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Biosensor-Based Evaluation of Liposomal Behavior in the Target Binding Process

MATTHIAS HÖPFNER,[1] ULRICH ROTHE,[1] AND GERD BENDAS[2]

[1]Department of Physiological Chemistry, Medical Faculty, University Halle, Germany
[2]Department of Pharmaceutics, University Bonn, Bonn, Germany

In this study we evaluated the quartz crystal microbalance (QCM) as a biosensor for a real-time investigation of liposomal binding, under dynamic flow conditions, onto target proteins immobilized at the sensor. The mass-sensitive frequency changes of quartz sensors allow for a quantification of the liposomal binding process. Furthermore, simultaneous damping analysis gives an insight into liposomal behavior, such as the degree of liposomal deformation or spreading at the target surface. In this study a series of liposomes was evaluated, differing in the kind and concentration of ligands interacting with appropriate target proteins. It became evident that an increase in homing device concentration accelerated deformation and flattening of liposomes, triggering a fusion process. Furthermore, liposomal deformation corresponded with the binding affinity of target molecules, comparing biotin/avidin with E-selectin/ligand interactions. Deformation could be emphasized using dioleoylphosphatidylethanolamine (DOPE) as a fusiogenic membrane component, while sterical stabilization by polyethylenglycol (PEG-PE) appeared in a low degree of deformation. Consequently, the online detection of liposomal target binding by QCM is an excellent facility to control and predict the liposomal behavior at the target site for increasing therapeutic potency.

Keywords biosensor, liposomal targeting, QCM

Introduction

The targeting of liposomes to tissues and organs has gained enormous therapeutic interest. While the accessibility of targets is an essential prerequisite for liposomes, therapeutic effects will result finally from the specific mode of liposomal interaction with target cells. In general, two main scenarios can be distinguished. Depending on the targeted cellular epitopes, on the liposomal drug load, or the pharmacological approach, (1) liposomes stay intact at the target surface, which allows uptake by cells for generating intracellular effects, or (2) liposomes disrupt at the target and release the drug load to achieve high local concentrations outside target cells. Although both options will overlap in practice,

Address correspondence to Prof. Dr. Gerd Bendas, Dept. of Pharmaceutics, University Bonn, An der Immenburg 4, 53121 Bonn, Germany; E-mail: gbendas@uni-bonn.de

the prediction of liposome–cell interactions would greatly increase therapeutic benefit. Theoretical knowledge exists on the influence of certain lipid species to control liposome characteristics, such as membrane-disrupting lipid for controlled fusion at the target site (Huang, 1994). However, liposome–cell interactions can not directly be detected, which complicates the correlation of liposomal design and targeting behavior.

Either electron microscopy or scanning force microscopy can excellently illustrate liposomal shapes on different surfaces, but these techniques do not allow real-time detection without artificial preparations. On the other hand, spectroscopic methods can detect processes, such as marker release or liposomal aggregation, but can not directly focus on the liposomal structure. Consequently, a technique would be desired that both quantifies the liposome–target interactions in real time, and simultaneously allows an insight into liposome behavior.

Recent advances in the development of biosensors in analytics might be a basis for an application in this field. Quartz crystal microbalances (QCM) are prominent mass-sensitive detectors based on the piezoelectric properties of quartz crystals. Electrical excitation of a thin quartz plate results in oscillations of the resonance frequency, which indirectly correlates with the mass load on its surface. Consequently, changes in mass can be detected in real time by monitoring frequency shifts. To apply QCM as a biosensor technique, the quartz electrodes have to be functionalized as biospecific layers. The QCM method has been established for a large number of analytical approaches, such as polymer surface characterization (Kurosawa et al., 2002; Mak, 1997), ligand-receptor interactions (Janshoff et al., 1997; Simonis et al., 2007), detection of conformational changes in DNA or drug-DNA interaction (Matsuno et al., 2001; Yamaguchi et al., 1993), or protein adsorption onto membranes (Christ et al., 2007).

A few QCM studies evaluated biophysical processes of liposomes (spreading or multilayer formation) onto different surface materials, such as hydrophobized gold or SiO_2, and completed postulations with further analytical techniques (Keller et al., 1995). For instance, a two-step mechanism of vesicle fusion was proposed; vesicles first adsorb to the surface and fuse with each other to form larger vesicles on the surface to subsequently rupture for bilayer fragment formation. Liposomal behavior was shown to be dependent on lipid species (Richter et al., 2003). Pignataro and others investigated the binding of biotinylated liposomes to surface-bound avidin under static conditions and illustrated liposomal deformation finally by AFM (Pignataro et al., 2000).

The involvement of damping analysis, which evaluates the flexibility of surface-bound layers, amplifies the analytical value of mass-dependent frequency changes. Differences between intact or flattened liposomes at the surface appeared detectable. On that basis, liposome fusion onto different materials was investigated with respect to liposomal size and concentration (Reimhult et al., 2003, 2006; Stalgren et al., 2001). Patel and Frank illustrated a rapid formation of dense surface bilayers using osmotically stressed vesicles in a QCM-dissipation study (Patel and Frank, 2006).

The QCM damping analysis should also have the potential to simulate liposomal behavior with respect to therapeutic targeting. The aim of this study was to investigate whether the properties of liposomes at simulated target sites can be detected by this technique to finally predict liposomal effects for therapeutic approaches. Therefore, we compared the binding of nontargeted liposomes with targeted liposomal binding onto model and therapeutically relevant target molecules under physiological shear flow conditions.

We showed that QCM damping analysis allows further insight into the targetability of liposomes with respect to liposomal appearance and the controlled prediction thereof.

Materials and Methods

Reagents were obtained from the following sources: soy phosphatidylcholine (SPC) was a kind donation of Lipoid AG (Ludwigshafen, Germany), 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE), methoxy-polyethylenglycol-phosphatidylethanolamine (PEG-PE 2000), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), N-glutaryl-phosphatidylethanolamine (NgPE), N-biotinyl-dipalmitoyl-phosphatidylethanolamine, and N-[biotinyl(polyethylenglycol)-2000]-dipalmitoyl-phosphatidylethanolamine were purchased from Avanti Polar Lipids (Alabaster, Alabama, USA). Glycolipid with a sLex moiety attached to a dialkylglycerol via a spacer of 9 ethoxy units [17-(1,2-di-O-hexadecyl-sn-glycer-3-oxy)-3,6,9,12,15-pentaoxaheptadec-1-yl]-O-(triethylammonium-5-acetamido-3,5dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonat)-(2-3)-(β-D-galactopyranosyl)-(1-4)-[α-L-fucopyranosyl)-(1-3)]-2-acetamido-2-deoxy-β-D-glucopyranosid was synthesized by Dr. Gege (University Konstanz, Germany) as described (Gege et al., 2000). Recombinant human E-selectin-Fc chimera were purchased from R&D Systems, Wiesbaden, Germany. Purified antihuman CD62E were from Becton Dickinson GmbH, Heidelberg, Germany. Cyanuric chloride, avidin, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) were purchased from Sigma, Deisenhofen, Germany. 6-Mercaptohexan-1-ol was purchased from Fluka, Neu-Ulm, Germany, and chloroform was from Riedel de-Haen, Seelze, Germany. All salts and buffers were of analytical grade.

Preparation of the Quartz Crystal Surface

Quartz crystals were cleaned using piranha solution (H_2O_2 conc./H_2SO_4 conc. 1/3) and subsequently rinsed with demineralized water. After repeating this procedure three times, the quartz crystals were rinsed with ethanol and dried under a stream of nitrogen. The cleaned quartz crystals were added to a 1 mM chloroform solution of 6-mercaptohexan-1-ol for about 12 h at room temperature (rt) to form a thiol-functionalized monolayer. After rinsing the quartz crystals briefly with ethanol and drying them under a stream of synthetic air, the quartz crystals were put into a chloroform solution of cyanuric chloride (1 % mass/volume) for 2 h at rt, again followed by ethanol washing and drying. The cyanuric chloride acts as a reactive linker onto the immobilized 6-mercaptohexan-1-ol for coupling avidin or the E-selectin-Fc chimera. A solution of 20 μL avidin (1 μg/μL in 10 mM borate buffer, pH 8.8) or 20 μL E-selectin-Fc chimera in PBS (0.2 μg/μL) and 20 μL borate buffer (pH 8.8) was put on the upper gold surface of the quartz sensors. After 2 h at rt, the quartz crystals were rinsed once again with demineralized water and dried under a stream of nitrogen.

QCM Measurements

QCM measurements were performed with a LiquiLab21 quartz crystal microbalance (ifak e.V., Barleben, Germany). The measurement chambers (volume of about 100 μL) were made of polycarbonate, as schematically illustrated in Fig. 1. A peristaltic pump assured both flow conditions and the transport of analyte solutions to the quartz crystal surface. Quartz crystals (10 MHz resonance frequency, 14 mm diameter) were inserted into the measurement chambers. PBS buffer was used as flow medium. The experiments were performed under a volume flow rate of 270 μL min^{-1}. The quartz crystals were equilibrated under flow conditions for 30 minutes to reach a constant resonance frequency. The indicated volumes of liposomes, corresponding to final concentrations of about 0.5 mM, were added under constant flow conditions. The changes of frequency were computer-monitored in real time.

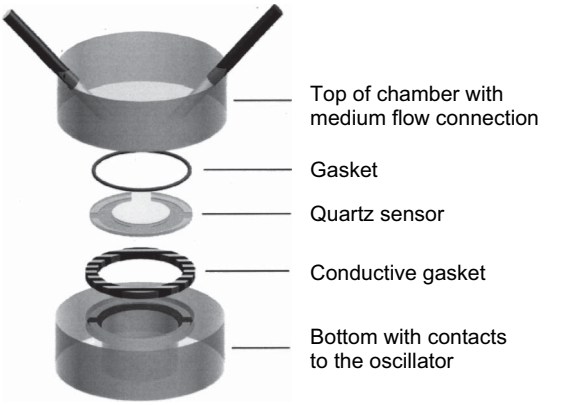

Figure 1. Schematic illustration of the flow chamber device for biosensor measurements.

Liposome Preparation

Liposomes were prepared from SPC with the indicated fractions of $mPEG_{2000}$-PE, N-glut-PE, biotin-PE, biotin-PEG-PE, or sLex-lipid by hydrating the dried films with PBS buffer (pH 7.4) to reach a final lipid concentration of 10 mM. Unilamellar liposomes were prepared with a Mini-extruder, (Avanti Polar Lipids), from multilamellar vesicles extruded 19 times through a 200 nm polycarbonate membrane, 19 times through a 100 nm polycarbonate membrane, and 10 times through a 50 nm polycarbonate membrane (Whatman Nuclepore 18 mm polycarbonate membrane, Richmond, USA). The resulting diameter of the liposomes was detected by PCS (Malvern Autosizer).

To couple the E-selectin antibody to the liposomal surface, 5 mol% N-Glut-PE (NgPE) were incorporated into the liposomal bilayer and activated by EDC. Protein coupling was performed as previously described in a 1:1000 protein:lipid molar ratio (Kessner et al., 2001) controlled by protein assay (Peterson, 1977) and phosphate assay (Ames and Dubin, 1960).

Results and Discussion

Binding Characteristics of Nontargeted Liposomes onto Surfaces

QCM is a mass-sensitive biosensor system. The binding of analytes onto the gold-coated sensor surface induces a decrease in oscillation frequency, which correlates with bound mass according to Sauerbrey's equation, where Δf is the change in frequency, f_0 is the oscillation frequency (10 MHz), A is the sensing area of the quartz (0.282 cm^2), μ_q is the shear-modulus (2.947×10^{11} g/cm s^2), ρ_q is the density of the quartz (2.648 g/cm^3) and Δm is the bound mass on the quartz surface, resulting in a constant C_f of 1.24 ng/Hz for used quartz sensors.

$$\Delta f = -\frac{2 f_0^2}{A\sqrt{\mu_q \rho_q}} \Delta m = -C_f \Delta m \qquad (1)$$

In order to investigate whether this device is suitable for the characterization of liposomal binding processes at target surfaces, plain SPC LUV with a diameter of about 100 nm were selected for first analyzing the adhesion process at the sensor surface. The QCM measurements were performed in a flow chamber to simulate physiological blood flow conditions.

After equilibrating the resonance frequency of the quartz under flow, addition of the indicated amount of liposomes into the medium, and subsequent binding onto the gold surface can be followed in real time by the drop in frequency (black) in Fig. 2a. Liposome adsorption is controlled by achieving a force equilibration between hydrophilic contact formation and increased curvature. The binding process, which corresponds to a frequency drop of about 276 Hz ± 32 Hz, is complete within 3 minutes. According to Eq. 1, the frequency change corresponds to a mass load of about 4.3 μg/cm^2. Considering the MW and head group size of the phospholipid molecules, SPC bilayer formation at the

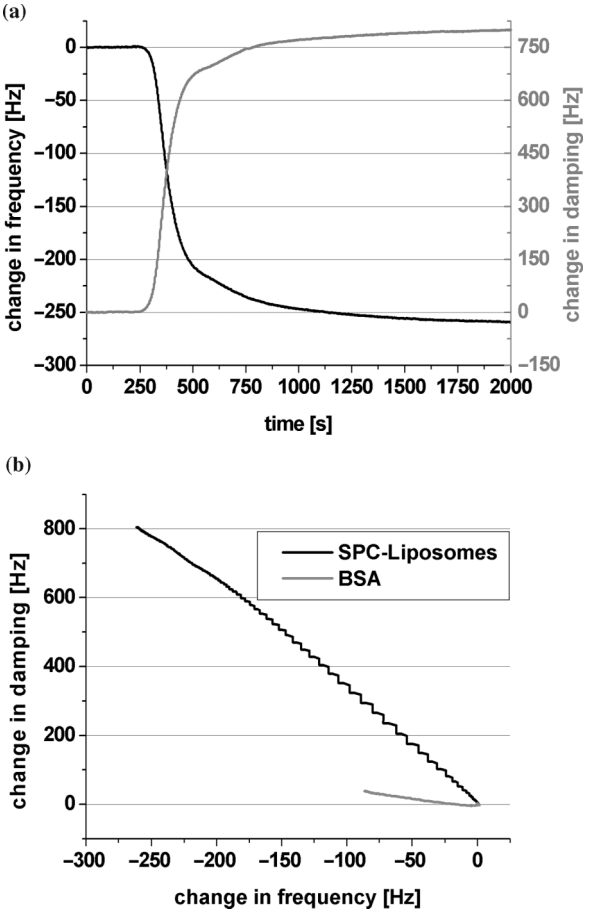

Figure 2. (a) Example of a measurement course of the QCM biosensor. The binding of a certain analyte onto the sensor surface can be quantified in real time by following the frequency drop (black line). The resulting increase in surface viscoelasticity correlates with damping frequency (grey line); (b) The plotting of frequency versus damping changes and the resulting D/f slopes allow interpretations on the appearance of surface-bound structures.

sensor surface would induce a frequency drop of about 128 Hz, or 2.0 μg/cm^2, respectively. This indicates either that liposomes fuse spontaneously to multilayers at the sensor, or a certain number of liposomes bind intactly.

Further information on the properties of liposomes should result from the damping analysis. Since a high viscoelasticity of bound analytes induces an increase in damping frequency, a differentiation of intact liposomes, deformed flattened ones, or fused bilayer formation appears possible in detecting damping. The increase in damping frequency of about 788 Hz ± 142 Hz is illustrated as a grey line in Fig. 2a. To consider this behavior as independent of analyte concentration, the ratio of damping and frequency change has been described as a valuable parameter following the time course of binding. As illustrated in Fig. 2b, a continuous D/f slope of 2.83 ± 0.4 is evident. This contradicts a liposomal spreading or fusion process, which would be apparent in a time-dependent decrease of the slope, for example according to the two-step model (Keller et al., 1995). Furthermore, the higher viscoelasticity of intact liposomes, for example higher D/f ratios versus dense surface layers, became evident when comparing a D/f value of 0.6 ± 0.2 of an adsorbed BSA layer at the sensor.

In order to test the analytical value of the damping analysis and the D/f ratio for characterizing liposomal behavior, we modified the liposomes as well as the sensor surface for further "nontargeted" liposome binding approaches.

The principle of sterical stabilization by PEGylating liposomes implies that unspecific adsorption would be diminished (Woodle 1992). Consequently, the deformation of PEGylated liposomes onto the hydrophilic gold surface should also be reduced. This is strongly supported by considering the data in Table 1.

An increase in sensor hydrophobicity by self-assembling an octadecanthiol layer also reduces the binding intensity of non-PEGylated liposome and indicates intact and less deformed vesicles compared to the hydrophilic gold surface. As anticipated, membrane fusion onto hydrophobic surfaces did not occur. Obviously, shear force conditions avoid tight membrane contacts, which would trigger fusion processes.

In order to induce stronger vesicle deformation or membrane spreading, charge-induced interactions between liposomes and the sensor surface were aspired. Therefore, the interaction of DOTAP liposomes with the negatively charged surface of mercaptohexadecanic acid-modified sensors was analyzed. The high intensity of binding and the low D/f value indicate massive binding and strong deformation and flattening of the liposomes.

These data indicate that the QCM device is a promising tool to follow time-dependent interactions of different types of liposome quantitatively. The damping analysis, that is the

Table 1

Adsorption behavior of different liposome types onto unmodified and modified gold sensors. Frequency changes illustrate the intensity of binding, whereas D/f slope indicates the liposomal appearance at the surface. Data represent means of at least 3 independent experiments ± SD

Sensor surface	Liposomes	Frequency change [Hz]	D/f slope	Postulated liposome behavior
Unmodified gold	SPC	788 ± 142	2.83 ± 0.4	deformed
Unmodified gold	SPC/PEG-PE	15 ± 4	3.51 ± 0.7	less deformed
Hydrophobic modified	SPC	34 ± 13	4.35 ± 0.2	not deformed
Negatively charged	DOTAP	938 ± 23	2.29 ± 0.6	flattened

D/f ratio, is a successful parameter to derive sensitive information about liposomal behavior in the binding process, as depicted in Table 1.

Binding Characteristics of Avidin-Targeted Liposomes

In order to investigate liposomal behavior in a simulated receptor-mediated targeting process, we selected the biotin/avidin system as established, high affinity binding partners. This should provide an insight into the role of liposomal homing devices in the targeting process.

Therefore, different amounts of biotinylated lipids were incorporated into SPC liposomes, and the interaction with avidin-coated sensor surfaces was analyzed. The obtained frequency drops and calculated D/f ratios are summarized in Table 2.

It is evident that the binding intensity of liposomes corresponds with biotin concentration. Surprisingly, the strongest frequency drop—that is, the surface binding—was obtained using liposomes with 20 mol% biotin-PE. The higher biotin-PE concentration of 30 mol% obviously reduced the binding. An interpretation of this fact could be derived from the values of the D/f quotients. While the D/f values dropped slightly up to a concentration of 5 mol% biotin-PE, which indicates no pronounced liposome deformation, higher biotin amounts induced drastic reductions. It might be assumed that the increased flattening of the liposomes up to 20 mol% biotin-PE passes to liposomal spreading at exceeding biotin concentrations. This could explain both the low D/f value of 0.98 and the lower frequency shift, since liposomal fusion and subsequent release of entrapped content would reduce the bound mass. This indicates in general that a high concentration of high-affinity liposomal ligands is able to change or finally disrupt the liposomal integrity interacting with the sensor surface.

However, liposomes for targeting approaches often obtain a sterical stabilization by grafted PEG chains. In order to investigate the interplay between PEGylation with the previously described liposomal distortion in the binding process, we applied various biotinylated liposomes carrying 2 mol% or 5 mol% PEG-PE for avidin binding studies. The data obtained are summarized in Table 3.

The frequency changes confirm that PEGylation in general diminishes liposome interaction with the avidin surface; compare this with data in Table 2. This might be

Table 2

Binding of biotinylated liposomes onto avidin-modified sensors with respect to liposomal biotin concentration. The decrescent D/f slope data indicate a correlation of liposome deformation and biotin concentration. Data represent means of at least 3 independent experiments ± SD

Concentration of biotin-PE [mol %]	Liposome size [nm]	Frequency change [Hz]	D/f-slope
0.1	96 ± 4	18 ± 12	3.43 ± 0.57
1	92 ± 5	68 ± 26	3.38 ± 0.58
5	100 ± 8	76 ± 20	3.22 ± 0.07
10	96 ± 3	266 ± 63	1.99 ± 0.28
20	92 ± 2	359 ± 78	1.42 ± 0.16
30	91 ± 5	215 ± 59	0.98 ± 0.01

Table 3
The influence of sterical stabilization of biotinylated liposomes by PEG-PE on binding and deformation onto avidin-coated sensor surfaces. Data represent means of at least 3 independent experiments ± SD

Conc. of biotin [mol %]	Conc. of PEG-PE [mol %]	Liposome size [nm]	Frequency change [Hz]	D/f-slope
5	2	93 ± 6	7 ± 1	4.55 ± 0.75
10	2	95 ± 5	20 ± 16	4.15 ± 0.11
20	2	92 ± 6	38 ± 11	3.01 ± 0.66
30	2	98 ± 8	118 ± 36	3.72 ± 0.05
5	5	91 ± 5	28 ± 10	4.12 ± 0.01
30	5	90 ± 8	40 ± 10	4.02 ± 0.70

related to the bulkier hydrophilic surface, which reduces the feasibility of biotin at the liposomal body interacting with avidin. Nevertheless, keeping PEG content constant, increasing biotin concentrations also induces higher liposome binding. In addition to reduced binding processes, the D/f values indicate that the addition of PEG circumvents liposomal deformation. This correlates with the PEG amount, comparing the 30 mol% biotin liposomes without PEG (0.98), 2 mol% (3.72), and 5 mol% PEG-PE (4.02).

In order to investigate whether the failing distortion in the presence of PEG is a general matter of membrane stabilization or a result of shielded ligand accessibility, we inserted a PEG-lipid with terminal biotin moiety. These liposomes should combine a sterical stabilization with excellent accessibility of high-affinity ligands. The data in Table 4 clearly indicate a stronger binding ability and a strong flattening of liposomes corresponding to biotin concentration. The deformation of 30 mol% PEG-biotin liposomes is comparable to non-PEGylated ones. This confirms that PEGylation does not change the membrane characteristics itself, such as fluidity or stiffness, but reduces surface interactions.

The findings of the biotinylated liposomes with respect to membrane deformation are schematically summarized in Fig. 3.

Binding Behavior of Therapeutically Relevant, Selectin-Directed Liposomes

In previous studies we introduced E-selectin, an inflammatory specific endothelial receptor involved in leukocyte emigration, as a promising target for liposomal drug delivery strategies

Table 4
Binding of liposomes with PEG-grafted biotin onto avidin-coated sensors. Deformation is illustrated by reduced D/f quotients. Data represent means of at least 3 independent experiments ± SD

Conc. of biotin PEG-PE [mol %]	Liposome size [nm]	Frequency change [Hz]	D/f-slope
5	91 ± 5	192 ± 17	2.31 ± 0.33
30	93 ± 4	111 ± 2	1.26 ± 0.12

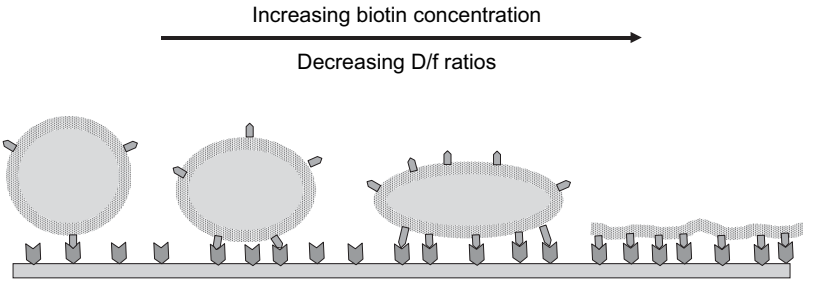

Figure 3. Schematic summary of liposomal deformation in the binding process with respect to liposomal ligand density and the analytical evaluation by calculating D/f slopes.

at inflammatory sites (Kessner et al., 2001). Either liposomal disruption, for increased local drug concentrations, or controlled cellular uptake of liposomes would be of therapeutic interest depending on the specific drug load. In order to simulate this situation and to modify liposomal characteristics to accomplish one or the other, we prepared E-selectin directed immunoliposomes by coupling antiE-selectin mAb onto N-glut-PE anchors in SPC LUVs. The protein coupling yield was around 35 µg/µmol total lipid, which corresponds roughly with the attachment of about 30 antibodies per liposome considering a liposomal size of 94 nm. The flow interaction of these liposomes using sensor-immobilized E-selectin-Fc chimera resulted in a frequency drop of about 32 Hz; the D/f value of 3.77 indicates intact, less deformed vesicles at the E-selectin layer. However, the lower affinity of IgG/antigen interactions compared to biotin/avidin binding cannot be correlated with these findings with respect to ligand density, accessibility, and affinity, since the number of coupled antibodies is rather low and differs individually within the liposomal population.

E-selectin is a carbohydrate-binding protein with binding specificity for the tetrasaccharide sialyl LewisX (sLex). The very low affinity of sLex to E-selectin is described to be in the millimolar range (Jacob et al., 1995). However, multimeric presentation of sLex onto liposomal surfaces should increase avidity (Stahn et al., 1998) and has been described for different therapeutic approaches (Hirai et al., 2007; Zeisig et al., 2004). We incorporated sLex-based lipids into SPC LUV and analyzed E-selectin binding onto the sensor. In contrast to biotinylated liposomes, which bind immediately from the flow medium to the sensor, sLex liposomes need a much longer time of continuous flow to attach to target molecules. This process of slow binding saturation was followed for several hours; data in Table 5 are representative for a 6 hour period.

Table 5
Binding of sLex-modified liposomes onto E-selectin-coated sensors surfaces. Data represent means of at least 3 independent experiments ± SD

Conc. of sLex ligand [mol %]	Conc. PEG-PE [mol %]	Liposome size [nm]	Frequency change [Hz]	D/f-slope
1	–	98 ± 4	80 ± 13	4.60 ± 0.34
5	–	102 ± 8	390 ± 72	3.74 ± 0.56
10	–	105 ± 6	133 ± 34	3.58 ± 0.22
5	2	96 ± 5	77 ± 21	5.54 ± 0.13

In absence of PEG-PE, the increase in sLex concentration from 1 to 10 mol% improved the binding. It is not easy to explain why liposomes with 5 mol% ligand bind more intensively than those with 10 mol% ligand. Maybe, aspects of multivalent ligand presentation and avidity increase have to be considered. However, the D/f values display a certain decrease corresponding to increased ligand densities. In comparison to biotin liposomes interacting with avidin, the degree of distortion is much smaller (10 mol% sLex corresponds to 0.1 mol% biotin, D/f values of 3.58 versus 3.43 respectively), which clearly confirms the impact of ligand binding affinity on liposomal deformation at the target site. As expected, the incorporation of PEG-PE into sLex liposomes further reduces binding ability and avoids any distortion of liposomal membranes. Consequently, sLex-targeted liposomes of this design would stay intact at the endothelial surface, disposed for endocytotic uptake by E-selectin (Kessner et al., 2001).

In order to design sLex-targeted liposomes for a controlled drug release at the endothelial surface, we replaced SPC by DOPE, which has a strong tendency to adopt an inverse hexagonal (H_{II}) phase. But in combination with other "functional" lipids that display bulkier head group regions and an inverse cone shape, DOPE liposomes can be generated. Those mixtures have been used for a controlled rupture of liposomes due to shape changes of functional lipids after certain trigger mechanisms (Kirpotin 1996; Wright 1992), such as pH-change (Simoes et al., 2004) or target contact (target-sensitivity) (Ho et al., 1986). One principle of target-sensitivity has been postulated to be the lateral diffusion and local concentration of bilayer-stabilizing ligands in the liposomal target contact area, thus destabilizing the DOPE liposomes at the converse site (Huang 1994). We aimed at this principle of target-sensitivity by incorporating 5 mol% sLex lipid into DOPE. The bulky tetrasaccharide head group, spacered by hydrophilic ethoxy units, allows the preparation of DOPE liposomes with a particle size of 96 ± 8 nm, which displayed sufficient size stability during a 10 day period.

In contrast to the SPC-sLex liposomes, DOPE-sLex liposomes bind immediately to the E-selectin layer from the flowing medium. It might be assumed that the smaller head group of PE compared to PC allows easier accessibility of the ligand, or perhaps a higher lateral diffusion of ligands (within the DOPE matrix) to the contact region increases avidity.

Frequency and damping changes were followed for a 12-hour period. It was evident that maximal binding was achieved after 2 hours, with a frequency change of about 1200 Hz displaying strong binding ability. Up to this time, damping analysis generates D/f values of about 3.36, indicating slightly deformed vesicles. Following frequency for a longer time, frequency increased again, which should correspond to partial desorption of adhered liposomes. But the damping data render continuously decreasing D/f values up to 1.91, which might be related to a flattening or partial spreading of the liposomes. However, as evident from the data in Table 1, the D/f value of 1.91 is not equivalent to bilayer formation, but would indicate strongly flattened liposomes. Since liposome flattening would not influence bound mass and thus the resonance frequency, we postulate superimposed processes of liposome deformation and bilayer formation with subsequent content release. This is supported by recent experiments on liposomal targeting of endothelial E-selectin. Only sLex/DOPE liposomes, but not sLex/SPC liposomes displayed an aqueous marker release after cell contact (data not shown).

Conclusions

The QCM biosensor is an attractive device for obtaining insights into liposomal appearance in the targeting process. Besides providing an exact quantification of liposomal binding

onto immobilized targets to confirm and optimize targeting approaches, the biosensor allows for a detailed analysis of liposomal behavior. Since therapeutic effects depend on the mode of liposomal interaction with target cells, a prediction and potential directing of liposomal appearance will facilitate therapeutic potency. In general, liposomes tend to deform at the target surface upon binding, correlated with target affinity and concentration of liposomal homing devices. This process can even be triggered to end up in a bilayer fusion and subsequent content release by choosing specific parameters like membrane destabilizing lipids or high ligand concentrations. We illustrated that PEGylated liposomes have high conformational stability on simulated target surfaces and therefore are convenient for cellular uptake. The online detection of liposomal behavior might help optimize these carriers for certain therapeutic approaches.

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