TiO_x/Ti films, Figure S4: Ex-situ XPS Ti2p high-resolution spectrum of the TiO_x/Ti film, Table S2. Results of the Ti 2p deconvolution, Figure S5: Ex-situ XPS O1s high-resolution spectrum of the TiO_x/Ti film, Figure S6: Ex-situ XPS C1s high-resolution spectrum of the TiO_x/Ti film, Figure S7: QCM-D results (overtones 3 to 9) for PAK(128–144) adsorption at the Au surface, Figure S8: QCM-D results (overtones 3 to 9) for PAO(128–144) adsorption at the Au surface, Figure S9: QCM-D results (overtones 3 to 9) for PAK(128–144) adsorption at the SiO₂ surface, Figure S10: QCM-D results (overtones 3 to 9) for PAO(128–144) adsorption at the SiO₂ surface, Figure S11: QCM-D results (overtones 3 to 9) for PAK(128–144) adsorption at the TiO_x/Ti

surface, Figure S12: QCM-D results (overtones 3 to 9) for PAO(128–144) adsorption at the TiO_x/Ti surface.

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References

- Hedrick, T.L.; Adams, J.D.; Sawyer, R.G. Implant-associated infections: An overview. *J. Long Term Eff. Med. Implant.* **2006**, *16*, 83–99. [\[CrossRef\]](#)
- Busscher, H.J.; van der Mei, H.C.; Subbiahdoss, G.; Jutte, P.C.; van den Dungen, J.J.A.M.; Zaat, S.A.J.; Schultz, M.J.; Grainger, D.W. Biomaterial-associated infection: Locating the finish line in the race for the surface. *Sci. Transl. Med.* **2012**, *4*, 153rv10. [\[CrossRef\]](#)
- Laxminarayan, R.; Duse, A.; Wattal, C.; Zaidi, A.K.M.; Wertheim, H.F.L.; Sumpradit, N.; Vlieghe, E.; Hara, G.L.; Gould, I.M.; Goossens, H.; et al. Antibiotic resistance—The need for global solutions. *Lancet Infect. Dis.* **2013**, *13*, 1057–1098. [\[CrossRef\]](#)
- Levy, S.B.; Marshall, B. Antibacterial resistance worldwide: Causes, challenges and responses. *Nat. Med.* **2004**, *10*, S122–S129. [\[CrossRef\]](#) [\[PubMed\]](#)
- Taubes, G. The bacteria fight back. *Science* **2008**, *321*, 356–361. [\[CrossRef\]](#) [\[PubMed\]](#)
- Alt, V. Antimicrobial coated implants in trauma and orthopaedics—A clinical review and risk-benefit analysis. *Injury* **2017**, *48*, 599–607. [\[CrossRef\]](#) [\[PubMed\]](#)
- Salwiczek, M.; Qu, Y.; Gardiner, J.; Strugnelli, R.A.; Lithgow, T.; McLean, K.M.; Thissen, H. Emerging rules for effective antimicrobial coatings. *Trends Biotechnol.* **2014**, *32*, 82–90. [\[CrossRef\]](#) [\[PubMed\]](#)
- Souli, M.; Galani, I.; Giamarellou, H. Emergence of extensively drug-resistant and pandrug-resistant Gram-negative bacilli in Europe. *Euro. Surveill.* **2008**, *13*, 19045. [\[CrossRef\]](#)
- Livermore, D.M. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: Our worst nightmare? *Clin. Infect. Dis.* **2002**, *34*, 634–640. [\[CrossRef\]](#)
- Willyard, C. The drug-resistant bacteria that pose the greatest health threats. *Nature* **2017**, *543*, 15. [\[CrossRef\]](#)
- Craig, L.; Forest, K.T.; Maier, B. Type IV pili: Dynamics, biophysics and functional consequences. *Nat. Rev. Microbiol.* **2019**, *17*, 429–440. [\[CrossRef\]](#) [\[PubMed\]](#)
- Craig, L.; Pique, M.E.; Tainer, J.A. Type IV pilus structure and bacterial pathogenicity. *Nat. Rev. Microbiol.* **2004**, *2*, 363–378. [\[CrossRef\]](#)
- Hahn, H.P. The type-4 pilus is the major virulence-associated adhesin of *Pseudomonas aeruginosa*—A review. *Gene* **1997**, *192*, 99–108. [\[CrossRef\]](#)
- Giltner, C.L.; van Schaik, E.J.; Audette, G.F.; Kao, D.; Hodges, R.S.; Hassett, D.J.; Irvin, R.T. The *Pseudomonas aeruginosa* type IV pilin receptor binding domain functions as an adhesin for both biotic and abiotic surfaces. *Mol. Microbiol.* **2006**, *59*, 1083–1096. [\[CrossRef\]](#) [\[PubMed\]](#)
- Craig, L.; Taylor, R.K.; Pique, M.E.; Adair, B.D.; Arvai, A.S.; Singh, M.; Lloyd, S.J.; Shin, D.S.; Getzoff, E.D.; Yeager, M.; et al. Type IV Pilin Structure and Assembly. *Mol. Cell* **2003**, *11*, 1139–1150. [\[CrossRef\]](#)
- Hench, L.L. The story of Bioglass. *J. Mater. Sci. Mater. Med.* **2006**, *17*, 967–978. [\[CrossRef\]](#)
- Geetha, M.; Singh, A.K.; Asokamani, R.; Gogia, A.K. Ti based biomaterials, the ultimate choice for orthopaedic implants—A review. *Prog. Mat. Sci.* **2009**, *54*, 397–425. [\[CrossRef\]](#)
- Sehnal, D.; Bittrich, S.; Deshpande, M.; Svobodová, R.; Berka, K.; Bazgier, V.; Velankar, S.; Burley, S.K.; Koča, J.; Rose, A.S. Mol* Viewer: Modern web app for 3D visualization and analysis of large biomolecular structures. *Nucleic Acids Res.* **2021**, *49*, W431–W437. [\[CrossRef\]](#)
- Lear, S.; Cobb, S.L. Pep-Calculator: A set of web utilities for the calculation of peptide and peptoid properties and automatic mass spectral peak assignment. *J. Comput. Aided Mol. Des.* **2016**, *30*, 271–277. [\[CrossRef\]](#)
- Gasteiger, E.; Hoogland, C.; Gattiker, A.; Duvaud, S.; Wilkins, M.R.; Appel, R.D.; Bairoch, A. Protein Identification and Analysis Tools on the ExPASy Server. In *The Proteomics Protocols Handbook*; Walker, J.M., Ed.; Humana Press: Totowa, NJ, USA, 2005; pp. 571–607. ISBN 978-1-58829-343-5.
- Yang, Y.; Knust, S.; Schwiderek, S.; Qin, Q.; Yun, Q.; Grundmeier, G.; Keller, A. Protein Adsorption at Nanorough Titanium Oxide Surfaces: The Importance of Surface Statistical Parameters beyond Surface Roughness. *Nanomaterials* **2021**, *11*, 357. [\[CrossRef\]](#)
- Nečas, D.; Klapetek, P. Gwyddion: An open-source software for SPM data analysis. *Open Phys.* **2012**, *10*, 181–188. [\[CrossRef\]](#)

23. Amirzada, M.R.; Tatzel, A.; Viereck, V.; Hillmer, H. Surface roughness analysis of SiO₂ for PECVD, PVD and IBD on different substrates. *Appl. Nanosci.* **2016**, *6*, 215–222. [[CrossRef](#)]
24. Yang, Y.; Yu, M.; Böke, F.; Qin, Q.; Hübner, R.; Knust, S.; Schwiderek, S.; Grundmeier, G.; Fischer, H.; Keller, A. Effect of nanoscale surface topography on the adsorption of globular proteins. *Appl. Surf. Sci.* **2021**, *535*, 147671. [[CrossRef](#)]
25. Hanke, M.; Yang, Y.; Ji, Y.; Grundmeier, G.; Keller, A. Nanoscale Surface Topography Modulates hIAPP Aggregation Pathways at Solid–Liquid Interfaces. *Int. J. Mol. Sci.* **2021**, *22*, 5142. [[CrossRef](#)]
26. Hajiraissi, R.; Hanke, M.; Gonzalez Orive, A.; Duderija, B.; Hofmann, U.; Zhang, Y.; Grundmeier, G.; Keller, A. Effect of Terminal Modifications on the Adsorption and Assembly of hIAPP(20–29). *ACS Omega* **2019**, *4*, 2649–2660. [[CrossRef](#)] [[PubMed](#)]
27. Hajiraissi, R.; Hanke, M.; Yang, Y.; Duderija, B.; Gonzalez Orive, A.; Grundmeier, G.; Keller, A. Adsorption and Fibrillization of Islet Amyloid Polypeptide at Self-Assembled Monolayers Studied by QCM-D, AFM, and PM-IRRAS. *Langmuir* **2018**, *34*, 3517–3524. [[CrossRef](#)]
28. Himpfel, F.J.; McFeely, F.R.; Taleb-Ibrahimi, A.; Yarmoff, J.A.; Hollinger, G. Microscopic structure of the SiO₂/Si interface. *Phys. Rev. B* **1988**, *38*, 6084–6096. [[CrossRef](#)] [[PubMed](#)]
29. Benkoula, S.; Sublemontier, O.; Patanen, M.; Nicolas, C.; Sirotti, F.; Naitabdi, A.; Gaie-Levrel, F.; Antonsson, E.; Aureau, D.; Ouf, F.-X.; et al. Water adsorption on TiO₂ surfaces probed by soft X-ray spectroscopies: Bulk materials vs. isolated nanoparticles. *Sci. Rep.* **2015**, *5*, 15088. [[CrossRef](#)]
30. Dixon, M.C. Quartz crystal microbalance with dissipation monitoring: Enabling real-time characterization of biological materials and their interactions. *J. Biomol. Tech.* **2008**, *19*, 151–158. [[PubMed](#)]
31. Futera, Z. Amino-acid interactions with the Au(111) surface: Adsorption, band alignment, and interfacial electronic coupling. *Phys. Chem. Chem. Phys.* **2021**, *23*, 10257–10266. [[CrossRef](#)]
32. Kyte, J.; Doolittle, R.F. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **1982**, *157*, 105–132. [[CrossRef](#)]
33. Canning, J.; Tzioumis, N.; Tzoumis, N.; Beattie, J.K.; Gibson, B.C.; Ilagan, E. Water on Au sputtered films. *Chem. Commun.* **2014**, *50*, 9172–9175. [[CrossRef](#)]
34. Zhang, R.M.; Snyder, G.H. Dependence of formation of small disulfide loops in two-cysteine peptides on the number and types of intervening amino acids. *J. Biol. Chem.* **1989**, *264*, 18472–18479. [[CrossRef](#)]
35. Krieger, F.; Möglich, A.; Kiefhaber, T. Effect of proline and glycine residues on dynamics and barriers of loop formation in polypeptide chains. *J. Am. Chem. Soc.* **2005**, *127*, 3346–3352. [[CrossRef](#)]
36. Ley, K.; Christofferson, A.; Penna, M.; Winkler, D.; Maclaughlin, S.; Yarovsky, I. Surface-water Interface Induces Conformational Changes Critical for Protein Adsorption: Implications for Monolayer Formation of EAS Hydrophobin. *Front. Mol. Biosci.* **2015**, *2*, 64. [[CrossRef](#)]
37. Dove, P.M.; Craven, C.M. Surface charge density on silica in alkali and alkaline earth chloride electrolyte solutions. *Geochim. Cosmochim. Acta* **2005**, *69*, 4963–4970. [[CrossRef](#)]
38. McUmber, A.C.; Randolph, T.W.; Schwartz, D.K. Electrostatic Interactions Influence Protein Adsorption (but Not Desorption) at the Silica-Aqueous Interface. *J. Phys. Chem. Lett.* **2015**, *6*, 2583–2587. [[CrossRef](#)] [[PubMed](#)]
39. Meissner, J.; Prause, A.; Bharti, B.; Findenegg, G.H. Characterization of protein adsorption onto silica nanoparticles: Influence of pH and ionic strength. *Colloid Polym. Sci.* **2015**, *293*, 3381–3391. [[CrossRef](#)] [[PubMed](#)]
40. Chu, C.-H.; Sarangadharan, I.; Regmi, A.; Chen, Y.-W.; Hsu, C.-P.; Chang, W.-H.; Lee, G.-Y.; Chyi, J.-I.; Chen, C.-C.; Shiesh, S.-C.; et al. Beyond the Debye length in high ionic strength solution: Direct protein detection with field-effect transistors (FETs) in human serum. *Sci. Rep.* **2017**, *7*, 5256. [[CrossRef](#)] [[PubMed](#)]
41. Ackler, H.D.; French, R.H.; Chiang, Y.-M. Comparisons of Hamaker Constants for Ceramic Systems with Intervening Vacuum or Water: From Force Laws and Physical Properties. *J. Colloid Interface Sci.* **1996**, *179*, 460–469. [[CrossRef](#)]
42. Kosmulski, M. The pH-dependent surface charging and points of zero charge: V. Update. *J. Colloid Interface Sci.* **2011**, *353*, 1–15. [[CrossRef](#)]
43. Sauerbrey, G. Verwendung von Schwingquarzen zur Wägung dünner Schichten und zur Mikrowägung. *Z. Physik* **1959**, *155*, 206–222. [[CrossRef](#)]
44. Nobbmann, U. Characterization down to Nanometers: Light Scattering from Proteins and Micelles. In *Mesoscale Phenomena in Fluid Systems*; Case, F., Alexandridis, P., Eds.; American Chemical Society: Washington, DC, USA, 2003; pp. 44–59, ISBN 9780841238671.