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Bacterial adhesion onto materials with specific surface chemistries under flow conditions

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Abstract *Staphylococcus epidermidis* adhesion onto materials with specific chemical functionalities, under flow, was investigated by using surfaces prepared by self-assembly of alkyl silane monolayers on glass. Terminal methyl (CH_3) and amino (NH_2) groups were formed by chemical vapor deposition of silanes, at elevated temperature. Carboxyl (COOH) terminated groups were prepared by further modification of NH_2 groups with succide anhydride and positively charged NH_2 groups by adsorption of poly-L-lysine hydrobromide. Hydroxyl (OH) terminated glass was used as control. Surface modification was verified by contact angle measurements, atomic force microscopy and X-ray photoelectron spectroscopy. A parallel plate flow chamber was used to evaluate bacterial adhesion at various shear rates. Adhesion was found to be depended on the monolayer's terminal functionality. It was higher on the CH_3 followed by the positively charged NH_2, the non-charged NH_2 groups, the COOH and minimal on the OH-terminated glass. The increase in the material surface free energy significantly reduced the adhesion of a hydrophilic bacterial strain, and this is in accordance with the predictions of the thermodynamic theory. However, the increase in the shear rate restricted the predictability of the theory and revealed macromolecular interactions between bacteria and NH_2- and COOH-terminated surfaces.

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1 Introduction

In spite of non-septic conditions during the surgical procedure and systematic administration of antibiotics, bacterial adhesion and subsequent biofilm formation impede the materials' long-term use [1, 2]. *Staphylococcus epidermidis* has been identified as a predominant cause of infection in the presence of a medical device, due to its ability to form large biofilms [3].

Bacterial adhesion is mediated by interactions between the material and the bacterial surfaces [4]. Both specific (i.e., receptor–ligand), in the case of protein/cell coated surfaces, and nonspecific (i.e., colloidal-type) interactions contribute to the ability of the bacterial cell to attach to the biomaterial surface. However, their relative contribution is not completely understood [5].

Modifications of the surfaces of polymers via plasma-processing techniques, in order to improve their biocompatibility, usually produce on the surface numerous functional groups and chemical crosslinks [6–8], while treatments often cause severe degradation of the surface, leading to increased roughness as well as to surface heterogeneity [7]. Time-dependent conformational rearrangements of these surfaces may also be observed [8].

Recently, much interest has arisen in self-assembled monolayers (SAMs), with the goal of developing molecular-level control over surface properties. SAMs formed by the adsorption of terminally functionalized alkyltriethoxysilanes [EtO_3Si–R] onto hydroxylated silicon and glass surfaces [9] are structurally the best ordered interfaces currently available for studying the interaction of cells and proteins with substrates of different surface chemistries, and provide the capability of circumventing many of the aforementioned experimental uncertainties [10].

Since it has been shown that bacterial adhesion is influenced not only by the surface chemistry but by roughness and configuration as well [11], we prepared surface chemistries with similar topography and roughness.

Moreover, since the process of bacterial adhesion to indwelling medical devices is associated in most cases with flow of body fluids [12], physical forces such as shear generated by local haemodynamics may modulate the adhesion process.

In this direction, this study attempts to answer in a fundamental way, by using the simplest possible chemistries, the following questions: Does the surface chemistry influence *S. epidermidis* adhesion? How does adhesion depend on shear rate and on the relative contribution of physicochemical and hydrodynamic interactions? Does the *S. epidermidis* adhesion behavior agree with the trends predicted by the thermodynamic theory?

These questions were addressed by quantitative measurement of bacterial adhesion on surfaces in laminar flow as a function of fluid shear rate.

2 Materials and methods

2.1 Preparation of substrata

2.1.1 Chemicals

Glass slides were purchased from Knittel Gläser, octadecyltriethoxysilane [ODS: $H_3C(CH_2)_{17}Si(OCH_2CH_3)_3$] from Gelest Inc., aminopropyltriethoxysilane [APTES: $H_2N(CH_2)_3Si(OCH_2CH_3)_3$], poly-L-lysine, succide anhydride, hexane, N,N-dimethylformamide (DFM) and methylene iodide from Sigma–Aldrich, toluene, HNO_3, H_2SO_4, H_2O_2, Na_2HPO_4 and KH_2PO_4 from Merck, glycerol anhydrous from Fluka, ethanol absolute and NaOH from Carlo Erba.

2.1.2 Preparation of self-assembled monolayers

Glass slides were hydrolyzed and oxidized by immersion in NaOH aqueous solution 5 M for 1 h, followed by soaking in fresh piranha solution (3:1 sulfuric acid 98%/hydrogen peroxide 30%) for 1 h. The hydroxyl (OH)-terminated glass substrates were prepared immediately prior to silanization or kept under water till use in order to prevent the ageing effect [8].

The OH-terminated glass slides were coated with self-assembled monolayers terminated by CH_3 or NH_2, following the vapor phase method [13]. Briefly, the glass slides were placed together with a glass cup filled with ODS or APTES into a Teflon container. The container was sealed and placed in an oven maintained at 150°C, for 3 h, in the case of ODS, and at 90°C, for 1 h, in the case of APTES. Subsequently, the CH_3-substrates were rinsed with hexane and the NH_2-substrates were sonicated in dehydrated ethanol and toluene, in NaOH (1 mM) and HNO_3 (1 mM) to remove excessively absorbed APTES molecules. The COOH-terminated substrates were obtained by immersing the NH_2-terminated substrates in 0.1 M succide anhydride in DFM for 24 h and rinsing with DMF [14]. The positively charged NH_2-terminated substrates were prepared by immersing the OH-terminated glass in 0.01 (w/v) poly-L-lysine in water for 3 h [15].

2.2 Bacterial strain and culture conditions

The bacterial strain used in this study was the reference type culture *Staphylococcus epidermidis* ATCC 35984, that is slime-producing. Before each experiment, bacteria were subcultured onto Tryptic Soy Agar (TSA, Difco Laboratories, Detroit, USA) for 24 h at 37°C. Stationary phase cells were obtained by incubating bacteria from the TSA, in Tryptic Soy Broth (TSB) for 18 h at 37°C in a rotary shaker at 120 r.p.m. Cells were harvested by centrifugation and re-suspended in phosphate buffered saline (PBS) 0.1 M, pH 7.4 at a concentration of 3×10^8 colony forming units (CFUs)/ml, according to the McFarland standard (BioMerieux, SA Lyon, France).

2.3 Material and bacterial surface characterization

The topography and roughness (Ra) of the surfaces were examined by means of a Multimode AFM (Nanoscope III, Veeco) in contact mode.

The wettability of the surfaces was determined by measuring the contact angles of three probe liquids with different polarities, using the CAM 100 goniometer and the KSV 100 software (KSV Instruments Ltd). The probe liquids were ultrapure water, methylene iodide and glycerol. Measurements were made at room temperature and ambient humidity using the sessile drop technique [16]. In the case of the bacterial cells, the measurements were performed on bacterial layers deposited on membrane filters according to the method described by Busscher [16].

Measured contact angles of the three probe liquids were converted into surface free energies and their components, according to the Lifshitz–van der Waals Acid–Base (LW–AB) approach of the Thermodynamic Theory [17], using the equation

$$(1+\cos\theta)\gamma_L = 2\left(\sqrt{\gamma_S^{LW}\gamma_L^{LW}}\right) + \sqrt{\gamma_S^{+}\gamma_L^{-}} + \sqrt{\gamma_S^{-}\gamma_L^{+}} \quad (1)$$

in which γ_S^{LW} is the Lifshitz–van der Waals component of the surface free energy, γ_S^{+} is the electron acceptor and γ_S^{-} the electron donor parameters of the acid–base component of the surface free energy, where S is the substratum or the bacterial surface and L is the liquid.

The surface chemical composition of the substrates was determined by X-ray Photoelectron Spectroscopy (XPS). XPS data were obtained with a LHS-10 spectrometer (SPECS Scientific Instruments, Inc.). Two separate measurements were taken on different spots, for each substrate, for two separately prepared surfaces.

2.4 Dynamic bacterial adhesion assays

For evaluating bacterial adhesion under flow conditions the parallel plate flow chamber (PPFC), which is described in Stavridi et al. [18], was used. The configuration of the chamber is such that the sample is sandwiched between two plexiglas plates. Four syringes were placed in an automated syringe pump and connected to four different chambers.

Two shear rates were used: 50 and 2000 s^{-1}, because these correspond to the physiological ones for laminar flow in blood vessels [12]. Each experiment was performed three times. Each time the bacterial suspension used was from different bacterial culture and the substrates were from different silane modified glass slides.

2.5 Quantification of bacterial adhesion

After the adhesion experiments, the samples were fixed in glutaraldehyde, dehydrated by ethanol, sputter coated with gold and examined by a JEOL–JSM 6300 scanning electron microscope (SEM) [19]. Adherent bacteria were counted in three fields for each shear rate value and for each sample (three samples) by using the Image Pro Plus Analysis Software (Media Cybernetics). Magnifications of $\times$2000 were used.

2.5.1 Thermodynamic theory—Gibbs free energy change

According to the Lifshitz–van der Waals (LW) acid–base (AB) approach of the thermodynamic theory [17], the tendency of bacterial adhesion is expressed by the Gibbs free energy change [$\left(\Delta G_{\mathrm{adh}}^{\mathrm{LW-AB}}\right)$ $(\mathrm{J/m^2})$] of the process.

According to this approach, the total free energy of adhesion is the sum of the LW and AB adhesion energies, $\Delta G_{\mathrm{d0}}^{\mathrm{LW}}$ and $\Delta G_{\mathrm{d0}}^{\mathrm{AB}}$ respectively, and is calculated by using the following equation:

$$\begin{aligned}\Delta G_{\mathrm{adh}}^{\mathrm{LW-AB}} = {} & 2\left(\sqrt{\gamma_{\mathrm{B}}^{\mathrm{LW}}}-\sqrt{\gamma_{\mathrm{L}}^{\mathrm{LW}}}\right)\left(\sqrt{\gamma_{\mathrm{L}}^{\mathrm{LW}}}-\sqrt{\gamma_{\mathrm{S}}^{\mathrm{LW}}}\right) \\ & +2\left[\sqrt{\gamma_{\mathrm{L}}^{+}}\left(\sqrt{\gamma_{\mathrm{B}}^{-}}+\sqrt{\gamma_{\mathrm{S}}^{-}}-\sqrt{\gamma_{\mathrm{L}}^{-}}\right)\right. \\ & +\sqrt{\gamma_{\mathrm{L}}^{-}}\left(\sqrt{\gamma_{\mathrm{B}}^{+}}+\sqrt{\gamma_{\mathrm{S}}^{+}}-\sqrt{\gamma_{\mathrm{L}}^{+}}\right) \\ & \left.-\sqrt{\gamma_{\mathrm{B}}^{+}\gamma_{\mathrm{S}}^{-}}-\sqrt{\gamma_{\mathrm{B}}^{-}\gamma_{\mathrm{S}}^{+}}\right] \end{aligned} \quad (2)$$

2.6 Statistical analysis

The effects of the surface free energy and flow conditions on bacterial adhesion were statistically analyzed using the SPSS package for windows.

3 Results and discussion

3.1 Surface characterization

The AFM analysis of the substrates revealed that they present relatively smooth surfaces with similar average surface roughness. Table 1 summarizes the mean values of the Ra for the various substrates and the results show that the adsorption of the specific silanes and poly-L-lysine did not significantly influence the surface morphology and Ra of the glass slides, enabling the examination of the effect of the surface chemistry and the flow conditions on the bacterial adhesion, in a direct manner.

Table 1 presents also the mean values of the experimentally measured contact angles θ (deg) of bacterial cells and the various substrates. The influence of Ra on the measured contact angles is considered negligible since all the substrates present quite small Ra and therefore the real

Table 1 Mean values and standard deviations of average surface roughness (Ra), water, methylene iodide (CH_2I_2) and glycerol contact angles (θ) and total surface free energy $\left(\gamma_{\mathrm{S}}^{\mathrm{LW-AB}}\right)$, its apolar $\left(\gamma_{\mathrm{S}}^{\mathrm{LW}}\right)$ and polar $\left(\gamma_{\mathrm{S}}^{\mathrm{AB}}\right)$ components, its electron donor $\left(\gamma_{\mathrm{S}}^{-}\right)$ and electron acceptor character $\left(\gamma_{\mathrm{S}}^{+},\right)$ of *S. epidermidis* and the various materials, as these are calculated according to the "Lifshitz–van der Waals acid–base" approach (three samples, three measurements for each one)

Sample	Ra (nm)	θ Water (deg)	θ CH_2I_2 (deg)	θ Glycerol (deg)	$\gamma_{\mathrm{S}}^{\mathrm{LW}}$ $(\mathrm{mJ/m^2})$	γ_{S}^{+} $(\mathrm{mJ/m^2})$	γ_{S}^{-} $(\mathrm{mJ/m^2})$	$\gamma_{\mathrm{S}}^{\mathrm{AB}}$ $(\mathrm{mJ/m^2})$	$\gamma_{\mathrm{S}}^{\mathrm{LW-AB}}$ $(\mathrm{mJ/m^2})$
ATCC 35984	–	23.1 ± 3.2	64.5 ± 4.1	24.2 ± 2.9	26.0	5.7	45.3	32.2	58.2
Glass	0.9 ± 0.2	10.0 ± 2.1	34.5 ± 2.4	19.0 ± 1.8	24.7	6.4	51.8	36.3	60.9
Glass–NH_2	1.2 ± 0.2	49.1 ± 3.3	36.8 ± 3.1	47.1 ± 2.1	28.0	2.6	29.4	17.5	45.5
Glass–lysine	1.5 ± 0.4	27.6 ± 1.7	31.1 ± 1.6	32.1 ± 1.3	43.8	1.1	41.5	13.3	57.1
Glass–COOH	1.4 ± 0.3	32.8 ± 1.9	30.9 ± 1.1	35.4 ± 2.2	43.8	0.9	38.3	11.9	55.7
Glass–CH_3	1.1 ± 0.3	93.0 ± 3.2	55.2 ± 2.1	71.6 ± 2.3	31.7	1.2	0.03	1.19	32.9

area of the surface is not significantly different than the geometric one [20]. The results presented in Table 1 show that the ATCC 35984 bacteria have low water and glycerol contact angles, indicating that their character is polar. Moreover, all treatments significantly increased the measured water and glycerol contact angles, in comparison to the OH-terminated glass.

Since the standard deviations of the contact angle measurements are relatively low, the mean values are used, for computational reasons, to calculate the LW $\left(\gamma_S^{LW}, \gamma_B^{LW}\right)$, and the AB $\left(\gamma_S^+, \gamma_S^-, \gamma_B^+, \gamma_B^-\right)$ components of the total free energy of the bacteria and the substratum surfaces $\left(\gamma_S^{LW-AB}\right)$, according to the "LW–AB" approach. These results are summarized in Table 1 and show that the bacteria and the OH-terminated glass appear to be polar with higher γ_S^{AB} than γ_S^{LW}, whereas the CH_3-terminated SAMs are rather hydrophobic with much higher γ_S^{LW} than γ_S^{AB}. The NH_2- and COOH-terminated SAMs, as well as the positively charged NH_2-groups, prepared by the adsorption of poly-L-lysine, are moderately hydrophobic with the AB component lower than that of the OH-terminated glass but much higher than that of CH_3-terminated SAMs. The LW component of the various bacteria and material surface free energy does not vary as much as the AB component.

Therefore, the increase in the free energy of the various samples is mainly due to the significantly enhanced polar component, indicating that the ATCC 35984 bacteria, the OH and to a less extent the other surfaces, except the CH_3-terminated, have polar character. Moreover, the γ_S^- of the bacteria and all the substrates, apart from the CH_3-terminated, is much greater than the γ_S^+. This may suggest that these surfaces have a strongly monopolar surface or that they favor electron-donating or Lewis base properties. In contrast, the γ_S^{AB}, γ_S^-, γ_S^+ of the CH_3-terminated SAM appear low, reflecting its apolar character.

XPS confirmed the modification of the glass surfaces by the silanes and poly-L-lysine. Table 2 contains the elemental percentages of C, N, O and Si. The results show that the amount of carbon increases for all substrates, in comparison to the OH-terminated glass slides, due to the polymethylene chains of the coatings. The amount of carbon to the OH-terminated glass can be attributed to surface contamination. As anticipated, the amount of oxygen decreases for all substrates, in comparison to the OH-terminated glass, and this explains the decreased polar character observed by the contact angle measurements and the surface free energy calculations. Furthermore, the presence of the N1s XPS peak is an indication that the glass slides were successfully modified in the case of the NH_2, the COOH and the positively charged NH_2-terminated groups. This peak consists of one chemical component with binding energy of 399.5 eV, in the case of the NH_2 and the COOH-terminated groups, indicating that they are not protonated. Whereas, in the case of the poly-L-lysine, the peak consists of two chemical components with binding energies of 399.5 and 401.8 eV. This indicates that part of the NH_2-terminated groups is positively charged.

Table 2 XPS elemental composition for the various substrates (two samples)

Sample	C (%)	N (%)	O (%)	Si (%)
Glass	8.9 ± 0.1	0	64.4 ± 0.1	26.7 ± 0
Glass–NH_2	14.6 ± 0.2	1 ± 0.1	58.4 ± 0.4	26.0 ± 0.1
Glass–lysine	20.2 ± 0.3	2 ± 0.4	59.5 ± 0.2	18.3 ± 0.3
Glass–COOH	18.7 ± 0.2	1 ± 0.2	60.2 ± 0.2	20.1 ± 0.2
Glass–CH_3	34.8 ± 0.4	0	44.5 ± 0.2	20.7 ± 0.1

3.2 Correlations between bacterial adhesion—surface free energy and components

Table 3 presents the combined effect of the surface chemistry and shear rate on bacterial adhesion. The results show that, under both shear rates, bacteria adhered more to the CH_3, the substrate with the lowest surface free energy (γ_S^{LW-AB}), acid–base (γ_S^{AB}) and electron donor (γ_S^-) components, followed by the positively charged NH_2, the non-charged NH_2 groups, the COOH and minimal on the OH-terminated glass, the substrate with the highest γ_S^{LW-AB}, γ_S^{AB} and γ_S^-.

However, the thermodynamic theory could not explain why bacteria adhered to a higher extent onto the lysine-terminated glass, although this appeared more polar, in comparison to the NH_2-terminated substrate. This could be

Table 3 The number of adherent bacteria/cm^2 (N) for shear rates 50 and 2000 s^{-1} and the Gibbs free energy of adhesion $\left(\Delta G_{d0}^{LW-AB}\right)$ its apolar $\left(\Delta G_{d0}^{LW}\right)$ and polar $\left(\Delta G_{d0}^{AB}\right)$ components, as these are calculated according to the "Lifshitz–van der Waals acid–base" approach

Sample	N*E6, 50 s^{-1}	N*E6, 2000 s^{-1}	ΔG_{d0}^{LW} (mJ/m^2)	ΔG_{d0}^{AB} (mJ/m^2)	ΔG_{d0}^{LW-AB} (mJ/m^2)
Glass	1.77 ± 0.54	1.02 ± 0.17	−0.3	20.1	19.8
Glass–NH_2	3.78 ± 0.27	2.84 ± 0.28	−0.5	13.9	13.4
Glass–lysine	4.37 ± 0.14	3.95 ± 0.23	−1.7	21.3	19.6
Glass–COOH	2.53 ± 0.12	1.49 ± 0.23	−1.7	20.2	18.6
Glass–CH_3	6.64 ± 1.00	4.25 ± 0.74	−0.9	−9.7	−10.5

explained by the attractive electrostatic interactions, which are not encountered by the thermodynamic theory, between the negatively charged bacteria [21] and the positively charged lysine-terminated substrates.

In an attempt to compare our results with other literature findings, we observed that there are controversies concerning the effect of the substratum surface free energy and its polar component on adhesion. In our previous study [8] we observed that *S. epidermidis* adhesion onto He and He/O_2 treated PET was reduced, in comparison to PET, due to the increase in the surface free energy and polar component, However, Bakker et al. demonstrated that strains isolated from a given niche, whether medical or marine, utilize different mechanisms in adherence, through selective pressures [22]. Therefore, generalization is probably impeded by the complexity of the bacterial cell surface at the nanometer level and by the various experimental approaches—static and dynamic—that are used in order to examine bacterial adhesion and retention.

Decrease in the number of adherent bacteria, for all materials, was observed when the shear rate increased from 50 to 2000 s^{-1}. This decrease was significantly different ($P < 0.01$), for all substrates, apart from lysine.

3.3 Thermodynamic theory—shear—number of adherent bacteria

In an attempt to examine if the pronounced effect of the total free energy of the substratum surfaces and its polar component on bacterial adhesion can be explained by the thermodynamic models, the "LW–AB" approach of the thermodynamic theory was used to calculate the Gibbs free energy changes of adhesion $\left(\Delta G_{d0}^{LW-AB}\right)$ of the bacteria interacting with the various substrates, and these are presented in Table 3. ΔG_{d0}^{LW-AB} is decoupled in each case to its components; ΔG_{d0}^{LW} and ΔG_{d0}^{AB}.

This approach results in negative ΔG_{adh}^{LW-AB} values for the bacteria interacting with the CH_3 SAM. Therefore, adhesion should be favored to the CH_3 SAM, to the substrate that presents the lowest γ_S^{LW-AB} and γ_S^{AB} values, and this is in agreement with the experimental results. Moreover, as it is observed in Table 3, the driving force for the negative or positive ΔG_{adh}^{LW-AB} values is the ΔG_{d0}^{AB} component, since the values of ΔG_{d0}^{LW} are low in comparison to the ΔG_{d0}^{AB} ones, for all the possible combinations.

By plotting the number of adherent bacteria/cm^2 (N) as a function of the total free energy of adhesion $\left(\Delta G_{d0}^{LW-AB}\right)$, it's ΔG_{d0}^{AB} and ΔG_{d0}^{LW} components, for both shear rates (plots not presented), we observed that N was negatively correlated with ΔG_{d0}^{LW-AB} and ΔG_{d0}^{AB} ($P < 0.001$), whereas it was not significantly correlated with ΔG_{d0}^{LW} ($P > 0.001$) due to the small differences in the ΔG_{d0}^{LW} values that the various materials present. This indicated that the predominant interactions between the bacteria and the various substrates are the acid–base.

Moreover, the correlation was better for the lower shear rate. This happened because, although according to the thermodynamic theory the lowest decrease in the bacterial adhesion should be observed onto the CH_3-terminated SAM, due to the low ΔG_{d0}^{LW-AB} values, the lowest decrease was experimentally observed onto the lysine-terminated glass followed by the NH_2-, the COOH- and the OH-terminated ones. The highest decrease in bacterial adhesion was observed onto the CH_3-terminated SAM. Therefore, the flow conditions strongly influence the number of attached bacteria in a way that restricts the predictability of the thermodynamic theory. These results are in agreement with those of Finlay et al. [23] who observed that although the highest number of *enteromorpha* zoospores adhered to the less polar SAMs surface, at high shear stress zoospores detached more easily from the less polar SAMs than from the polar ones. In contrast, Bayoudh et al. [24] observed that bacterial adhesion strength measurements were in agreement with the adhesion free energy calculations. Boks et al. [25] observed that it was more difficult to detach *S. epidermidis* from non polar surfaces than from polar, but the critical shear force to prevent the adhesion of two *S. epidermidis* strains to polar and non polar substrates was controversial.

According to our previous study [26] and the predictions of the Extended DLVO (XDLVO) theory which encounters the AB, the LW and the electrostatic interactions between bacteria and substrates, the bacteria may not manage to overcome the energy barrier and come into close contact to the substrate in the case of the CH_3 SAM, and this explains the high decrease in the number of the adherent bacteria under shear rate 2000 s^{-1}. In the case of the NH_2-terminated SAM, there is an interaction minimum between the bacteria and the NH_2 groups for large separation distances, but according to the XDLVO theory this interaction is much lower than the hydrodynamic forces.

These results indicate that the thermodynamic theory predicts the observed bacterial adhesion only in a qualitative manner and that along with the LW–AB and the electrostatic interactions, that the colloidal theories take into account [17, 21], the discrete bonds formed between bacteria and NH_2- and COOH-terminated surfaces, through bacterial surface-bound macromolecules, can enhance attachment or resist detachment of bacteria from the surface, as Ma and Dickinson [27] proposed. Therefore, macromolecular interactions are of significant importance and should be examined further.

4 Concluding remarks

We demonstrated that the material and bacterial surface free energy and the shear conditions significantly influence

the *S. epidermidis* adhesion to the various materials. The results were qualitatively predicted by the thermodynamic theory. However, simulated hemodynamic shear conditions identified limitations to this theory. Higher shear rates indicated the presence of other than the colloidal interactions between the bacteria and the substrates. Therefore, the driving forces for *S. epidermidis* adhesion to biomaterials may be considered a combination of interactions governed by physicochemical-macromolecular and physical forces dominated by shear. The macromolecular interactions apparently stem from the highly dynamic surface of the bacteria and their response to the environmental changes and is subject of further investigations.

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