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The adhesion and spreading of thrombocyte vesicles on electrode surfaces

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ABSTRACT

The interaction of thrombocyte vesicles with the surface of metal electrodes, i.e., mercury, gold and gold electrodes modified with self assembled monolayers (SAM), was studied with the help of chronoamperometry, atomic force microscopy, and quartz crystal microbalance measurements. The experimental results show that the interaction of the thrombocyte vesicles with the surface of the electrodes depends on the hydrophobicity of the latter: whereas on very hydrophobic surfaces (mercury and gold functionalized with SAM) the thrombocyte vesicles disintegrate and form a monolayer of lipids, on the less hydrophobic gold surface a bilayer is formed. The chronoamperometric measurements indicate the possibility of future applications to probe membrane properties of thrombocytes.

1. Introduction

Thrombocytes, also called blood platelets, are small cells which rapidly form a haemostatic plug at sites of vascular injury to prevent haemorrhage. They are also involved in the occlusion of blood flow by the formation of a primary thrombus and are involved in tissue injury, inflammation and wound healing, as well as in attracting and binding of leucocytes. They have ultrastructural, physiological and biochemical characteristics different from those of other cells. They have no nucleus, only small amounts of rough endoplasmic reticulum, no Golgi complex and no centrioles, but they contain intracellular granules enclosed by a membrane [1]. These granules are divided in at least three types: alpha, dense and lysosomal, each of which contains certain kinds of polypeptides [2]. They play an important role in primary haemostasis, blood coagulation and in occlusive vascular diseases [3]. In the case of damaged blood vessels, platelets adhere to subendothelial tissue via von Willebrand factor and collagen, undergo shape change and activation, release ADP and procoagulatory coagulation factors, and recruit other platelets, finally forming a haemostatic plug. In the activated platelet, the membrane exposes by flip-flop mechanism the inner membrane layer to the outside. The exposed phospholipids form the catalytic surface for activation of the coagulation cascade which finally results in generation of thrombin, formation and crosslinking of fibrin, which leads to consolidation of the haemostatic plug.

The adhesion of thrombocytes on different substrates has been recently the subject of study in order to determine the blood compatibility of several materials with different physical surface properties [4–8]. Among others, the role played by the hydrophobicity of the material has been determined to be of high importance to produce or avoid platelet adhesion on such surfaces. In the present work, the differences in the mechanism of adhesion of platelet vesicles on hydrophilic and hydrophobic substrates was studied, and the chronoamperometric method described in previous publications [9–12] is shown to be a valuable tool to study the adhesion of platelet vesicles on hydrophobic substrates.

In previous studies of liposomes [9–12] we could show that the interaction between a charged mercury surface and multi- and unilamellar liposomes leads to capacitive signals that give a detailed insight into the mechanism of adhesion and spreading. The kinetics of these processes resembles the kinetics of vesicle fusion. Here we describe experiments with thrombocyte vesicles. These vesicles are closed unilamellar shells formed from intracellular granule membranes and outer cell plasma membranes when membranes are mechanically disrupted [13,14]. They form spontaneously when the membrane is broken. The vesicles retain little or no cytosol and intracellular organelles. Very similar to liposomes, thrombocyte vesicles dispersed in isotonic aqueous potassium chloride solutions also produce adhesion-spreading signals on mercury electrodes, and here we report the characteristic features of that process.

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2. Experimental

Potassium chloride (Suprapur®, MERCK, Darmstadt, Germany) was exclusively used as electrolyte. The water was purified in a Millipore unit. Before measuring, the suspensions were deaerated for 20 min with high-purity nitrogen. Electrochemical measurements were performed with an AUTOLAB PGSTAT 12 (Eco Chemie, Utrecht, Netherlands) interfaced to a P4 PC in conjunction with an electrode stand VA 663 (Metrohm, Herisau, Switzerland). A multimode mercury electrode served as working electrode, the auxiliary electrode was a platinum rod, and an Ag|AgCl (3 M KCl, E=0.208 V vs. SHE) electrode was used as reference electrode. The surface area of the mercury drop was 0.48 mm^2, as determined by weighting 50 drops. The chronoamperometric measurements were performed within 1 s with sampling each 50 µs.

Concentrated suspensions of isolated blood cells (erythrocytes, MRP5 and V33 cells) and thrombocyte vesicles (with the plasma membrane of thrombocytes separated from the other subcellular fractions (granules and lysosomes)) were prepared following a novel procedure described in a recent publication [15]. The concentration of the thrombocyte vesicle suspension was around 3×10^{11} vesicles per mL. The suspensions were stored at −20 °C and used within a week. For the chronoamperometric experiments, the necessary amount of suspension was diluted in 25 ml of isotonic KCl solution. Chronoamperometric measurements were performed at room temperature and with varying the potential in 100 mV steps between −1.0 and 0.0 V vs. Ag|AgCl. For each potential 10 measurements were performed. In all measuring curves a peak search with the program Signal Counter [16] was used to identify the peak shaped signals. Unless stated otherwise, only peaks with an area larger than 0.8 pC were counted and analyzed, since smaller peaks cannot be distinguished from background noise with the necessary statistical confidence.

A QCM200 quartz crystal microbalance digital controller with a QCM25 5 MHz crystal oscillator (Stanford Research Systems, Stanford, USA) was used to perform the quartz crystal microbalance measurements. The surface area of the crystal was 0.4 cm^2. Before the measurements, the crystals were treated with piranha solution (one part 30% hydrogen peroxide, and 3 parts concentrated sulphuric acid) in order to remove any adsorbed substances, rinsed with deionized water and dried under nitrogen flow. The crystals were used immediately after cleaning to avoid degradation of the hydrophilic surface. For the study of the adhesion on hydrophobic substrates, the crystals were kept overnight in a solution of n-hexanethiol in ethanol (20% volume) in order to form a hydrophobic self assembled monolayer (SAM). Previous to the measurements, the microbalance was let to equilibrate overnight in 175 mL of an isotonic KCl solution and 85 µL of the concentrated thrombocyte vesicles suspension were added afterwards. Then the system was kept unaltered over a period of 30 to 50 h, recording the changes in the oscillation frequency and the motional resistance each 0.25 s. Two kinds of experiments were performed: the adhesion of thrombocyte vesicles at clean hydrophilic gold, and the adhesion of thrombocyte vesicles at the hydrophobic SAM modified gold.

Surface images were recorded with a Multimode Atomic Force Microscope (AFM) with Nanoscope IIIa controller and "E" scanner (Digital Instruments, Santa Barbara, CA). The "E" scanner exhibits a maximum scan area of 10×10 μm^2, a vertical range of 2.5 µm and was height calibrated using a TGZ01 grating (MicroMasch, Estonia; step height 26 nm) and linearized using a PG grating (Digital Instruments; 1 µm pitch). The images were recorded with tapping mode in liquid using standard tapping mode cantilevers (DNP-S, Veeco, Dourdan, France). Images were obtained at the same position but following increasing adsorption times (scan size 8 µm×8 µm).

Due to the adsorption of thrombocyte vesicle parts (membranes, membrane proteins etc.) one expects to find structures with a height of the order of several nanometres. The root mean square (RMS) roughness of the gold films is unfortunately approx. 3 nm. Hence the usage of such films for AFM imaging is not suitable in our case. Therefore we created ultraflat gold surfaces according to Stamou et al. [17] as follows: Gold was evaporated onto a RCA cleaned silicon wafer. Then RCA cleaned microscope slides were glued onto the gold film using an UV cureable glue (NO 68, Norland Adhesives, Cranbury, NJ). Ultraflat gold surfaces are then obtained by breaking the slide from the silicon wafer. The adhesion of gold to the glue is much better than to the silicon wafer, leading to a complete transfer of the gold film from the silicon to the slide. Silicon is atomically flat and thus this transfer leads to very flat surfaces (RMS roughness approx. 1 nm).

The slides were broken from the wafer directly before measurement. The surface was mounted into the fluid cell of the AFM and allowed to equilibrate for at least 1 h in Millipore water before a reference image was recorded. Then the fluid cell was filled with thrombocyte vesicle suspension in isotonic KCl (approx. 10^8 vesicles mL^{-1}) and images were recorded every 20 min.

3. Results

Whereas suspensions of thrombocyte vesicles exhibit a frequency of adhesion events of 13 s^{-1} (50 µL of concentrate in 25 mL of isotonic KCl; at E=−0.9 V vs. Ag|AgCl), suspensions of erythrocytes, V33 and MRP5 cells gave only very sporadic adhesion signals with frequencies between 1 and 2 s^{-1}. It is very unlikely that these sporadic signals are caused by the erythrocytes, V33 and MRP5 cells, since their concentration was as high as that of the thrombocyte vesicles. Therefore we take these few signals as caused by contaminations, possibly thrombocytes. All further studies were performed with thrombocyte

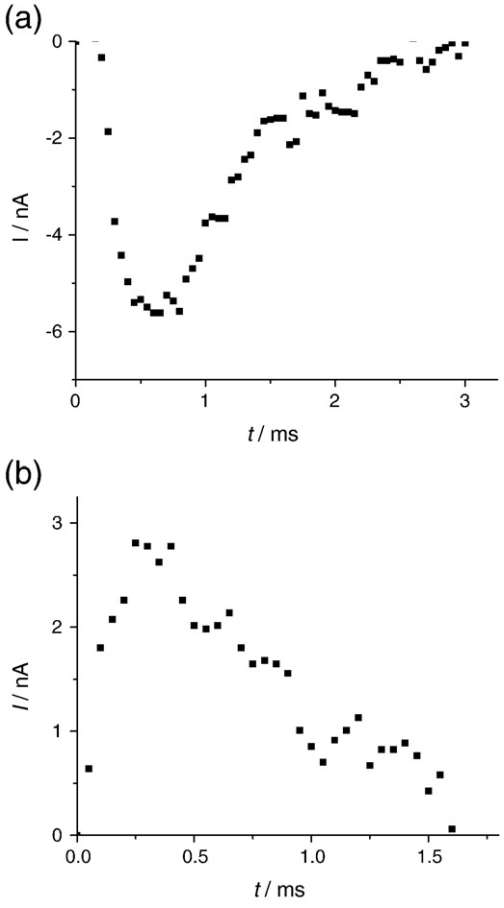

Fig. 1. Capacitive peaks obtained by performing chronoamperometry in a thrombocyte vesicle suspension (50 µL of concentrate in 25 mL of isotonic KCl) at, a) −0.4 V, b) −0.6 V vs. Ag|AgCl.

vesicles only. For a better understanding of the interaction of the mercury surface with the thrombocyte vesicles, the potential of the mercury electrode was varied and the frequency and sign of the detected capacitive signals was determined. Fig. 1 shows capacitive peaks arising from individual adhesion-spreading events at different electrode potentials.

Obviously, positive peaks are detected at potentials negative of the point of zero charge (pzc: −0.45 V vs. Ag|AgCl) and negative peaks at potentials positive of the pzc. Fig. 2 depicts a plot of the frequency of these peaks (i.e., the frequency of measurable adhesion-spreading events of thrombocyte vesicles on the mercury surface) versus electrode potential.

Very interestingly, completely like in case of liposomes [9–11], the peaks change their sign at the pzc, i.e., they are practically not detectable at the pzc. This key experiment cannot be understood when the thrombocyte vesicles would only adhere at the mercury surface keeping their own characteristic surface charge. If that would happen, the signals should behave as in case of charged clay particles [18] and marine algae [19], i.e., they should change their sign at a potential that is different from the pzc, i.e., at a potential at which the surface charge of the mercury exactly compensates the surface charge of the thrombocyte vesicles. Since the surface charge of thrombocytes is −2.45 μC cm^{-2} [20], and assuming it to be similar to that of the used thrombocyte vesicles, this would correspond to an electrode potential positive of pzc, exactly −0.359 V vs. Ag|AgCl for the used electrolyte. These results allow only one conclusion: The thrombocyte vesicles disintegrate and form an adsorbed layer of the membrane constituting molecules. Only when that happens, adhesion peaks can be observed at potentials negative to the pzc [18,21]. Therefore we conclude that the thrombocyte vesicles interact with the hydrophobic mercury surface in a very similar manner like liposomes.

In order to determine the final state in which the thrombocyte vesicles are found after the adhesion-spreading on the hydrophobic mercury surface, and for determining the mechanism of adhesion of the thrombocyte vesicles on different substrates, quartz crystal microbalance (QCM) measurements were performed. Fig. 3 shows the changes of the oscillation frequency (Δf_o) and of the motional resistance (ΔR_m) when the microbalance is immersed in a thrombocyte vesicles suspension. Two different substrates were used in these experiments: (i) gold, having a hydrophilic surface, and (ii) gold covered with a hydrophobic self assembled monolayer (SAM).

The curves depicted in Fig. 3 clearly show that the adhesion-spreading process is different depending on the used substrate. Other QCM studies have demonstrated that the adhesion of cells on the crystal follows different mechanisms and produce different final states depending on the type of cells as well as on the used substrates [22,23]. If the cells are destroyed and form either a lipid bilayer or monolayer on the surface of the QCM crystal surface, the dissipation factor—and therefore the change in the motional resistance—should have a relatively low value, as a rigid adsorbed film that does not slip on the surface is formed. The weight increase detected by the

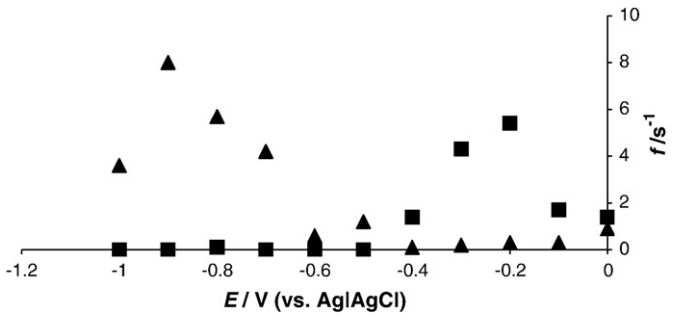

Fig. 2. Potential dependence of the peak frequency. Squares: Negative peaks. Triangles: Positive peaks.

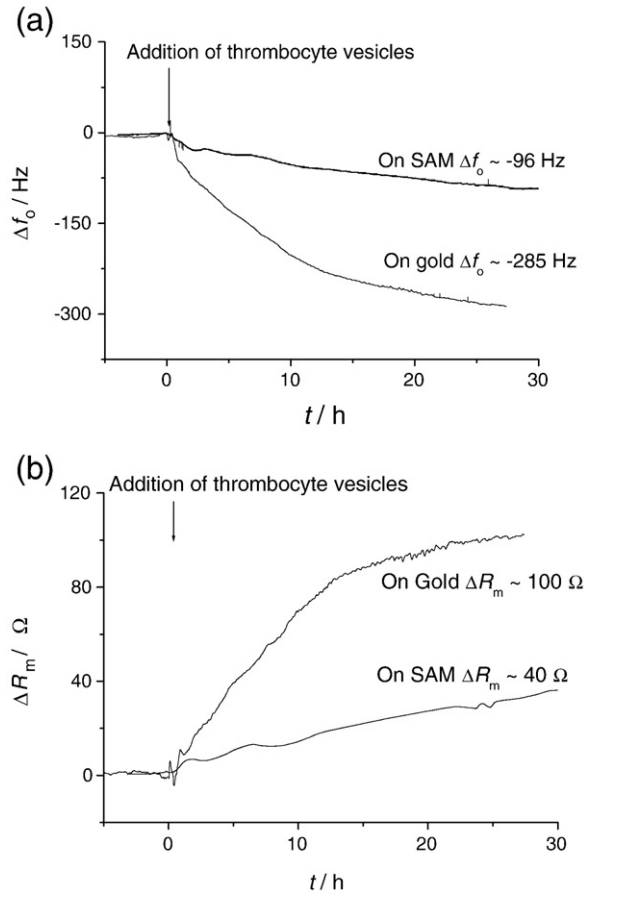

Fig. 3. Dependence of a) the oscillation frequency changes (Δf_o), and b) changes of the motional resistance (ΔR_m) on time as measured with a quartz crystal microbalance (QCM) for the thrombocyte vesicles adhesion on a hydrophilic (gold) and on an hydrophobic (SAM on gold) substrates.

microbalance would then, according to the Sauerbrey equation [24], be proportional to the change of the oscillation frequency Δf_o:

$$\Delta f_o = -\frac{2f_o^2}{\rho_q v_q}\Delta m = -\frac{f_o}{\rho_q t_q}\Delta m = -C\Delta m \quad (1)$$

where ρ_q and v_q are the specific density and the shear wave velocity of quartz, respectively, t_q is the thickness of the quartz plate, m is the mass per unit area of the formed film. Thus it follows that the following relation holds: $C=f_o(\rho_q t_q)^{-1}$. However, if the formed film is not rigid (adhesion of intact cells, for example), the change in the oscillation frequency would be proportional to the substrate-adhering material contact area and would correspond only to an *effective* mass, rather than to the actual mass of all the cells, vesicles or other soft materials attached to the crystal [22]. Changes of the oscillation frequency will also be affected by the viscoelastic properties of the material, by changes of its morphology and by interfacial interactions between the substrate and the material [25]. In the case of the adhesion of cells, both the membrane and the cytoplasm may cause deviations from the Sauerbrey equation, and, therefore, it is not easy to determine quantitatively the actual mass deposited on the crystal [23,26,27]. Soft materials and/or thick layers of materials, such as cells and vesicles, produce also a large dissipation factor when they attach to the surface, as they are less compact and subject to larger deformations under shear stress [28,29], what would result in a large increase of the motional resistance [30] (by hundreds or even thousands ohms). For this kind of films, Rodahl et al. [28] developed a model by which the shift of the resonance frequency and that of the dissipation factor were analyzed together to get information on the

adsorption process of viscoelastic films when the QCM is oscillating in a liquid. Based on that model, ΔD_s versus Δf_o curves have to be analyzed. Here, ΔD_s is the shift in the dissipation factor, which is proportional to the resistance change ΔR_m ($D_s = \omega C_m R_m$, where ω is the angular frequency at series resonance and C_m is the motional capacitance). These curves are taken as "fingerprints" of the cell-surface interactions [23,28].

The analysis of the adhesion on the hydrophilic gold surface will be discussed first. The ΔR_m vs. Δf_o curve for this case is shown in Fig. 4. The relationship is almost linear, with a small but clear discontinuity at $\Delta f_o = -45$ Hz. At frequency differences above −250 Hz the dR_m/df_o is slightly decreasing.

As the shift of the oscillation frequency Δf_o is related to the attached mass and/or the coverage of the gold surface, the obtained results show that the process causes always the same dissipation per mass/coverage unit (considering always a direct proportionality between the dissipation factor and the motional resistance). This coupling of the changes of the oscillation frequency and the motional resistance indicates changes of the viscosity of the contacting liquid resulting from an increase of the number of substrate-thrombocyte vesicle contacts [27], suggesting a uniform adhesion process with time. Wegener et al. [31,32] have reported that the epithelial cell line MDCK-I and the Swiss-3T3 fibroblast produce a frequency shift of the order of what we observed during the adhesion of thrombocyte vesicles (320 ± 20 Hz and 240 ± 15 Hz, respectively), while they report a rather large change of the motional resistance (755 ± 35 Ω and 280 ± 20 Ω respectively). These responses arise from the number of cell-substrate contacts and the resulting contact area [33,34]. 3T3 fibroblasts are rather large cells (surface area = 745 ± 30 μm^2) that are known to touch the substrate only at small areas, with the rest of the basal plasma membrane further apart, while the smaller epithelial cells (surface area = 270 ± 15 μm^2) attach firmly to surfaces with most of the basal plasma membrane in contact with the substrate. Thrombocytes are known to be smaller than these cells, and they are also known to spread on several substrates giving rise to large contact areas. When they spread (as intact cells), they may have a contact area of up to 80 μm^2. The platelet vesicles used in this study are smaller than intact thrombocytes, and a large number of them would be required to cover the whole area of the oscillator The determined change in the oscillation frequency (285 Hz); however, is lower as would be expected for the large number of vesicles needed to cover the whole electrode if they would behave as in the cases described above. On the other hand, the shift of the motional resistance (100 Ω) is also too low to account for the close packed adhesion of spread intact thrombocyte vesicles. Three main possibilities can be considered: (*i*) the surface of the resonator is just partially covered, (*ii*) the thrombocyte vesicles attach but do not spread , (*iii*) they not only spread, but a strong gold-membrane interaction produces the rupture of the plasma membrane and its adsorption as a rigid lipid bilayer with other membrane components embedded in the lipid matrix. The attachment of intact thrombocyte vesicles is very unlikely to occur without giving rise to larger resistance shifts. Even with the larger 3T3 fibroblasts the reported shift is three times what is observed in the present case. Considering incomplete coverage, the change of the motional resistance is too small to account for such a large change of the oscillation frequency (see, for comparison, the data for the epithelial cell line mentioned above). The formation of an adsorbed lipid bilayer with incorporated proteins and other membrane components is more likely to occur. Such bilayer formation can explain the comparatively small change of the motional resistance and also the rather large change in the oscillation frequency. The bilayer membrane of the thrombocyte vesicles is composed of phospholipids (with tail lengths of 18 to 20, and up to 24 carbon atoms), a great amount of proteins (1.7 g of protein per gram of lipid), and a high concentration of cholesterol (20–30% molar fraction of the total lipid content) forcing the lipids to pack closely together [1,14,35–40]. These properties of the membrane cannot account completely for the rather large mass density calculated using the Sauerbrey equation (5 μg cm^{-2} for the recorded Δf_o). However, it is necessary to recall that this relationship is not applicable to viscoelastic systems as the one studied here. The fluidity of the bilayer and its fluctuations on the surface of the gold substrate very likely affect the recorded values of Δf_o.

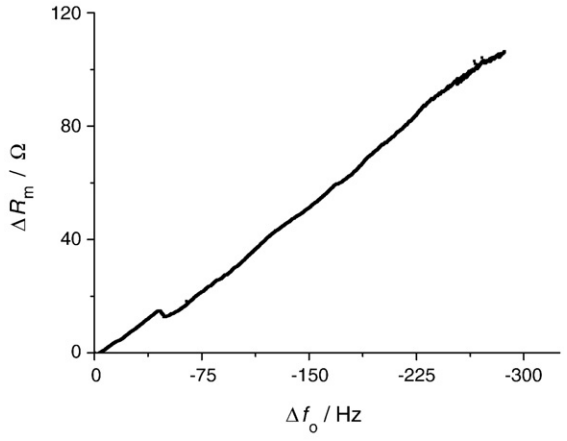

Fig. 4. Changes of the motional resistance (ΔR_m) versus oscillation frequency changes (Δf_o) for the adhesion of thrombocyte vesicles on a QCM with a hydrophilic gold surface.

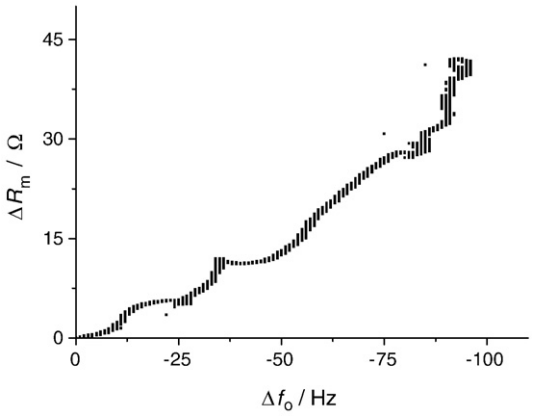

Fig. 5. Changes of the motional resistance (ΔR_m) versus oscillation frequency changes (Δf_o) for the adhesion of thrombocyte vesicles on a QCM with a hydrophobic SAM on gold surface.

Fig. 5 shows the fingerprint curve ΔR_m vs. Δf_o for the case of the adhesion of thrombocyte vesicles on a hydrophobic surface (the SAM covered gold layer).

The curve exhibits striking differences with respect to that shown in Fig. 4: It is non-linear and it shows distinct steps of different height and steepness. The vertical lines resulting from time intervals with zero Δf_o change are due to very slow processes (defining the rate of the process as the rate of coverage, that is, the change of Δf_o with time). It is obvious that fast and slow processes alternate, the former corresponding to those with small or negative values of dR_m/df_o, and the latter corresponding to large changes of the motional resistance with respect to Δf_o. According to previous reports on QCM studies of cell adhesion [22,23], high values of dR_m/df_o are related to the adhesion of non-spreading cells with a very small contact area, while lower values of dR_m/df_o account for processes in which the contact area increases faster than the energy dissipation (which would occur during spreading). According to these reports, the mechanism of cell adhesion involves the initial physical contact, the secretion of micro-exudates to drive the spreading, the actual spreading of the cell (leading to changes in the QCM signal), a modification of the adhesion properties (strength, number of proteins involved, etc.), and changes

of the cell cytoskeleton. All these processes have an effect on the QCM responses.

Table 1 shows a summary of the steps observed in Fig. 5, the change in the oscillation frequency and motional resistance observed during each step and the average values of the slope dR_m/df_o. According to the data given in Table 1, a fast process (A) with low or no change in the motional resistance is observed at the beginning of the measurement. In that segment, the slope dR_m/df_o is rather small, meaning that the process involves the coverage of a large area and a low energy dissipation. Such behaviour can be explained either by an adsorption of a rigid layer, or by an adsorption of cells with a large contact area where the rest of the membrane is still further away from the surface. However, spread thrombocytes or thrombocyte vesicles are almost flat, meaning that the area which is not in direct contact with the gold surface is close enough to it as to be capable of generating a large increase in the motional resistance. This first step may then account for the adsorption of other components of the thrombocyte vesicle suspension. In fact, AFM studies on a gold surface showed that during the first hour of exposure of the substrate to the thrombocyte vesicles, small islands of adsorbed material are formed which do not correspond to thrombocyte vesicles, as their area is too small (Fig. 6-a), although their height corresponds to that of an adsorbed lipid bilayer (Fig. 6-b).

In the QCM measurements, this segment of the ΔR_m vs. Δf_o is similar for both, hydrophilic and hydrophobic substrates. The second step (B in Table 1) is slow and has a large value of dR_m/df_o. Such behaviour is indicative of an attachment and spreading of intact thrombocyte vesicles with a large contact area and a large portion of the plasma membrane close enough to the electrode as to generate a large dissipation. Notice that the process is relative slow, as the suspension is quite diluted and the number of thrombocyte vesicles reaching the resonator and overcoming the activation energy necessary for this first adhesion is low. Once the thrombocyte vesicles are attached, all other processes involved in the mechanism of cell adhesion occur concurrently. The following segment (C) involves a very fast decrease on the oscillation frequency while no significant change in the resistance is observed. In analogy with the behaviour of liposomes on mercury, it is possible that this step is the opening of that part of the bilayer being in direct contact with the substrate, i.e., that this step involves the rearrangement of the lipid molecules in such way as to expose their lipophilic tails to the SAM surface. This will produce a larger area of contact, detected as an increase in the oscillation frequency, as the inner lipid monolayer will find its way to the surface, whereas the motional resistance will not change as the source of the energy dissipation (the membrane not in contact with the surface) remains unaltered or undergoes only slow changes. This process occurs almost immediately after the attachment, but at the beginning the supply of thrombocyte vesicles by diffusion is fast causing the change in the motional resistance to be larger than the change in the oscillation frequency (segment B). Once the supply is slowed down, the bilayer opening controls the process (segment C). Segment D corresponds to the saturation of the surface. Less thrombocyte vesicles can attach or open, and a rearrangement of the membrane that is not yet in contact with the surface is responsible for the increase of the motional resistance. Such rearrangement, again comparing it with the behaviour of liposomes, must be related to the stretching of the membrane, the formation of a pore and the squeezing out of its contents. These rearrangements are coupled with the filling of the intercellular space, covering the surface that was still free of adsorbed material (segment E) and which will correspond to a spreading process analogue to that described before for the liposomes. The small but clear decrease in the motional resistance at the beginning of segment E, suggests the formation of a rigid adsorbed film and supports the mentioned assumption. Segments F and G represent the saturation of the surface, meaning that there may be free space for the thrombocyte vesicles to attach, but not enough space for spreading, neither for intact vesicles nor for lipid monolayers. This proposed mechanism agrees well with what is observed in the chronoamperometric measurements. However, it is necessary to

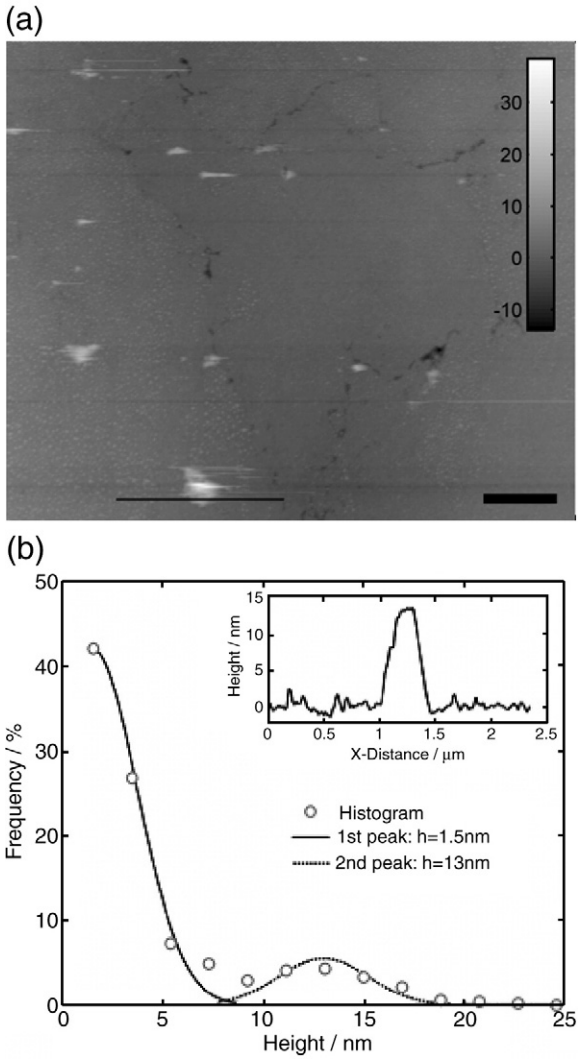

Fig. 6. a) Topography of an ultraflat gold surface (RMS roughness approx. 1 nm) 20 min after immersion in a thrombocyte vesicle suspension in isotonic KCl (approx. 10^8 vesicles mL^{-1}). The colour bar gives the height of the structures in nm and the scale bar (bold black line at the bottom) corresponds to 1 µm. The adsorbed particles exhibit an irregular shape. They appear as white areas due to the colour coding. b) Histogram of the maximum height observed in the 512 rows forming the AFM image on the left. The first peak measures the substrate height, the second the average adsorbed particle height, which is 13 nm. The inset gives an exemplary height profile of the surface (section along the narrow black line of Fig. 6-a) and confirms that the second peak in the histogram is created by the adsorbed particles. Furthermore this section shows that the adsorbed particles constitute only a fraction of a thrombocyte bilayer (diameter >2 µm was found with an optical microscope).

Table 1
Summary of the different segments forming the QCM response for the adhesion of thrombocyte vesicles on a SAM modified gold surface

Step	Δt/h	$(dR_m/df_o)/(\Omega s)$*	$\Delta R_m/\Omega$	$\lvert\Delta f_o\rvert$/Hz	$(\lvert\Delta f_o\rvert/\Delta t)$/Hzh^{-1}
A	0.4	0.14	1.1	8.6	21.5
B	1	0.57	3.5	6.4	6.4
C	0.2	0.09	0.9	7.9	39.5
D	5.5	0.75	6.2	12	2.2
E	3.1	0.12	1.4	16.1	5.2
F	19.8	0.55	14.6	27	1.4
G	17.9	1.1	13.95	13.3	0.74

* from absolute values of Δf_o.

point out that the QCM response for the adhesion of a great quantity of thrombocyte vesicles covers hours, whereas the chronoamperometric signals arising from the adhesion of one single thrombocyte vesicle have a duration of just some milliseconds. The processes involved in the individual thrombocyte vesicle adhesion and spreading must be also fast, and they all occur concurrently.

The recorded change of the oscillation frequency for the adsorption on SAM is 34% of that determined for the adsorption on gold. Assuming that a bilayer is formed on gold, and a monolayer on SAM, it was expected to record exactly a decrease of 50% for the latter case. However, it is very likely that in the bilayer some soluble molecules (small proteins, short fatty acids, carbohydrates) are kept, that are released when the bilayer is destroyed. Furthermore, the rupture of the bilayer will cause transmembrane proteins to rearrange in order to minimize the contact of their hydrophobic parts with the electrolyte solution. They will accommodate parallel to the membrane plane, occupying a larger surface area. As the concentration of protein in the membranes is quite high, this rearrangement, together with the solubility of some membrane components, may easily account for the recorded lower oscillation frequency. Also, the viscosity of the adsorbed lipid monolayer may differ from that of a bilayer, affecting also the recorded value of Δf_o. The rather high frequency shift on a gold surface coupled with a relatively small change in the motional resistance is indicative of a rather rigid film, and thus the possibilities are either a lipid bilayer or a lipid monolayer. As on SAM a smaller decrease of the oscillation frequency is determined, it is safe to assume that a bilayer forms on gold and a monolayer on SAM.

In previous publications [10,11] we have shown that the integrated current transients of the adhesion and spreading of liposomes obtained using chronoamperometry can be modelled with the following simple equation:

$$Q = Q_0 + Q_1(1-\exp(t/\tau_1)) + Q_2(1-\exp(t/\tau_2)) \quad (2)$$

in which the right side represents three processes: a very fast "touching" or "docking" process given by the first term (called "interaction-docking"), a first order process with a time constant τ_1 which is caused by the initial rearrangement of the molecules in the initial contact configuration (here called the bilayer opening step), and finally a second first order process with a time constant τ_2 resulting from the breaking up of the membrane, its spreading on the electrode surface and the formation of an adsorbed monolayer (here called the rupture-spreading step).

Fitting the signals of thrombocyte vesicles with Eq. (2) shows that for electrode potentials negative with respect to the pzc, the precision of fitting is acceptable (Fig. 7-b); however for potentials positive vs. the pzc, the average errors of the fitting parameters are unacceptable large, i.e., the model is most probably wrong (cf. Table 2, and Fig. 7-a in which the fitting of a charge transient for one adhesion-spreading event is given as an example). Since the thrombocyte vesicles possess a negative surface charge [20], their attachment on a positively charged electrode surface will certainly involve an attractive coulombic interaction, which may slow down the disintegration process and completely change the kinetics of adhesion and spreading. For a negatively charged electrode, an initial repulsion of the negatively charged thrombocyte vesicles must be effective; however, the disintegration of the cell membrane and the adsorption of the hydrophobic tails of the lecithin molecules will quickly and irreversibly initiate the interaction-docking, bilayer opening and rupture-spreading of the membrane on the mercury surface. The time constant of bilayer opening τ_1 is in the range of 8.5×10^{-5} to 2.2×10^{-4} s, and the time constant of rupture-spreading τ_2 is in the range of 3.8×10^{-4} to 8.5×10^{-4} s. The data are rather similar to the time constants found for unilamellar and multilamellar DMPC liposomes [10,11].

Table 2
Average error of the fitting parameters for the signals obtained at different potentials

Potential	Signals integrated	Average error on Q_0	Average error on Q_1	Average error on τ_1	Average error on Q_2	Average error on τ_2
0.0 V	5	1000%	400%	178%	250%	320%
−0.1 V	8	321%	195%	101%	19%	118%
−0.4 V	4	1250%	13.5%	10.3%	5%	5%
−0.6 V	5	140%	44%	78%	15%	36%
−0.8 V	9	471%	52%	30%	16%	18%
−1.0 V	9	1250%	41%	37%	6%	13%

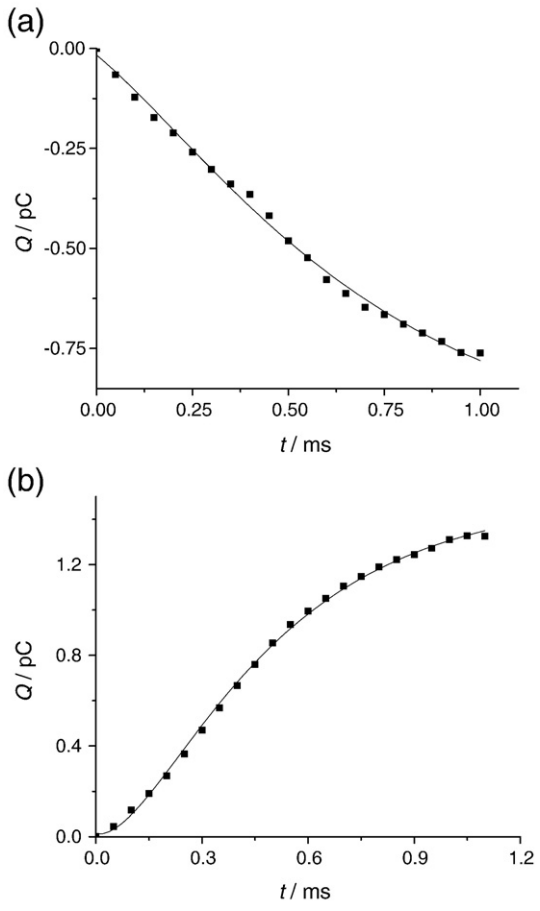

Fig. 7. Charge vs. time transients at potentials of a) 0.0 V, and b) −0.6 V vs. Ag|AgCl. Solid lines represent the fitted curves.

4. Conclusions

The described experiments allow drawing the following conclusions:

(i) Thrombocyte vesicles interact with the hydrophobic surface of a mercury electrode in a way that is similar to the interaction of lecithin liposomes with the same surface, i.e., their membrane disintegrates irreversibly and the membrane constituents spread on the electrode surface.

(ii) Thrombocyte vesicles belong to a very small group of biological vesicles and cells showing such adhesion-spreading behaviour on mercury. Only some other cells (e.g., the alga *Dunaliella tertiolecta* [19]) exhibit similar interactions, although none of them has been reported to actually disintegrate as in the present case.

(iii) It is easy to speculate that the well-known exceptional flexibility of thrombocyte membranes is the reason that they are able to undergo the observed irreversible adhesion-spreading events on mercury.

At this stage of studies it is not possible to say whether the observed irreversible adhesion-spreading events also proceed on other very hydrophobic surfaces. Possible candidates would be solid and liquid paraffins, teflon, etc. Future studies should be focused on that question. Another direction of future research is to study how certain diseases will affect the kinetics of adhesion-spreading events of thrombocyte vesicles. As it is known that some diseases change the membrane of thrombocytes, a measurable effect can be expected.

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