Electronic Supplementary Material (ESI) for Lab on a Chip

Supporting information:

Single cell 3-D platform to study ligand mobility in cell-cell contact

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Materials and Methods: Microwell fabrication and functionalization

Microwell fabrication Arrays of microwells with different geometries (circles, squares, triangles, rectangles, spindles, etc.), different lateral dimensions (81 μm^2 to 900 μm^2 projected area) and a depth of 10 μm were first produced in silicon using standard photolithography with the negative photoresist SU8 (MicroChem) and replicated into a PDMS master (negative structure). To achieve thin (compatible with inverted stage microscopy), microstructured PDMS films with positive structure, the arrays were replicated a second time. The replication and surface modification was performed as described in [1, 2]. Briefly, after fluorosilanization of the PDMS master, the second replication resulted in the final microwell structure in PDMS.

Microwell functionalization After air plasma treatment which converted the PDMS surface to a hydrophilic, SiO_2-like, thin layer, the plateau areas were passivated with PLL-*g*-PEG using an inverted microcontact printing technique [2]. Briefly, a flat PLL-*g*-PEG loaded hydrogel was placed on the structured substrate resulting in conformal contact between the stamp and the plateau, but not the insides of the well. The contact transferred PLL-*g*-PEG to the plasma treated PDMS plateau surface, rendering it resistant against protein [3] and vesicle adsorption [4]. After passivation of the plateau with PLL-*g*-PEG, the sample was exposed to 0.5 mg/ml of extruded vesicles for 10 min at room temperature for the DOPC and at 37°C for the MPPC vesicles. The phospholipid vesicles ruptured on the SiO_2-like surfaces to form a SPB inside the 10 μm deep microwells but not on the passivated plateau where the vesicles were repulsed by the PLL-*g*-PEG layer. The samples were rinsed with PBS after vesicle incubation.

Coverage and mobility of SA layer

Mobility

On SPBs on PDMS, the SA was mobile. Furthermore, the diffusion coefficients of lipid and SA on SPBs on PDMS were of the same order of magnitude. For oxidized PDMS, a lipid diffusion coefficient was 0.1 ± 0.04 $\mu m^2/s$ with a mobile fraction of 83 ± 4 % was found. This diffusion coefficient was lower than reported in the literature. Phillips *et al*. reported a lipid diffusion coefficient of 2.2 ± 0.9 $\mu m^2/s$ for PC on oxidized PDMS [5]. By comparison, a low lipid diffusion coefficient of 0.28 ± 0.04 $\mu m^2/s$ with a mobile fraction of 86 ± 1 % was measured for the same preparation on SiO_2 (Table S1). Rossetti *et al*. measured a lipid diffusion coefficient of 1.7 ± 0.3 $\mu m^2/s$ for NBD-PC on SiO_2 [6]. The diffusion coefficient of SA bound to SPBs on PDMS was 0.09 ± 0.01 $\mu m^2/s$ with a mobile fraction of 88 ± 2 %, while SA on SPBs on SiO_2 showed slow and incomplete fluorescence recovery. The mobility of SA on the PDMS-supported platform ensures that E-cad/Fc ligands bound to the SA will also be mobile since the E-cad/Fc do not appear to form lateral aggregates. The similar diffusion coefficient to that of the lipids is expected since liposomes tethered to a SPB exhibited diffusion coefficients related only to the anchor mobility and not to vesicle size [7].

Table S1 Mobility of DOPC (NBD-PC labeled) SPB and streptavidin (Alexa 633 labeled) on PDMS and SiO_2 determined by FRAP. Diffusion coefficient and mobility of 5 % bDOPE/DOPC SPB on SiO_2 and oxidized PDMS, and for the streptavidin adsorbed to bDOPE on top of the same SPBs.

		Diffusion coefficient [$\mu m^2/s$]	Mobile fraction [%]
SiO_2	SPB	2.8 ± 0.4	86 ± 1
	Streptavidin	N/A	N/A
PDMS	SPB	1.0 ± 0.4	83 ± 4
	Streptavidin	0.9 ± 0.1	88 ± 2

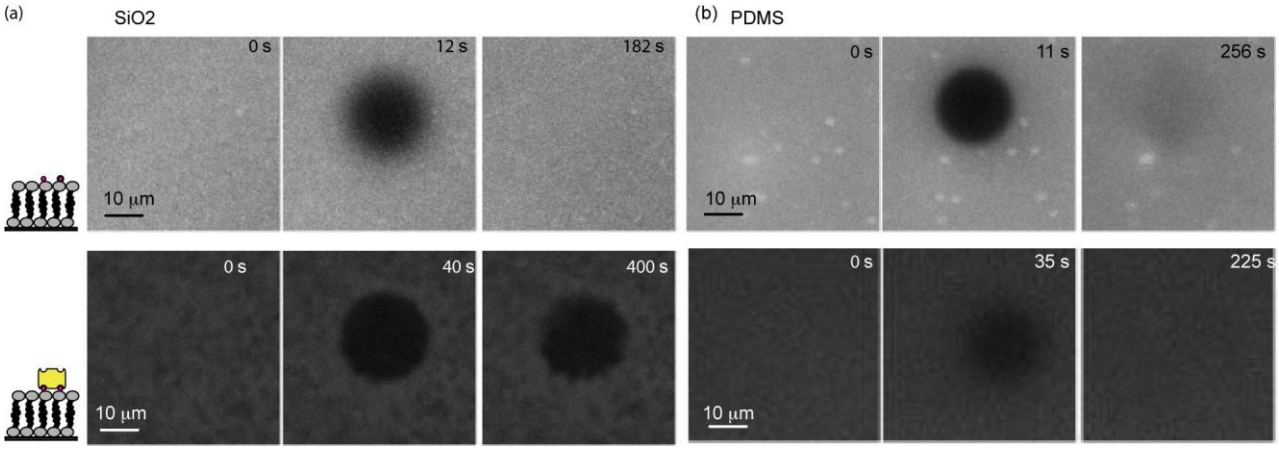

Figure S1 ***Mobility of SPB and streptavidin on PDMS and SiO_2.*** (a) shows the FRAP studies on SiO_2 with 5% biotinylated DOPC. After SPB formation, the fluorescently labeled SPB was investigated for its mobility and shows a fluorescent recovery after 182s. After streptavidin addition, the streptavidin layer was investigated by FRAP and showed a non-mobile layer. (b) shows the same experiment, but on a PDMS surface. Here we can see that not only the SPB undergoes fluorescent recovery but also the streptavidin in the top layer is mobile.

Coverage

QCM-D measures the mass of the adsorbed molecules *and* hydrodynamically trapped water in the film. Therefore, the mass measured by QCM cannot directly be calculated into a percentage of a close-packed monolayer. Reimhult *et al*. used simultaneous QCM-D and surface plasmon resonance (SPR) measurements to demonstrate that SA on a 5 % bPOPE/POPC SPB bound a mass of water equal to 250 ng/cm^2 [5], which corresponds to 56 % of the 440 ng/cm^2 total mass. The same ratio of coupled water in our case yields a SA layer equivalent to 64 % of a close-packed SA monolayer. The SPB was further functionalized with bIgG and finally E-cad/Fc by sequential rinsings and additions. "At roughly 80 ng/cm^2 hydrated mass of E-cad/Fc the surface density of the cadherin is on the order of 1000 molecules per μm^2 assuming an average hydrodynamically coupled mass of membrane bound proteins. This surface concentration is slightly higher than the baseline level of E-cadherin expression for non-interacting cells (~100-1000 molecules/μm^2 for L929 cells) [8], and thus at a reasonable level to mimic the cadherin concentration of the environment of an adhering cell.

The adsorption of SA on a SPB on PDMS and on SiO_2 was monitored by QCM-D. The QCM-D curves after SA injection showed a different response in the frequency and dissipation on PDMS compared to on SiO_2, indicating the different viscoelastic behavior of the SA layers in the two preparations (representative curves are shown in Figure S1). The frequency changes indicated that more SA adsorbed on SPBs on SiO_2 (540 ± 12 ng/cm^2) than on SPBs on PDMS (463 ± 28 ng/cm^2) for the same conditions (Figure S2a). On SiO_2, the dissipation of SA adsorbed on 5 % bDOPE/DOPC SPB increased and then decreased slightly, while the frequency initially decreased quickly and then continued to a slower, uniform decrease (Figure S2b). This is indicative of the initial formation of a dissipative non-rigid protein arrangement, followed by the formation of a more rigid layer [9]. On PDMS, the dissipation curve increased monotonically and saturated together with the frequency. Johannsmann *et al.* recently demonstrated that similar peaks in the dissipation for adsorbed proteins could be explained by a soft linker to the adsorbed protein in conjunction with hydrodynamic interactions which decreases as the proteins pack more densely [10]. These features are present for SA binding to a lipid bilayer. Our results thus indicate that SA packed more densely on an SPB on SiO_2 than on PDMS and likely formed a close-packed, at least partially jammed layer on SiO_2.

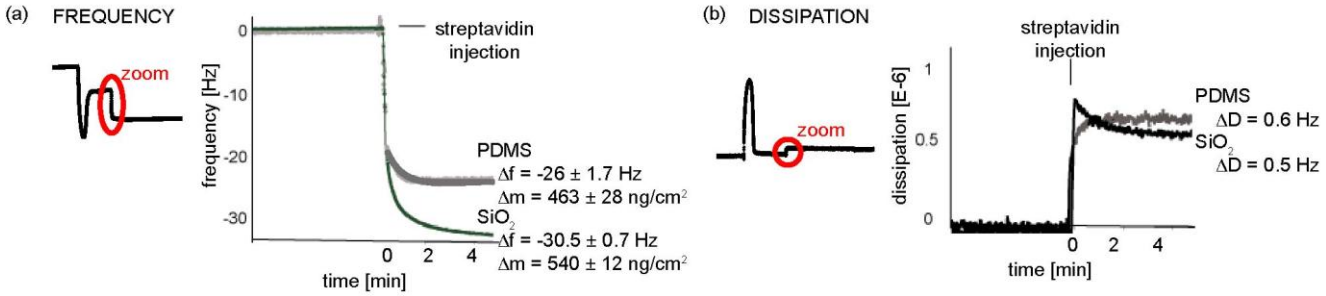

Figure S2. Frequency and dissipation changes after streptavidin injection on a 5% bDOPE/DOPC SPB on PDMS and SiO_2. (a) The frequency change in the QCM-D measurement indicates that more streptavidin adsorbed on the SiO_2 which would result in a more close-packed, immobile streptavidin layer. (b) The QCM-D measurements show a peak in dissipation for the SiO_2 surface after streptavidin injection in contrast to for PDMS indicative of more steric hindrance in a denser packed layer on SiO_2 [10]. The mean and SEM were calculated from a minimum of 3 independent measurements.

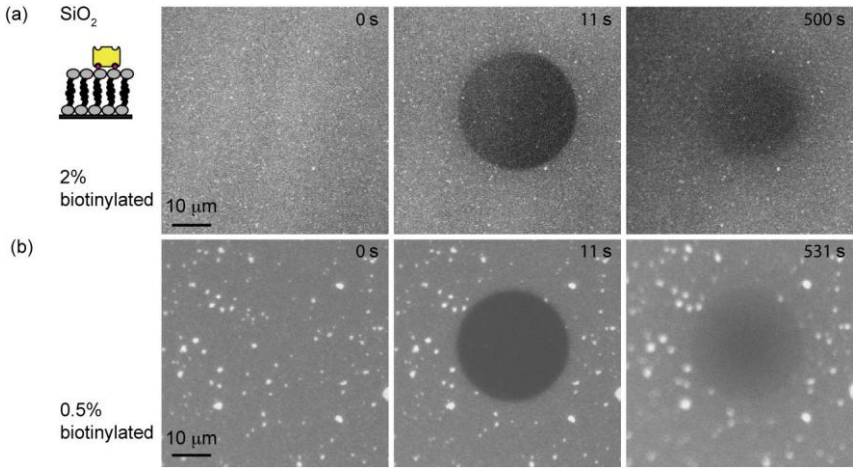

Figure S3 ***Influence of percentage of bDOPE on mobility of SA layer*****.** FRAP was performed to determine the lateral mobility of a SA layer on top of a bDOPE/DOPC SPB. (a). The SA layer on 2% bDOPE/DOPC was only able to recover some of the bleached fluorescence signal through lateral streptavidin diffusion by very slow lateral diffusion. (b) SA on 0.5% bDOPE/DOPC showed a higher and faster recovery of the fluorescence signal, indicating that the SA was laterally mobile.

Roughness values of PDMS and SiO_2

Table S2 ***Roughness values of PDMS and SiO_2*** Roughness parameters for different surfaces determined by AFM. The oxidized substrates were treated with air plasma for 30 s.

Roughness	PDMS master	PDMS wells - oxidized	PDMS spin coated – oxidized	SiO_2
R_{max}	3.8	6.8	2.5	4.9
R_{min}	- 5.9	-2.0	-4.3	-3.2
R_{rms}	0.9	0.63	1.2	0.73
Skewness	0.06	-0.26	0.42	-0.25
Kurtosis	0.9	0.86	0.27	0.89

The mobility of SA on PDMS could be influenced by parameters such as surface roughness [11]. The roughness of the PDMS surface might be critical for larger diffusing species like SA by, e.g., either disrupting formation of ordered domains or causing pinning. The roughness values of the PDMS master, the PDMS microwell structure after plasma treatment, the spin coated PDMS after plasma treatment, and glass slides coated with SiO_2 were all measured by AFM (Table S2), but neither qualitative nor quantitative inspection of the micrographs explained the difference seen in the lateral mobility of the SA. The surface roughness is similar by most measures, and it is even higher on SiO_2 than on PDMS. Roughness unlikely explains the differences in SA adsorption behavior since one would expect greater SA mobility of SA on SiO_2 than on PDMS in that case.

Influence of temperature on mobility of MPPC SPB

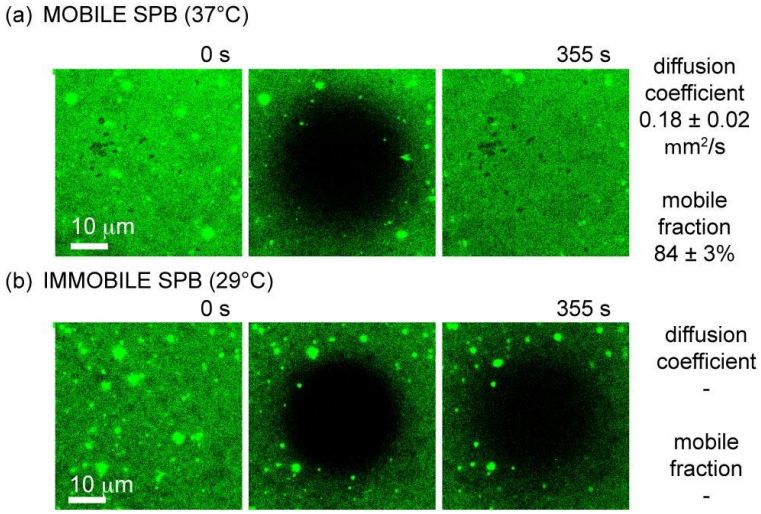

Figure S4 ***Influence of temperature on mobility of MPPC SPB.*** (a). The FRAP experiment at 37°C shows a recovery of the fluorescence signal of the SPB demonstrating full mobility of the SPB. (b) At 29°C, no fluorescence recovery of the bleached spot in the SPB could be detected, demonstrating no mobility of lipids and attached ligands.

Cell viability on E-cadherin functionalized SPB

A potential problem using SPBs for cell culture platforms is that cell-adhesive ligands linked to a viscous SPB might not resist the nanonewton forces generated by the cell, thus limiting cell spreading [12]. To study whether the SPBs functionalized with E-cad/Fc can support cell adhesion and growth, a cell live/dead assay was performed. Cells were cultured for 24 h on an E-cad/Fc functionalized on a planar SPB. Afterwards, the cells were stained with calcein AM and ethidium homodimer to determine if the cells were still viable (fig. S5). Cell survival was not diminished by the mobility of the ligand presentation. Therefore, we can assume that SPBs functionalized with cell-adhesive ligands can be used as model systems without reducing cell survival.

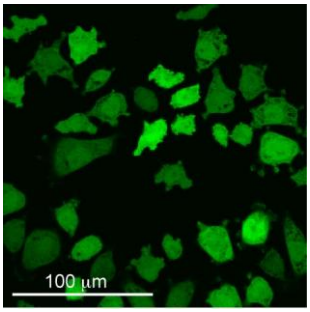

Figure S5 ***Cell survival on E-cad/Fcd functionalized SPB.*** Live/dead staining was performed for CHO cells cultured on an E-cad/Fc functionalized SPB for 24 hours. The calcein AM (green) cells are the living cells.

References

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