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Biophysical Chemistry 91 (2001) 273–279

Biophysical Chemistry

www.elsevier.com/locate/bpc

Charge transfer during staphylococcal adhesion to TiNOX® coatings with different specific resistivity

Albert T. Poortinga, Rolf Bos[1], Henk J. Busscher*

Department of Biomedical Engineering, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands

Received 16 February 2001; received in revised form 14 May 2001; accepted 16 May 2001

Abstract

Adhesion of the bacterial strain *Staphylococcus epidermidis* 3399 to titanium-oxy-nitride (TiNOX®) substrata with different specific resistivities was studied in a parallel plate flow chamber, while simultaneously measuring the electric potential of the substrata. During adhesion, bacteria either donated or accepted electrons to the substrata depending on the specific resistivity of the substratum and bacteria that had donated electrons to the substratum adhered more strongly than bacteria that had accepted electrons from the substratum. These results demonstrate that electron transfer plays a role in bacterial adhesion to conducting surfaces, which has hitherto been neglected.

Keywords: Bacterial adhesion; Parallel plate flow chamber; Metal surfaces; Charge transfer; Conductivity; DLVO-theory

1. Introduction

Microbial adhesion to metal surfaces occurs in a wide variety of different situations such as on food equipment, in tap water distribution systems, ship hulls and on metallic biomaterial implants inside the human body [1,2]. Microbial adhesion to non-conducting surfaces such as polymers and glass has been extensively studied and is frequently described using the classical DLVO (named after Derjaguin, Landau, Verweij and Overbeek) theory in which Lifshitz–Van der Waals and electrostatic interactions are distinguished [3]. The extended DLVO approach [4] also distinguishes Lewis acid–base interactions in addition to the classical DLVO interactions [5]. In contrast, physico–chemical descriptions of microbial adhesion to conducting surfaces, such as metals, are less common [6,7] and little is known

* Corresponding author. Tel.: +31-50-363-3140; fax: +31-50-363-3159.

E-mail address: h.j.busscher@med.rug.nl (H.J. Busscher).

Received 16 February 2001; received in revised form 14 May 2001; accepted 16 May 2001

[1]Present address: Friesland Coberco Dairy Foods, Corporate Research, Harderwijkerstraat 41006, 7418 BA Deventer, The Netherlands

0301-4622/00/$ - see front matter
PII: S0301-4622(01)00177-6

on whether microbial adhesion to conducting surfaces proceeds according to the same mechanisms as operative for non-conducting surfaces. In conducting (or semi-conducting) materials, free electrons are present which give rise to short-range electron exchange interactions [8], also called metallic bonds.

The bacterial cell surface consists of a variety of different chemical groups, most notably proteins [9]. Proteins have been described as being semi-conducting [10,11] and consequently the bacterial cell surface possesses free electrons. In addition, proteins contain electrochemically active groups and electron transfer from carboxylate functional groups in proteins during adsorption to metals has been described by Omanovic and Roscoe [12], while Bolz and Schaldach [13] showed that the conformation of adsorbed fibrinogen on metallic implants was determined in part by electron transfer to a semi-conducting surface. Adhesion of encapsulated bacteria to steel surfaces was accompanied by cathodic currents, attributed to ionic or electron exchange between the steel and the encapsulating polymer [14]. Poortinga et al. [15] used a parallel plate flow chamber to simultaneously measure bacterial adhesion to a conducting surface and the change in both the electric potential and capacitance of the surface during adhesion and concluded that bacteria exchange up to several percent of their total surface charge [16] during adhesion.

Hitherto, attempts to discourage bacterial adhesion have been undertaken by modifying the surface properties of the substratum surfaces, i.e. making them more or less hydrophobic or altering their zeta potentials. Inhibition of charge transfer during bacterial adhesion, e.g. by influencing the specific electrical conductivity of the substratum surfaces, may be an entirely new approach to manipulate bacterial adhesion with applications ranging from biomedical implants to surfaces in food industry. The aim of this paper is to study the role of charge transfer during bacterial adhesion to conducting surfaces. To this end, staphylococcal adhesion to titanium–oxy-nitride substrata with different specific resistivity will be measured in a parallel plate flow chamber.

2. Materials and methods

2.1. Bacterial strain

Staphylococcus epidermidis 3399 was cultured in brain heart infusion broth at 37°C in ambient air. For each experiment, *S. epidermidis* 3399 was inoculated from blood agar plates in a batch culture for 24 h. This culture was used to inoculate a second culture which was grown for 16 h prior to harvesting. Bacteria were harvested by centrifugation (5 min at 10 000 g), washed twice with demineralized water and resuspended to a concentration of 6×10^8 bacteria/ml in a potassium phosphate buffer (25 mM, pH 7.0).

2.2. Substratum materials and characterization

As substrata, reflective titanium–oxy-nitride coatings on glass were used, obtained from TiNOX®, München, Germany. Coatings were prepared by physical vapour deposition and the specific resistivity was controlled by varying the nitrogen and oxygen content of the samples. Five TiNOX® samples with different resistivity were used. The specific resistivities of the different samples were measured using a four-point method and ranged from 6.5×10^1 to 2.1×10^4 $\mu\Omega$ cm (Table 1). The ITO (indium–tin-oxide)-coated glass plates were cleaned by a 2-min sonication in methanol, followed by thorough rinsing with tap water and 2-min sonication in Millipore® filtered, deionized water. The open circuit electric potential V_0 of the clean TiNOX® samples in contact with a 25-mM potassium phosphate buffer solution was measured relative to a Ag/AgCl reference electrode (Ref201, Radiometer Copenhagen, Lyon, France) using an electrometer (Radiometer Kopenhagen PHM research pH meter, impedance $> 10^{12}$ Ω) (see also Table 1). Note that the open circuit potential differs widely over the different samples. For each experiment, new TiNOX® samples were used.

2.3. Bacterial deposition experiments

Bacterial deposition was measured in a parallel plate flow chamber described in detail before [17].

Table 1
The specific resistivity of the different TiNOX® samples together with the open circuit potential V_0 of the clean TiNOX® samples in contact with 25 mM potassium phosphate buffer, with respect to an Ag/AgCl reference electrode

Sample	Specific resistivity ($\mu\Omega$ cm)	Open circuit potential V_0 (mV)
1	6.5×10^1	−126
2	2.5×10^2	24
3	7.8×10^2	−49
4	4.6×10^3	−214
5	2.1×10^4	−30

Under the experimental conditions, V_0 was reproducible within 10 mV.

The top plate of the flow chamber consisted of transparent, conducting ITO-coated glass (Philips Components, Heerlen, The Netherlands) with the coated side in contact with the solution. As bottom plate, TiNOX® plates were used, with their coated side in contact with the solution (see Fig. 1). The top and bottom plate had an area in contact with the solution of 29 cm^2 and they were separated by 0.6 mm thick spacers.

Deposition to the bottom plate of the flow chamber was observed with a CCD-MXR camera (High Technology, Eindhoven, The Netherlands) mounted on a metallurgical microscope [18]. A pulse-free laminar flow of the bacterial suspension (0.02 ml/s) was created by hydrostatic pressure, while the suspension was recirculated using a peristaltic pump. From the initial, linear increase in time of the number of bacteria per unit area deposited to the bottom plate, the initial deposition rate j_0 was determined by a least-squares fitting procedure. Bacterial deposition to the bottom plate was studied for 4 h and the density of adhering bacteria after 4 h (n_{4h}) determined. From the spatial distribution of adhering bacteria, the so-called blocked area A_1 was determined, which is the area of the substratum that becomes inaccessible to other bacteria because of the presence of an adhering bacterium [19]. The total number of adhering bacteria per unit area as a function of time was fitted to [20]:

$$n(t) = j_0\tau(1 - \exp(-t/\tau)) \quad (1)$$

Eq. (1) can be used to derive the desorption probability of adhering bacteria β.

$$\beta = 1/\tau - j_0 A_1 \quad (2)$$

where the bacterial desorption probability β is assumed to be constant in time. The reciprocal desorption probability β^{-1} yields an indication of the strength of bacterial adhesion to a substratum surface. The adhesion strength was furthermore assessed at the end of each experiment by passing 10 consecutive air bubbles through the flow chamber, exerting high detachment forces on the adhering bacteria [21,22]. Subsequently, the percentage of adhering bacteria retained on the substratum was determined as another measure of the strength of bacterial adhesion.

All deposition experiments were done in triplicate at room temperature (21–23°C).

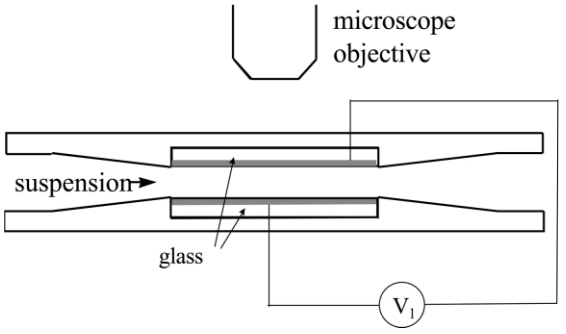

Fig. 1. Schematics of the parallel plate flow chamber equipped with coated glass plates (gray shaded area) in contact with the solution. The top plate is coated with ITO, while the bottom plate is TiNOX® coated. All other parts are made of polymethylmethacrylate, unless indicated otherwise. V_1 denotes an electrometer measuring the potential difference between the top and bottom plate.

2.4. Measurement of charge transfer during deposition

Before and during deposition, the potential difference V between the ITO-coated glass plate and the TiNOX® plate was measured and deposition experiments were started when this potential difference had reached an equilibrium value.

The potential difference was measured using an electrometer. From the initial, linear change in voltage difference between the ITO and the TiNOX® plate in time during bacterial deposition, an initial voltage change $(dV/dt)_{t=0}$ was determined. Care was taken that the electrical contacts were not in contact with the solution but only in contact with the top and bottom plate. The simultaneous measurement of the initial voltage change and the initial bacterial deposition rate enables calculation of the initial voltage change per adhering bacterium, $(dV/dn)_{t=0}$.

3. Results

Fig. 2a gives an example of the number of bacteria adhering to a TiNOX® sample, while Fig. 2b presents the corresponding potential difference $V(t)$ between the TiNOX® sample and the ITO plate as a function of time. The number of staphylococci adhering to the ITO top plate was negligible in each experiment indicating that the ITO–solution interface had constant properties during a deposition experiment and can thus, be considered as a reference electrode. Bacterial adhesion induces a change in the potential measured (compare Fig. 2a,b) which varies with the number of adhering bacteria (Fig. 2c). The potential change induced per adhering bacterium (dV/dn) decreases during the deposition process and eventually becomes zero.

Fig. 3 shows various characteristics of the bacterial deposition process as a function of the initial change in substratum potential $(dV/dn)_{t=0}$ per adhering bacterium. A positive value of $(dV/dn)_{t=0}$ indicates that the electric potential of the substratum increases upon bacterial adhesion, i.e. that the substratum donates electrons to adhering bacteria. The initial deposition rates and numbers of bacteria adhering after 4 h both decrease when electrons are donated to the bacteria upon adhesion (samples 2, 3 and 5). Adhering staphylococci could better withstand the detachment forces exerted by a passing air bubble when electrons were donated by adhering bacteria to the substratum and the percentage of sta-

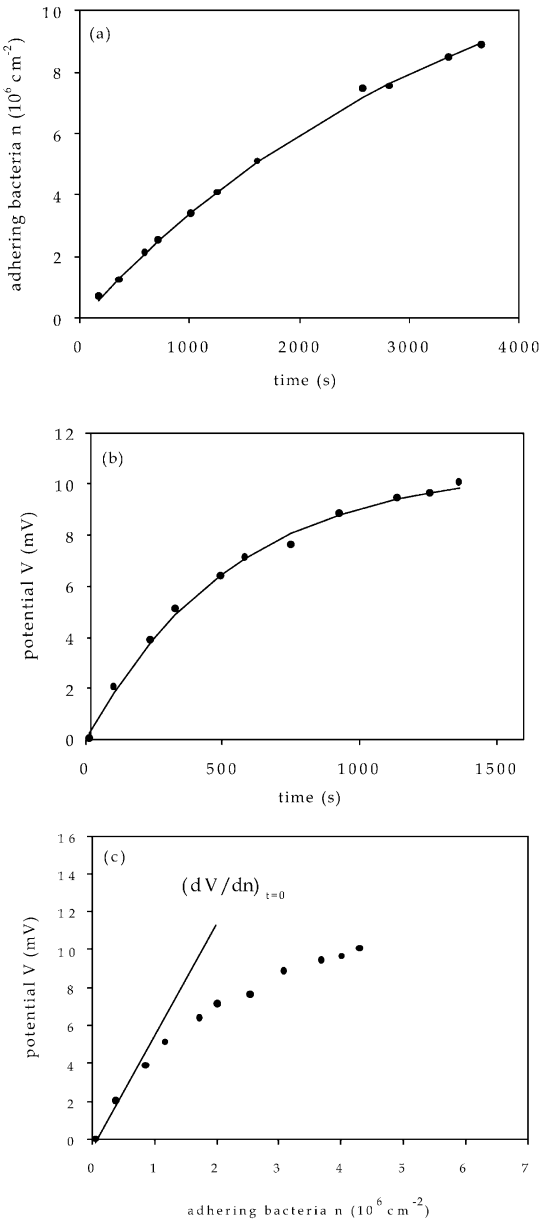

Fig. 2. Deposition of *S. epidermidis* 3399 to TiNOX® sample 5 from 25 mM potassium phosphate buffer under flow. (a) Number of bacteria $n(t)$ adhering to the TiNOX® sample as a function of time. (b) Potential difference, V, between the TiNOX® sample and the ITO during deposition as a function of time. Potential difference has been rescaled to give a zero potential difference at $t = 0$. (c) Potential difference between the TiNOX® sample and the ITO as a function of the number of bacteria adhering to the TiNOX® sample. $(dV/dn)_{t=0}$ indicates the change in potential difference per initially adhering bacterium.

phylococci retained decreased linearly with $(dV/dn)_{t=0}$, provided sample 2 with the positive open circuit potential is ignored. Similarly, the reciprocal desorption probability is highest when the bacteria had donated electrons to the substratum and decreases linearly with electron acceptance by the bacteria (again with the exception of sample 2).

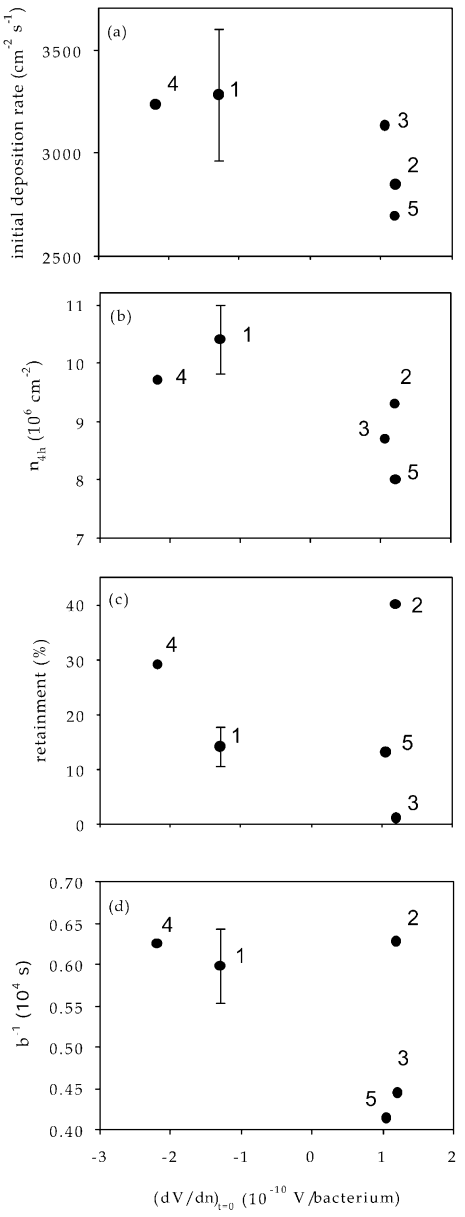

Fig. 3.

4. Discussion

In this paper, deposition of *S. epidermidis* 3399 to (semi)conducting titanium–oxy-nitride substrata with different specific resistivities was studied, while simultaneously measuring the substratum potential. In a previous paper [15], we showed that the change in substratum potential as a function of the number of adhering bacteria is a measure for the amount of charge transferred between the substratum and the bacterium during adhesion. Electrons could either be donated to or accepted by a substratum, depending on the bacterial strain and the ionic strength used. This study shows that charge transfer between the substratum and the adhering bacteria also depends on the specific resistivity of a substratum.

Charge transfer takes place over a range shorter than 0.5 nm [8] and may originate from either from double layer overlap and charge re-arrangement in solution or charge transfer between the substratum and the bacterial cell surface in case of direct contact. Our interpretation of the data is based on the direct contact hypothesis, as supported by recent observations by Baier [23] that biofilms appear as crystals on semi-conducting

Fig. 3. Deposition of *S. epidermidis* 3399 to different TiNOX® substrata from 25 mM potassium phosphate buffer under flow as a function of charge transfer. Numbers correspond with samples, described in Table 1. Error bars denote the average standard deviation over 3 separate experiments. (a) Initial deposition rate j_0 as a function of the change in substratum potential per initially adhering bacterium. Differences between extremes (sample 4 and 5) significant at $P = 0.32$ (Student t-test). (b) Number of adhering bacteria after 4 h of deposition n_{4h} as a function of the change in substratum potential per initially adhering bacterium. Differences between extremes significant at $P = 0.04$. (c) Percentage of bacteria retained after the passage of 10 consecutive air bubbles as a function of the change in substratum potential per initially adhering bacterium. Differences between extremes significant at $P = 0.01$. (d) Reciprocal desorption probability of adhering bacteria β^{-1} as a function of the change in substratum potential per initially adhering bacterium. Differences between extremes significant at $P = 0.01$.

surfaces. The crystalline nature of the biofilm was claimed to be due to charge transfer between the biofilm and electron-accepting lattice imperfections in the semi-conductor. For this to happen, bacteria need to be in direct contact with the substratum to allow charge transfer, as develops during their residence on a surface, explaining the weak relationship found between charge transfer and the bacterial initial deposition rate (Fig. 3a). The occurrence of charge transfer as an interaction between bacteria and a conducting substratum was also found to only weakly influence numbers of adhering bacteria after 4 h (Fig. 3b). Likely, this indicates that the number of bacteria that adhere is not solely determined by interactions between adhering bacteria and the substratum but also by interactions between depositing and already adhering bacteria. Charge transfer does effect the strength of bacterial adhesion and bacterial adhesion parameters pertinent to the strength of adhesion, like the reciprocal desorption probability and the percentage of bacteria retained after the passage of a consecutive number of air bubbles, evidently does relate to charge transfer (Fig. 3c,d).

It is difficult to fully explain the influence of charge transfer on bacterial adhesion, but it is hypothesized that after electron transfer, charge in the conducting substratum will redistribute. As charges in the TiNOX® can move freely, whereas bacterial surface charges are more bound, adhering bacteria may decrease electrostatic repulsion by donating negative charges from the region of contact between their surface and the TiNOX® surface. This mechanism also explains the deviating behavior of sample 2. Streaming potential measurements (results not given) showed that only this sample is positively charged with respect to the solution and consequently donates electrons to adhering bacteria, therewith increasing the attractive electrostatic interactions between bacteria and this substratum, which results in a stronger bond.

In conclusion, it has been shown that charge transfer during bacterial adhesion depends on the specific substratum resistivity with an influence on the strength of bacterial adhesion. Bacteria adhere more strongly to substrata after a donation of electrons to the substratum. Control of charge transfer during bacterial adhesion, e.g. by influencing the specific resistivity of the substratum surfaces, may, thus be an entirely new approach to manipulate bacterial adhesion with applications ranging from biomedical implants [1] to surfaces in food industry [24].

Acknowledgements

Supply of TiNOX® samples by TiNOX, Gesellschaft für Energieforschung und Entwicklung mbH, München, Germany is gratefully acknowledged.

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