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Adhesion Kinetics of Functionalized Vesicles and Mammalian Cells: A Comparative Study†

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The suitability of the quartz crystal microbalance technique (QCM) to monitor the formation and modulation of cell−substrate contacts in real time has recently been established. A more detailed analysis of the QCM response when living cells attach and spread on the resonator surfaces is, however, hampered by the chemical and mechanical complexity of cellular systems and the experimental difficulties to control one single parameter of cell−substrate contacts in a predictable way. In this study, we made use of liposomes as simple cell models and studied the interactions of these liposomes with the resonator surface. To mimic the specific interactions between cell and protein-coated substrate as given in cell culture experiments, we incorporated biotin-labeled lipids as "receptors" in the liposome shell and preadsorbed avidin on the resonator surface. The dissipational QCM (D-QCM) technology was applied to monitor the shifts in resonance frequency and energy dissipation during the adsorption of liposomes prepared with increasing amounts of biotin-labeled lipids. We also studied the adsorption kinetics of liposomes doped with biotin moieties that were attached to the lipid core by an alkyl spacer in order to increase the distance between liposome shell and resonator surface. A comparison of these data with the adhesion kinetics of mammalian cells as monitored by D-QCM is presented and discussed. Although the shifts in resonance frequency are very similar for intact liposomes and mammalian cells, the viscous energy dissipation is significantly higher when cells attach and spread on the resonator surface.

Introduction

The interactions of mammalian cells with in vitro surfaces have attracted more and more interest throughout the past decade. This increase of importance and attention originates from a broad range of biomedical applications that are based on cells immobilized on solid substrates in or ex vivo creating a biologically active interface.[1] The list of examples comprises the development of new implant materials that either support or impede the attachment and spreading of living cells dependent on their particular location and function within the animal body.[2,3] Another example is the development of neuron−silicon junctions that aim to control and sense neuronal activity by semiconductor elements.[4,5] The functionality of these devices is strongly dependent on a close adhesion of neuronal cells on the transducer surface.[4] But also experiments addressing fundamental biological processes such as wound healing, tumor metastasis, or leukocyte adhesion are often focused on studying the dynamics of cell−substrate adhesion in vitro as a measure for motility or transmigratory activity. Driven by these scientific endeavors, several new techniques have been developed that are capable of measuring the various aspects of cell−substrate interactions optically, electrically, or even mechanically. Total internal reflection aqueous fluorescence (TIRAF) [6,7] and reflection interference contrast microscopy (RICM)[8,9] have been used to image the adhesion area on solid substrates and to monitor its dynamics. Fluorescence interference contrast (FLIC) microscopy has paved the way to reliably determine and map cell−substrate separation distances with an accuracy down to a few nanometers or better.[10,11] Electrical impedance analysis has been applied to follow the kinetics of cell adhesion and dynamic changes in the cell−substrate adhesion zone of established cell layers when these are challenged with chemical, biological, or physical stimuli.[12,13] Elaborate arrays of field effect transistors were constructed

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to quantify the electrical properties of the interface between biological cell and inorganic substrate.[14]

The quartz crystal microbalance technique (QCM) was already well-known as an analytical tool to study physiological adsorption phenomena at the solid−liquid interface before its potential to study cell−substrate adhesion was recognized.[15] The QCM technique is based on a quartz resonator sandwiched between two metal electrodes that are used to excite mechanical shear displacements of the piezoelectric quartz disks electrically. When standing wave conditions are established for the acoustic shear wave traveling through the quartz material, the characteristic parameters of this shear oscillation, such as for instance the resonance frequency, respond very sensitively to any mechanical changes at the crystal surface. We[16,17] and others[18−21] recognized that the mechanical interaction of mammalian cells with the resonator surface was clearly detectable from readings of the resonance frequency. After it had been established that the shift in resonance frequency is linearly dependent on the surface coverage of the resonator, the QCM could be used as an integral sensor to monitor the kinetics of cell adhesion[16] but also the budding of exocytotic vesicles[22] or changes in the microtubule cytoskeleton.[23] However, to explore the full potential of the device for cell biological applications it is imperative to understand the contribution of subcellular components to the overall signal in detail. A pool of experimental observations exists indicating that the QCM is primarily sensitive to mechanical phenomena associated with the ventral, substrate-facing membrane and those molecular species involved in substrate anchorage.[24] It is, however, unclear how parameters such as surface density of cell−substrate adhesion spots, cell−substrate separation distance, or the topography of the cell−substrate adhesion zone influence the QCM signal.

The straightforward approach to explore the significance of one single parameter of the system for the readout of a transducer device is simply to change that particular parameter in a well-defined manner and to repeat the measurement. This is, however, very difficult if not impossible for living systems. Thus, we made use of chemically defined liposomes to serve as models for mammalian cells and studied their impact on the parameters of the shear displacement. The specific molecular interactions known to exist between living cells and surface-immobilized adhesive proteins are mimicked by incorporating various amounts of biotin-labeled lipids into the liposome shell and by preadsorbing a layer of avidin on the resonator surface. In this system, we were able to control the concentration of receptors in the liposome shell. By using biotin labels that are attached via an alkyl linker to the lipid headgroup, it was also possible to alter the distance by which they stick out of the bilayer plane of the liposome simulating an increase in liposome−substrate separation distance. Being aware of the limitations of this approach, it was our major objective to study the adhesion of these liposomes to solid substrates using the QCM device and to learn more about the QCM readout for adherent cells from these well-defined systems. Once the underlying mechanisms have been understood, the QCM approach may become a unique and multifunctional tool to study the interactions of mammalian cells with artificial surfaces noninvasively in a quantitative and integral manner.

Materials and Methods

Experimental Setup. To follow the adhesion kinetics of liposomes to solid surfaces, we made use of a special variant of the QCM technique that has been previously introduced by Kasemo and co-workers[25] and that is referred to as dissipational QCM (D-QCM). In this setup, an external voltage source excites the quartz crystal at its fundamental resonance frequency via the surface gold electrodes. When the shear displacement of the resonator is stationary, the voltage source is separated from the resonator by means of a computer-controlled relay. The time course of the free oscillation decay is then recorded by a digital oscilloscope and subsequently analyzed to extract the resonance frequency f and the characteristic decay time. The decay time, indicative of energy dissipation, is expressed as the dissipation factor D of the oscillation, which equals the inverse of the quality factor $Q = D^{-1}$. The entire process is continuously repeated, yielding both characteristic oscillation parameters with a time resolution of less than 10 s. The setup used in this study has been realized according to the instructions given in the pioneering work of Rodahl et al.[25] A PC-card (NI 5411) from National Instruments was employed as a 40 MS/s arbitrary waveform generator, while a NI 5911 PC-card with a flexible, high-speed, high-resolution digitizer with variable speed and resolution serves as an oscilloscope. The resolution of the NI 5911 PC-card ranges from 8 bits at 100 MS/s to more than 21 bits at 10 kS/s. A PIC microcontroller programmed in BASIC was used to switch the generator signal via a mercury relay. The software to run the equipment, including the microcontroller, and process the data has been written in LabView version 5.1. The experimental setup is schematically shown in Figure 1.Plano-plano AT-cut quartz resonators (KVG, Neckarbischofsheim, Germany) with a fundamental resonance frequency of 5 MHz and a diameter of 14 mm have been integrated into a self-made crystal holder that provides electrical contact to the surface electrodes without applying considerable mechanical stress. The crystal holder is placed in a temperature-controlled Faraday cage and connected to a continuous fluid flow system that allows injecting a compound of interest into the circulating buffer stream from the outside.

For the dynamic monitoring of liposome and protein adsorption, we applied the D-QCM technique as described above. The dynamics of cell adhesion were recorded by time-resolved impedance analysis using an experimental setup that has been described in detail elsewhere.[17,24,26] Impedance data were recorded using the continuous wave impedance analyzer SI-1260 (Solartron Instruments, Farnborough, U.K.). The resonator was excited with an ac voltage amplitude of 150 mV at 150 frequencies evenly spaced between 4.97 and 5.04 MHz. The raw data were analyzed by fitting the transfer function of the Butterworth−van Dyke equivalent circuit presented in Figure 1B to the recorded data. This analysis provides the motional resistance R_m and the motional inductance L_m, indicative of the viscoelastic properties of the surface load.[24] As outlined in the first paragraph of Results and Discussion, these parameters can be easily transformed to the corresponding resonance frequency f and the dissipation factor D as they are recorded using the D-QCM approach.

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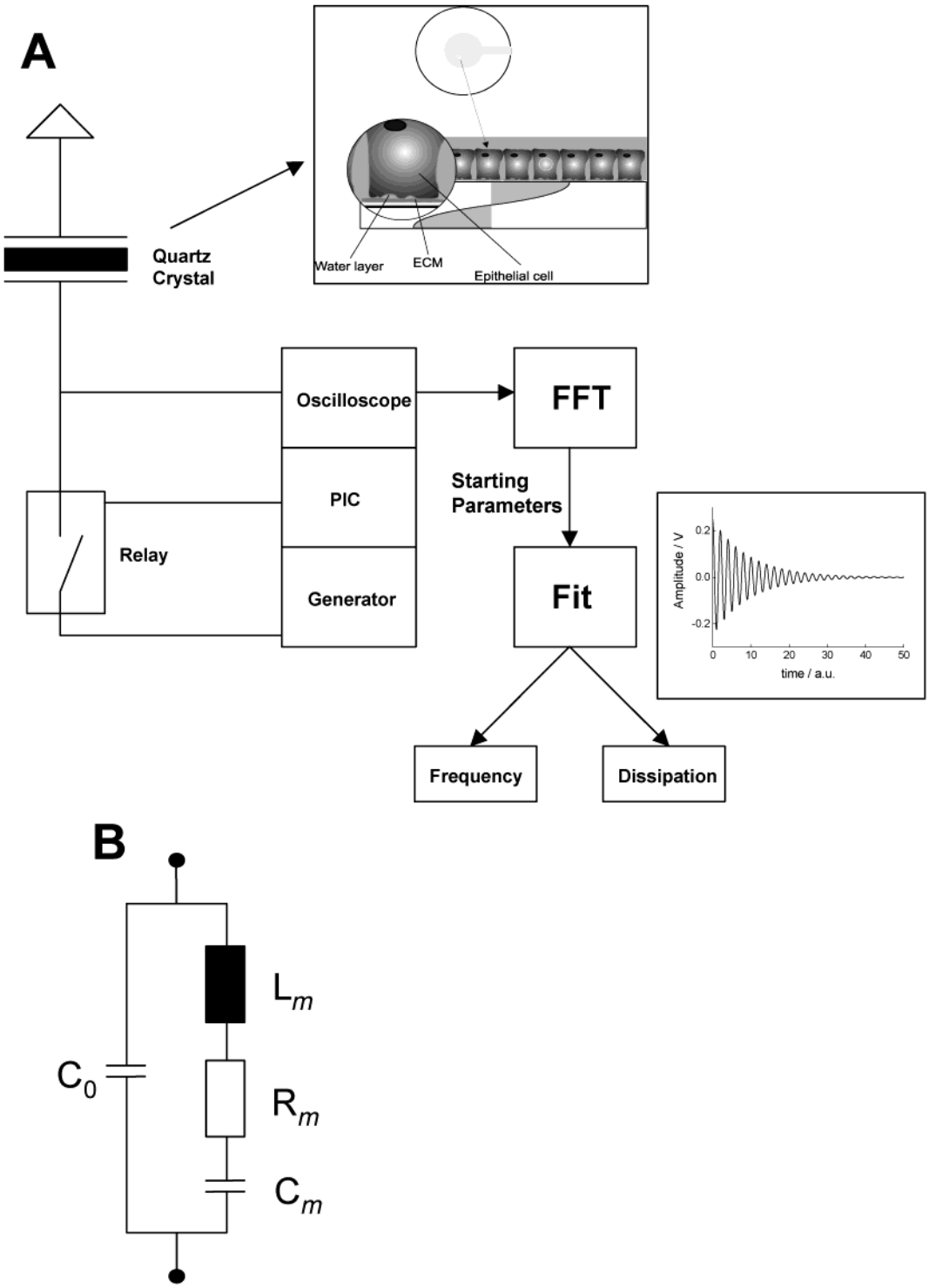

Figure 1. (A) Experimental setup to measure the resonance frequency *f* and the dissipation factor *D* of the shear oscillation according to the D-QCM technology. Details are given in Materials and Methods. (B) Butterworth−van Dyke equivalent circuit of a quartz crystal resonator near resonance. The subscript m denotes the impedance elements of the *motional branch* of the network that are electric analogues of the mechanical motion of the resonator.

Cell Culture. In experiments in which we studied the attachment and spreading of mammalian cells, we used strain II of the epithelial cell line MDCK (Madin−Darby canine kidney). These cells were routinely kept in MEM-Earle basal medium (Biochrom, Berlin, Germany) supplemented with 4 mM L-glutamine (Biochrom), 100 mg/L penicillin and streptomycin, respectively, and 10% (v/v) fetal calf serum (Gibco, Eggenstein, Germany). Stock flasks were grown in a humidified incubator with a 5% (v/v) CO_2 atmosphere. Cell suspensions were prepared from confluent monolayers by washing them twice in phosphate buffered saline (without divalent cations) and subsequent trypsinization in the presence of 1 mM EDTA. Cells were then collected by centrifugation (290*g*, 10 min, room temperature), resuspended in cell culture medium, counted with a Bürker hemacytometer, and seeded into the QCM chambers. The pentapeptides (Sigma, Deisenhofen, Germany) Gly-Arg-Gly-Asp-Ser (GRGDS) and Ser-Asp-Gly-Arg-Gly (SDGRG) were added in a final concentration of 1 mM to the seeding medium. After the QCM chamber had been mounted into the experimental setup, impedance data were continuously recorded with time.

Liposome Preparation. To model the adsorption of cells to the quartz resonator, we made use of liposomes prepared from 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) that were doped with increasing molar ratios of the biotin-labeled lipid

N-(biotinoyl)-1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine (biotin−DHPE) or *N*-((6-biotinoyl)-amino)hexyl)-1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine (biotin−X−DHPE). All lipids were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). The biotin moiety served as a receptor to anchor the liposome to avidin, preadsorbed to the resonator surface. The difference between the two dopants biotin−DHPE and biotin−X−DHPE is a C_6 spacer introduced between biotin and the lipid headgroup ethanolamine. All lipid components were dissolved in chloroform/methanol (1:3). Mixtures of DPPC and biotin−DHPE or biotin−X−DHPE, respectively, were prepared in molar ratios of 100:0, 99:1, 95:5, 90:10, 80:20, and 70:30. The solvent was evaporated in a flow of nitrogen at a temperature slightly above the main phase transition temperature of DPPC (41.5 °C). The films were subsequently dried in vacuo at 40 °C for 1 h. Multilamellar vesicles were formed by addition of buffer (1 mM Tris/HCl, pH 8.0) to the dried lipid films and repeated vortexing above the main phase transition temperature. These liposomes were then forced through a polycarbonate membrane with 100 nm pores of a LiposoFast miniextruder yielding large unilamellar vesicles doped with the biotin-labeled lipids.

Monitoring Specific Liposome Adhesion. To achieve specific adsorption of the liposomes to the resonator surface, the quartz resonators were first intensively cleaned in an argon plasma and then exposed to a 1 mM aqueous solution of 3-mercapto-propionic acid (MPA) for 20 min. Due to the strong chemical attraction between thiol groups and the gold films used as surface electrodes, MPA instantaneously forms a monomolecular layer on top of the quartz resonator that renders the surface negatively charged at pH 8. Under these conditions, the protein avidin, well-known for its biotin-binding capacities, adsorbs to the MPA-coated surface when offered from solution. Avidin (1 mg/mL in 1 mM Tris/HCl, pH 8) binding to the surface is driven by the electrostatic interactions between MPA (negative) and the protein (positive). This adsorption reaction was also monitored and verified using the QCM setup. Avidin adsorption generates a surface that is decorated with specific binding sites for the biotin-labeled liposomes. Adhesion of liposomes doped with different amounts of biotin-labeled "receptors" was followed by readings of the shift in resonance frequency and dissipation factor. Besides this real-time monitoring of the various adsorption processes, we also characterized the load on the resonator's surface by impedance analysis in the frequency regime of the fundamental quartz resonance.

Results and Discussion

Theoretical Considerations about QCM Measurements. In this study, two different operational modes of the QCM have been applied to characterize liposome and cellular adhesion to solid substrates. Since both approaches express the characteristics of the surface loads by individual but corresponding experimental parameters, we will give a condensed outline on QCM theory in this introductory paragraph for those readers not familiar with the details. As the quartz material is piezoelectric in nature, any mechanical shear displacement is coupled to an electrical potential difference between the crystal surfaces and vice versa. This piezoelectric coupling is exploited in two ways: (i) the mechanical oscillation is excited electrically and (ii) the parameters of the mechanical oscillation are mirrored in the oscillating potential difference between the crystal surfaces and are, thus, accessible from electrical measurements. Accordingly, the shear displacement of the resonator can be described by means of an electric equivalent circuit that is presented in Figure 1B. The series combination of the resistance R_m, the capacitance C_m, and the inductance L_m is the electrical analogue of the mechanical oscillation and is thus called the *motional* branch of the equivalent circuit. Since C_m is dominated by the elastic properties of the quartz material that are not altered by any adsorption to the resonator surface, C_m has a fixed value that can be calculated as detailed elsewhere.[26] The parallel capacitance C_0 has to be introduced for dielectric reasons but has no mechanical analogue with respect to the shear oscillation. This leaves the motional resistance R_m and the motional inductance L_m as the variable experimental parameters. These impedance elements are accessible from readings of the electrical impedance at frequencies closely embracing the resonance frequency of the crystal and subsequent equivalent circuit modeling. R_m is a direct measure for the dissipation of mechanical energy, whereas L_m is proportional to the mechanically stored energy. Since the dissipation factor D is defined as the ratio of dissipated energy to stored energy per cycle, D can be easily calculated from R_m and L_m according to $D = R_m/(2\pi f L_m)$ with the resonance frequency f. During a QCM experiment, shifts of f and L_m are rather small compared to their absolute values, so that D predominantly mirrors changes in dissipated energy (similar to R_m).

The resonance frequency f of the free oscillation (after the driving voltage source had been turned off, the resonator oscillates in parallel mode) can be calculated from the equivalent circuit parameters by

$$f = \frac{1}{2\pi}\sqrt{\frac{1}{L_m C_{total}}}$$

with $C_{total} = C_0 C_m/(C_0 + C_m)$. Since the capacitance C_{total} is constant in very good approximation, the resonance frequency in a D-QCM setup directly reflects changes in the motional inductance L_m or the stored energy. For simple systems, such as for instance a metal that is electrochemically deposited on the resonator surface, changes in L_m and f are directly proportional to the adsorbed mass as it is expressed in the well-known Sauerbrey equation.[27]

Analyzing adsorption processes by impedance analysis provides the parameters L_m and R_m to characterize the situation at the surface, whereas the QCM-D technology reads the resonance frequency f and the dissipation factor D.

Cell Adhesion Monitoring. Throughout the past years, we and several other groups have been studying the capabilities of the quartz crystal microbalance technique to monitor the establishment and modulation of cell−substrate interactions in vitro. It has been shown that the outstanding sensitivity, the excellent time resolution, and the quantitative nature of the recorded data make the QCM a very powerful tool also in cell biology.[16,18,19] In a more recent study,[16] we compared the adhesion kinetics of three different mammalian cell lines that were seeded as single-cell suspensions on 5 MHz quartz resonators. Formation of a complete monolayer of cells on the resonator surface induced shifts of the resonance frequency that were individually different for each particular cell type. For example, fibroblastic Swiss 3T3 cells induced frequency shifts of (240 ± 15) Hz compared to (530 ± 25) Hz for the epithelial cell line MDCK-II (these frequency readings were all recorded with an active oscillator circuit designed to read the frequency of minimum impedance, so that only trends but not absolute values are comparable to frequency readings presented here). Seeding more cells onto the resonator than fit into a complete monolayer did not result in an increased frequency response, indicating that only those cells contribute to QCM readings that are in direct contact with the resonator surface.[16] Taking these findings together, QCM readings apparently reflect the mechanical characteristics of the cell−substrate adhesion zone, that

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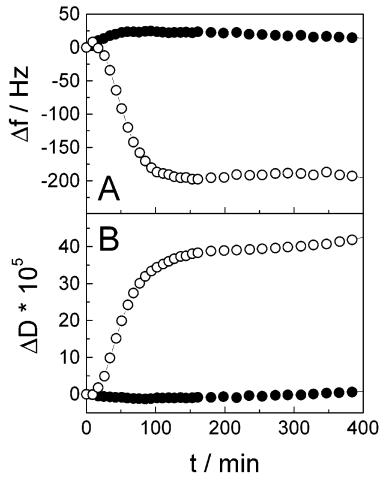

Figure 2. Shift of the resonance frequency Δf (A) and the dissipation factor ΔD (B) when suspensions of MDCK-II cells are seeded on the resonator surface in the presence of 1 mM of the pentapeptides GRGDS (filled circles) or SDGRG (open circles), respectively. The cells were seeded at a density of 5×10^5 cm^{-2} at time 0, and data acquisition was started immediately afterward. $T = 37$ °C.

may be individually different dependent on the cell type but are not understood in detail yet.

It was the objective of this study to gain more insight into the underlying mechanisms for this cell-type-specific QCM response. We first addressed the significance of specific molecular interactions between the cellular body and the resonator surface. In general, cell adhesion to in vitro surfaces requires the presence of an adhesive protein layer. When cells are seeded in a serum-containing medium, this layer is immediately formed by adsorption of proteins that are present in serum, most notably fibronectin or vitronectin. In these two adhesive proteins but also in many others there is a common binding site for cell surface receptors which is formed by the four amino acids Arg-Gly-Asp-Ser (RGDS). Thus, adding soluble peptides that contain the RGDS motif to a suspension of single cells strongly interferes with the specific adhesion of the cells to a protein-coated surface since the cell surface receptors (integrins) are competitively blocked. Figure 2 compares the time course of the shifts in resonance frequency (Figure 2A) and dissipation factor (Figure 2B) when equal numbers of MDCK-II cells were seeded on quartz resonators in the presence of either the pentapeptide GRGDS or the pentapeptide SDGRG (1 mM). Both peptides contain the same amino acids but in reverse order, so that only GRGDS is capable of specifically competing for the integrin binding sites. Since SDGRG carries the same charge density as GRGDS, it serves as a control to verify the specific interference with cell−substrate adhesion. In presence of 1 mM SDGRG (open circles), there is a strong decrease of the resonance frequency and a simultaneous increase of the dissipation factor shortly after injecting the cell suspension. This shift of QCM parameters is identical for conditions when no peptides have been added to the seeding medium at all (data not shown), indicating undisturbed adhesion of the cells to the resonator surface. In the presence of 1 mM GRGDS (filled circles), the time courses of both the resonance frequency and the dissipation factor do not show any significant changes indicating that cell−substrate interactions are strongly reduced if not entirely abolished. Accordingly, only cells capable of making specific molecular contacts to the surface induce a measurable QCM response. Thus, individual differences in the QCM readout induced by different cell species must be caused by the

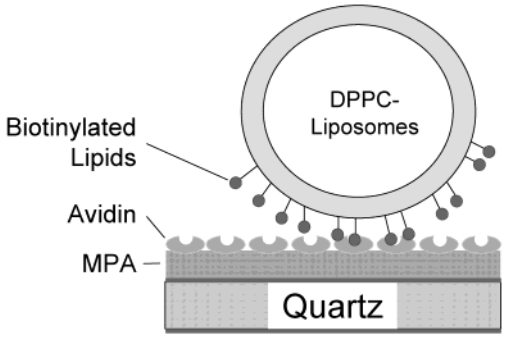

Figure 3. Modeling cellular adhesion by using liposomes ($d = 100$ nm) prepared from DPPC and biotinylated lipids as chemically defined cell models. A layer of avidin preadsorbed on the surface of the quartz resonator serves as an adhesive protein layer.

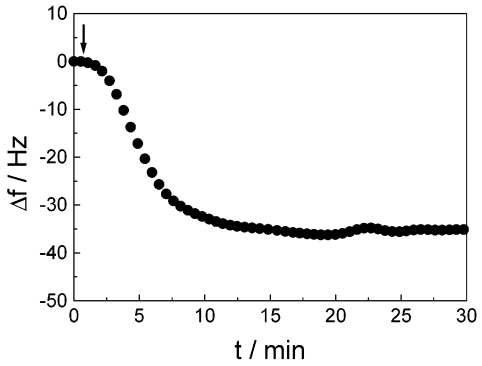

Figure 4. Shift of the resonance frequency Δf when avidin (1 μM) adsorbs onto a monomolecular layer of mercaptopropionic acid that had been immobilized on the surface of the quartz resonator. Addition of avidin is indicated by an arrow. $T = 25$ °C, pH 8.

number, the surface density, the length, or the individual mechanical strength of specific cell−substrate anchors.

Since it is impossible to systematically vary any of these parameters when working with living cells, we tried to simulate the situation at the quartz surface by using liposomes as cell models. Although different in their geometric dimensions by roughly one order of magnitude, the liposomes are supposed to model the cellular bodies as fluid-filled entities surrounded by a lipid double layer. We integrated biotin-labeled lipids into the liposome shells to function as cell surface receptors and pre-established a layer of avidin on the resonator surface in order to mimic the adhesive protein layer in cell adhesion experiments. Within this system, we were able to control the amount of specific receptors in the liposome shell by varying the molar ratio of bulk lipid to biotin-labeled lipid. The length of the substrate anchors was varied by using a biotin-labeled lipid in which the biotin moiety was not directly attached to the lipid headgroup like in biotin−DHPE but with a C_6 spacer in between (biotin−X−DHPE). The situation close to the surface is sketched in Figure 3.

Figure 4 shows a typical time course of the frequency shift when avidin was injected into the QCM chamber (arrow) that was loaded with a resonator that had been coated with a monomolecular layer of MPA prior to the experiment. The pH of the bathing buffer was set to 8.0 such that the MPA-coated surface was negatively charged whereas avidin (pI = 10) carries a positive net charge under these conditions. Adsorption of avidin to the resonator surface is driven by its electrostatic attraction to the MPA monolayer and can be followed in real time from the shift in resonance frequency. For final avidin concentrations of 1 μM within the circulating buffer, we

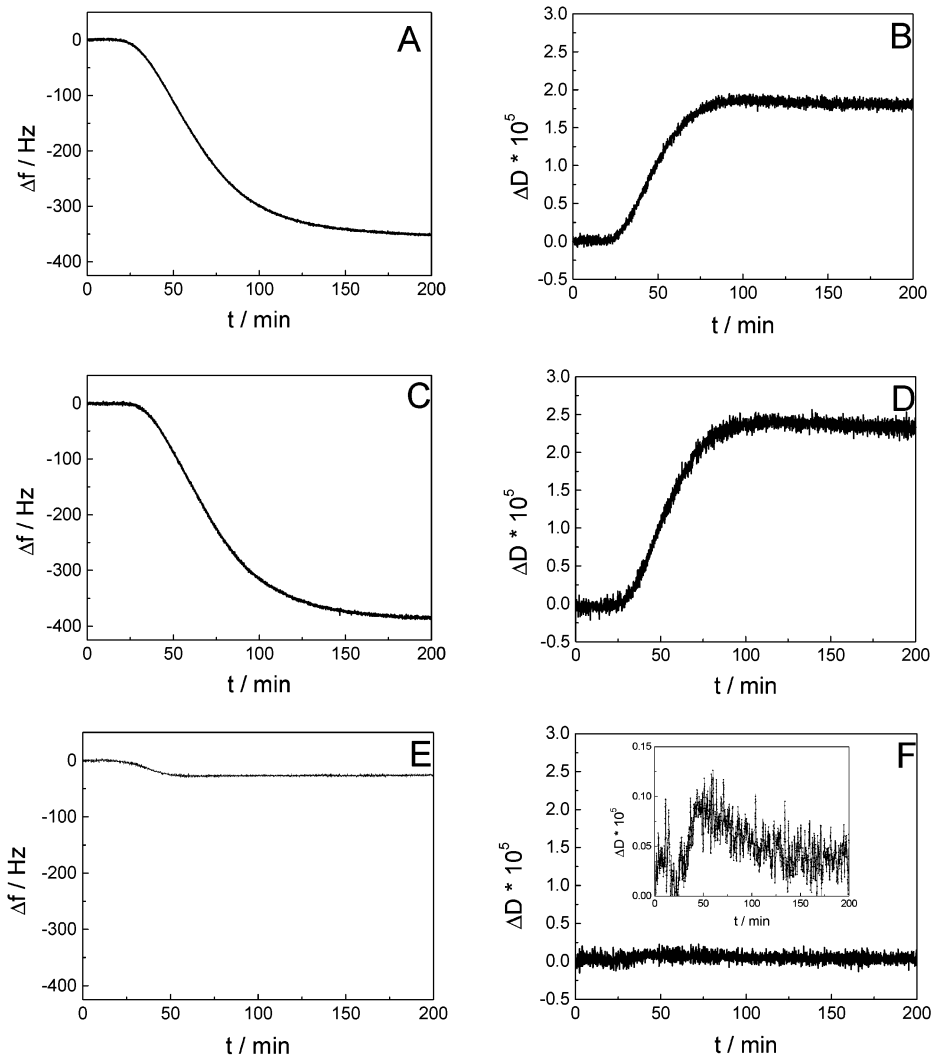

Figure 5. Shift of the resonance frequency Δf (A,C,E) and the dissipation factor ΔD (B,D,F) when liposomes prepared from DPPC and an increasing amount of biotin−DHPE are allowed to adsorb to a monomolecular layer of avidin immobilized on the resonator surface. The molar ratios DPPC/biotin−DHPE are 100:0 (A,B), 99:1 (C,D), and 70:30 (E,F). The blowup in panel F magnifies the subtle changes of the dissipation factor at the given conditions. T = 25 °C, pH 8.

observed frequency shifts on the order of 30−40 Hz, which corresponds approximately to a protein monolayer. According to Figure 4, protein adsorption is complete within 15 min. As expected for avidin adsorption, we did not record significant changes in the dissipation factor D (data not shown), confirming that the protein layer behaves like a rigid mass load and does not provide any significant viscoelastic energy losses.

After the avidin layer had been established on the resonator surface, the entire chamber was flooded with the protein-free buffer solution in order to remove all nonbound protein from the system. The resonator was equilibrated again, and then suspensions of large unilamellar DPPC liposomes doped with 0, 1, 5, 10, 20, or 30 mol % biotin−DHPE were injected into the circulating and stirred buffer yielding a final lipid concentration of 0.15 mg/ml. The interactions of the liposomes with the protein-coated surface were then followed by readings of the shifts in resonance frequency Δf and dissipation factor ΔD as a function of time. Figure 5A−F shows typical time courses as recorded for 0 mol % (A,B), 1 mol % (C,D), or 30 mol % (E,F) of the biotinylated lipid. Looking at the recorded frequency shifts first (Figure 5A,C,E), it is evident that pure DPPC vesicles and those doped with only 1 mol % biotin−X−DHPE show a rather strong frequency response which is in the order of 300−400 Hz and thus

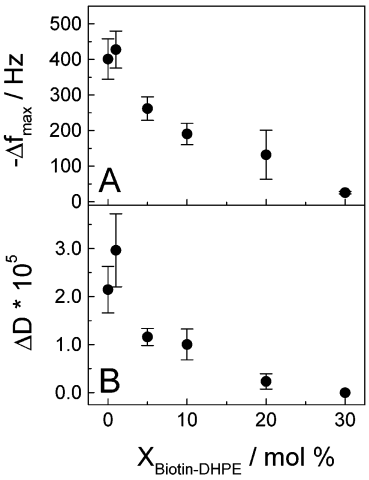

Figure 6. Shift of the resonance frequency Δf (A) and the dissipation factor ΔD (B) as a function of the molar fraction of biotin−DHPE in the liposome preparation (mean ± standard deviation (SD)). Data were extracted from experiments as shown in Figure 5.

very similar to the values that we recorded for the adhesion of living cells. High molar ratios of biotin-labeled lipids (Figure 5E), that were initially thought to model living

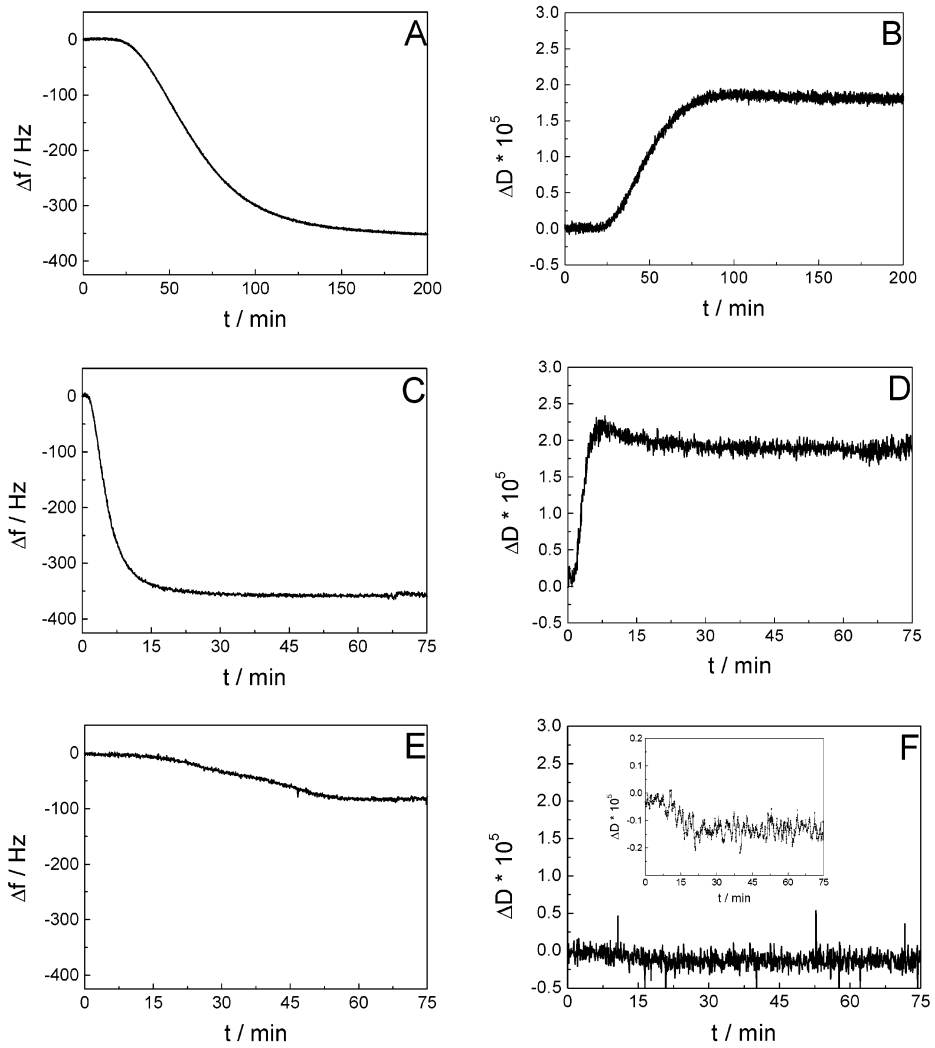

Figure 7. Shift of the resonance frequency Δf (A,C,E) and the dissipation factor ΔD (B,D,F) when liposomes prepared from DPPC and an increasing amount of biotin−X−DHPE are allowed to adsorb to a monomolecular layer of avidin immobilized on the resonator surface. The molar ratios DPPC/biotin−DHPE are 100:0 (A,B), 99:1 (C,D), and 70:30 (E,F). The blowup in panel F magnifies the subtle changes of the dissipation factor at the given conditions. $T = 25$ °C, pH 8.

cells with high amounts of cell surface receptors, induce rather moderate frequency shifts of less than 50 Hz. A very similar tendency is apparent from traces of the dissipation factor (Figure 5B,D,F). Undoped liposomes and those with only a minor amount of dopant show a strong increase of the dissipation factor in the order of $\Delta D = 2.5 \times 10^{-5}$. For liposomes prepared from DPPC and 30 mol % biotin−DHPE, the increase in energy dissipation is hardly significant. Figure 6A,B summarizes the results for the pool of data that we recorded on these systems. Although the frequency response of the liposome models with low dopant concentration was very similar to that of living cells, the shift of the dissipation factor is roughly one order of magnitude smaller compared to cell adhesion (Figure 2B).

As an independent control of these dynamic D-QCM measurements, we also performed impedance analysis of the loaded quartz resonators at frequencies closely embracing their fundamental resonance before and after liposome adhesion. Impedance analysis provides the most detailed characterization of the mechanical load on the resonator surface. It suffers, however, from a lack of sensitivity compared to the D-QCM technology. The impedance raw data were analyzed according to the Butterworth−van Dyke equivalent circuit providing the motional resistance R_m and the motional inductance L_m as quantitative parameters for energy dissipation and energy storage.[17] Although at the limits of sensitivity for the systems studied here, impedance measurements of the resonator before and after liposome adhesion independently confirmed that only those liposome preparations with low or zero dopant induced a significant energy dissipation characterized by an increase of the motional resistance in the order of 50 Ω. Increasing the molar ratio of biotin-labeled lipids reduced the change in motional resistance below the limit of sensitivity. Similar results were obtained for the motional inductance. Only liposomes with low or zero dopant incorporation showed a significant contribution to the motional inductance in the order of 10 μH, that was reduced to less than 3 μH for the highest amount of biotin−DHPE studied here.

Similar liposome model systems have been studied by scanning force microscopy (SFM).[28] Liposomes prepared from DPPC with increasing amounts of biotin−DHPE were allowed to adhere to a mica sheet precoated with the protein streptavidin that, similar to avidin, provides biotin binding sites. Using SFM, the authors were capable of imaging the shape of the liposome as long as the liposome concentrations were kept low for methodological reasons. With increasing dopant concentration, the recorded SFM images show a spreading of the liposomes to the surface

(28) Pignataro, B.; Steinem, C.; Galla, H. J.; Fuchs, H.; Janshoff, A. *Biophys. J.* **2000**, *78*, 487.

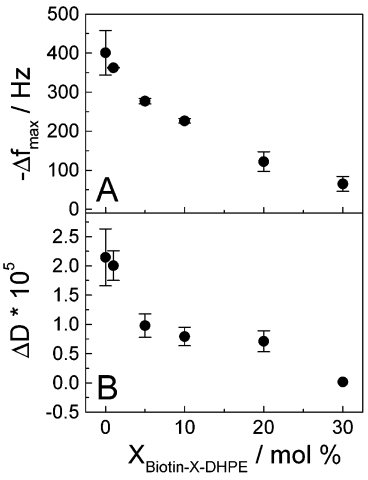

Figure 8. Shift of the resonance frequency Δf (A) and the dissipation factor ΔD (B) as a function of the molar fraction of biotin−X−DHPE in the liposome preparation (mean ± SD). Data were extracted from experiments as shown in Figure 7.

associated with a change in liposome morphology from almost spherical for low dopant concentrations to laterally more extended but less high for high amounts of dopant. With increasing concentrations of receptors in the liposome shell, the contact area of the liposome apparently increases. When the adhesion forces to the surface exceed the interactions forces between the individual lipids, as seems reasonable for high biotin concentrations, the liposome eventually ruptures and a lipid bilayer is formed on the surface. A surface-anchored lipid bilayer behaves like a rigid mass that synchronously follows the shear displacement of the resonator surface and does not induce viscous energy losses. This interpretation is consistent with our experimental findings (Figure 5F). Accordingly, viscous energy losses and the increased frequency response as observed for low or zero biotin−DHPE concentrations must be associated with the aqueous inner compartment of intact liposomes since low dopant conditions were shown not to compromise the integrity of the liposome but to induce only a moderate flattening of their morphology.[28] It seems reasonable that increased liposome spreading as observed for increasing receptor concentrations reduces the number of surface-bound liposomes and thus the total amount of surface-bound fluid. This may explain the gradual decrease that we observed for the shift in frequency and dissipation factor with higher concentrations of biotin (Figure 6A,B). Additionally, the acoustic shear wave may entirely penetrate flat liposomes and enter the bulk phase with considerable shear amplitude. In contrast, this may not be valid for almost spherical and significantly higher liposomes in which the shear wave dissipates more energy and loses amplitude.

The adhesion of living cells to the resonator surface is generally associated with very similar changes of the resonance frequency ($\Delta f = 100-500$ Hz dependent on the cell type), whereas changes of the dissipation factor are at least one order of magnitude higher, $\Delta D = 2 \times 10^{-4}$ to 7×10^{-4}. Consistent with these findings, we determined very similar values of the motional inductance L_m for cell and liposome systems (low dopant concentration) from impedance analysis. They are in the order of 2−10 μH. The motional resistances R_m of cellular systems are, however, significantly higher and range between 200 and 1500 Ω dependent on the cell type. It remains to be elucidated whether these differences are caused by the very unique mechanical composition of the cellular cytoplasm including the various cytoskeletal elements, by the distance between the ventral membrane and the surface, or by the extended dimensions of the cellular bodies compared to the used liposomes. Experiments performed with liposomes of different diameters (50−400 nm) did, however, not show any significant alterations of the recorded QCM response (data not shown).

We tried to address the impact of the membrane−substrate distance by studying the adhesion of liposomes prepared from DPPC and biotin−X−DHPE in which the biotin moiety is linked to the lipid headgroup by a C_6 spacer. Assuming an all-trans conformation, this spacer introduces an approximate distance of 0.5 nm between the biotin group and the lipid headgroup, which is the best estimate for the increase in membrane−substrate distance. Figure 7 shows typical time courses of the shifts in resonance frequency and dissipation factor for the three dopant concentrations 0, 1, and 30 mol %, whereas Figure 8A,B summarizes the entire pool of data for the biotin−X−DHPE system. Whereas the shifts in resonance frequency and dissipation factor are very similar for biotin−X−DHPE doped liposomes (Figure 8) compared to the biotin−DHPE system (Figure 6), the adhesion kinetics is rather different in the sense that liposome adhesion is much faster when biotin−X−DHPE molecules are used as receptors. Extracting the times necessary to induce a half-maximum change of Δf and ΔD, for instance for the 1% molar ratio of either dopant, returns 50−60 min for the biotin−DHPE system compared to 5−6 min for the biotin−X−DHPE receptors. From our data, we can only speculate about the reasons for this difference in adhesion dynamics. When the biotin group sticks out of the plane of the lipid headgroups, as given for the biotin−X−DHPE doped liposomes, this may sterically simplify the interaction of biotin with its binding site on the avidin protein and thereby provide a kinetic advantage of the binding reaction compared to that in liposomes doped with biotin−DHPE. The length of the spacer is, however, too small to induce significant changes with respect to the spreading behavior of the liposomes or other aspects of the mechanical surface load of the resonator. This was again confirmed by impedance analysis of the quartz resonators before and after adhesion of liposomes prepared with biotin−X−DHPE receptors that yielded very similar results as compared to those obtained with biotin−DHPE doped liposomes.

Conclusion

The experiments performed in the present study clearly indicate that the mechanical load imposed on shear wave resonators by mammalian cells cannot be modeled in its details by using liposome preparations as described above. Although one essential feature of cellular adhesion, specific molecular interaction with surface-bound proteins, was met by incorporating receptors into the liposome shell, the viscous energy dissipation in particular is rather different in the model compared to the living systems. However, these studies did reveal that the liquid environment that is entrained in the liposome interior and that models the cellular cytoplasm has a considerable impact on QCM parameters. Our further work will address and improve the limitations of the vesicular systems in order to get a highly controllable model for the adhesion of living cells.

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