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1 Thin phosphatidylcholine films as background
2 surfaces with further possibilities of
3 functionalization for biomedical applications.

4

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13

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16

1

2 ABSTRACT Non-specific adsorption is a crucial problem in the biomedical field. To
3 produce surfaces avoiding this phenomenon, we functionalized thin (7 - 180 nm)
4 poly(methylhydrosiloxane) (PMHS) network films at room temperature ($\approx 20^{\circ}\text{C}$) with
5 phospholipids (PL) bearing a phosphorylcholine head. Regardless of their mode of
6 preparation (casting or immersion), all surfaces appeared to be very hydrophilic with a captive
7 air-bubble contact angle stabilized around 40° . The thin films were protein-repellent in
8 phosphate saline buffer pH 7.4 according to analysis by normal scanning confocal
9 fluorescence. Neither was any adsorption or spreading of L- α -Phosphatidylcholine liposomes
10 on such films observed. In addition, amino functional groups could be easily attached to the
11 surface remaining available for further functionalization.

12

13 Keywords: biocompatibility; phosphorylcholine; phosphatidylcholine; biomaterials; PMHS;
14 anti-fouling

15

16

1

2 **1. Introduction**

3

4 The efficient functioning of sensors for biomedical analysis is dependent on the
5 integrity and accessibility of the surface-fixed molecule designed for molecular recognition,
6 on the one hand, and on the surroundings designed for neutral behavior, especially to avoid
7 nonspecific adsorption, on the other hand. Thus blocking buffers are proposed in diagnostic
8 kits with for instance albumin[1, 2]. In addition, fabrication of polymer films on solid
9 supports with defined structures and properties such as uniformity, stability, and
10 reproducibility is crucial in the development of thin-film chemical sensors. It is still difficult
11 to fabricate ultrathin polymer films that are uniform, continuously defect free, and stable.
12 Moreover, controlled growth of stable polymer films at the nanoscale level, which is
13 important in many applications, remains a challenge. Some control of film thickness,
14 uniformity, stability is needed to achieve reproducibility[3].

15 A crucial aspect in the design of biomaterials surfaces is to achieve control also over
16 the interfacial interactions between the synthetic material/device and the biological medium[4,
17 5]. Protein-resistant ("non-fouling") surfaces are particularly important in the context of
18 blood-contacting biomedical devices, and as non-interactive background for bio-diagnostic
19 surfaces. There is a very limited number of effective non-fouling surfaces available to meet
20 the challenges of practical applications [6]. Poly(ethylene glycol) (PEG) and oligo(ethylene
21 glycol) (OEG) have been widely used to resist nonspecific protein adsorption [7, 8] owing to
22 their ability to form a hydration layer via hydrogen bonds but the strength of these bonds
23 decreases as the temperature increases[9]. Moreover, PEG or OEG can decompose in the
24 presence of oxygen and transition metal ions[10]. Recently zwitterionic (sulfobetaine;
25 carboxybetaine) materials have been found to exhibit ultralow protein adsorption [11, 12]

1 (fibrinogen adsorption $< 5 \text{ ng cm}^{-2}$). This is due to the zwitterion structure which retains a
2 large amount of tenaciously held water independent of temperature because of hydration via
3 ionic solvation[13]. The interface is expected to be electrically neutral over a wide pH range
4 (5-9) with phosphate and sulfonate zwitterions while variations can be exploited with carboxy
5 groups. Coated surfaces with zwitterionic phospholipids have been shown to confer high
6 resistance to protein adsorption[14]. The mechanism of protein adsorption resistivity on the
7 phosphorylcholine modified surface is thought to be based on the interaction between water
8 and phosphorylcholine groups. It is reported that when the water fraction on the polymer
9 surface is maintained at a higher level, the proteins can contact the surface reversibly without
10 significant conformational changes[15]. The large amount of water around the
11 phosphorylcholine groups is thought to repel proteins and even prevent conformational
12 changes of the few adsorbed proteins[16-18].

13 Recently a zwitterionic trimethoxysilane was synthesized for direct grafting on
14 oxidized silicon wafers[19]. In the present work we applied the strategy of surface anchoring
15 a reactive poly(methylhydrosiloxane) (PMHS) network, as in the development of a sensor for
16 nitroaromatics[20], followed by covalent grafting of phospholipids bearing the zwitterionic
17 phosphorylcholine head on the polymer active sites (SiH) via the hydrosilylation reaction
18 (Fig. 1a). X-ray photoelectron analysis was used to follow the reaction. The captive air bubble
19 contact angle in water was measured to characterize the samples surface hydrophilicity.

20 We considered especially thin PMHS films (10-200 nm), extending the previous study
21 on films of thickness in the micrometer range[21], with the aim of creating low roughness
22 surfaces not exhibiting the grooves shown in that previous work but conserving their protein-
23 repellent character. Protein adsorption was analyzed by the normal scanning confocal
24 fluorescence technique.

25

1 2. Materials and Methods

2

3 2.1 Chemicals

4

5 Both the precursors methyldiethoxysilane $\text{HSi}(\text{CH}_3)(\text{OCH}_2\text{CH}_3)_2$ (DH) and
6 triethoxysilane $\text{HSi}(\text{OCH}_2\text{CH}_3)_3$ (TH) were purchased from ABCR (Karlsruhe, Germany) and
7 used as received. Water for substrate cleaning was obtained from a Milli-Q water purification
8 apparatus (Millipore). Absolute ethanol for sol-gel synthesis was of synthesis grade purity.
9 Trifluoromethanesulfonic acid $\text{CF}_3\text{SO}_3\text{H}$ was purchased from Aldrich. Toluene was distilled
10 before use for thin film hydrosilylation. The platinum-divinyltetramethyldisiloxane complex
11 in xylene (PC072) (platinum concentration of about 0.1 M assuming 2.4% (w) Pt in xylene),
12 also known as Karstedt's catalyst, was purchased from ABCR. 1,2-dilinoleoyl-sn-glycero-3-
13 phosphorylcholine (18:2 Cis) (PL) was purchased from AvantiPolarLipids. The molecule
14 bears the zwitterionic phosphorylcholine head and two unsaturated cis carbon-carbon bonds
15 on each of the two fatty chains. 3-(ethoxydimethylsilyl)-propyl amine (Aldrich, 588857) and
16 hexamethyldisilazane were used as received.

17

18 2.2 Proteins and labeling

19

20 Bovine serum albumin (A-7638), cytochrome C (C-2506), α -chymotrypsin (C-4129),
21 avidin (A9275) were purchased from Sigma-Aldrich; some avidin from Fluka (No. 11368).
22 Given their isoelectric point, at physiological pH 7.4, BSA ($67\,000\text{ g mol}^{-1}$, pI 5.6) is
23 negative, α -chymotrypsin ($25\,300\text{ g mol}^{-1}$, pI 8.1) slightly positive, avidin ($66\,000\text{ g mol}^{-1}$, pI
24 10) and cytochrome C ($12\,400\text{ g mol}^{-1}$, pI 10.2) strongly positive.

1 Labeling of proteins was performed with Alexa-fluor-594 succinimidyl ester (InvitroGen,
2 A30008). Typically 500 μL of protein solution were added to dry fluorophore Alexa-594 in
3 molar ratio 1:1 and allowed to react for 0.5 h at 20°C. Avidin was also labelled via previous
4 biotin-ethylenediamine hydrobromide (Sigma B9181) reaction with alexa succinimidyl ester
5 as described elsewhere [22]. The mixture was then put in an *ad hoc* microtube with filter
6 (Biospin P6) and centrifuged at 16 000 g for 1 min according to the supplier of the kit
7 (Labelling kit A10239, InvitroGen). The labeling ratio [Alexa] / [protein] (0.3-0.5) was
8 determined from the UV absorbances at 280 nm where both the label ($\epsilon_{280-\text{Alexa}} = 50\,400\text{ M}^{-1}$
9 cm^{-1}) and protein absorb and at 590 nm where only the label ($\epsilon_{590} = 90\,000\text{ M}^{-1}\text{ cm}^{-1}$)[23]
10 absorbs. Protein solutions were prepared in 10mM sodium phosphate buffer pH 7.4 with
11 0.15M NaCl in de-ionized water (MilliQ system, Millipore) and stored at 4°C.

12

13 *2.3 Liposome preparation and labeling*

14

15 One hundred milligrams of L- α -Phosphatidylcholine (Soy-20%) (Avanti Polar Lipids
16 Inc. 541601) was dissolved in 5ml of chloroform containing 100 μL of a 1,1'-dioctadecyl-
17 3,3,3',3'-tetramethylindodicarbocyanine perchlorate solution (Invitrogen Inc.) (1mg/ml in
18 methanol). After chloroform had been thoroughly removed under vacuum, phosphate buffered
19 saline (PBS, 10mM, pH 8.1, 150mM NaCl) was added and mixed in. Aggregated particles
20 were then sonicated until complete dissolution. The mixture was then taken through a freeze-
21 thaw procedure five times. This procedure involved freezing the solution by immersion in
22 liquid nitrogen, followed by thawing by immersion in 60 °C water. After the freeze-thaw
23 process, the vesicle suspension was divided into 10 samples of 500 μL , frozen once again by
24 immersion in liquid nitrogen and stored at -80°C. Before use, the vesicle suspension was
25 heated by immersion in 60°C water and regularized by extrusion through polycarbonate

1 membrane filters (Avestin Inc.) of pore diameter 400 nm mounted in a mini-extruder (Avestin
2 Inc.). We subjected the samples to 11 passes through the membrane.

3 The same procedure without the label DiD was applied to obtain unlabeled liposomes
4 whose size distribution was measured by photon cross-correlation spectroscopy (Nanophox,
5 Sympatec - France).

6

7 *2.4 Methods of preparation of the support*

8

9 *2.4.1 Substrate cleaning and activation*

10

11 Silicon wafers Si(100) (ACM, France) square strips of $2 \times 2 \text{ cm}^2$ were used as
12 substrates for spin-coating deposition. To bond covalently the PMHS thin films to native
13 oxide silica (thickness ca 2 nm) and to glass surfaces, they were cleaned and activated with
14 oxygen plasma for 60 seconds.

15

16 *2.4.2 PMHS film*

17

18 PMHS thin films were prepared at $22 \pm 1^\circ\text{C}$ by sol-gel polymerisation of DH and TH
19 as crosslinker, as described elsewhere[24]. This procedure gave layers of reproducible
20 homogeneity and thickness as controlled by ellipsometry (Plasmos SD 2300 ellipsometer
21 (München Germany)) [25]. We used refractive index $n = 1.4$ for PMHS.

22

23 *2.4.3 Grafting PMHS with phospholipids*

24

25 *a-by casting*: the phospholipid solution was prepared under argon. 20 mg of PL were
26 dissolved in 8 mL of dry toluene (2.5 mg / mL), with additional 7 μL of the Karstedt catalyst

1 (xylene solution of platinum divinyltetramethyldisiloxane complex). The molar ratio of
2 phospholipid over hydrogenosilane group SiH was 1.5. PMHS coated silicon wafers (2×2
3 cm^2) and glass surfaces (1.5 cm^2) were covered by 200 μL and 80 μL of phospholipid solution
4 respectively. The samples were kept for one hour under a cap to slowly evaporate the solvent
5 and then left in open air for quick evaporation.

6 *b-by immersion*: silicon wafers were immersed in the phospholipid solution (2.5 mg/mL)
7 for one hour.

8 For both methods, the samples were then rinsed successively in different
9 toluene/chloroform mixtures from 100/0 to 0/100 (% vol.) with a gap of 20% and dried under
10 a stream of Argon. We will refer to the PMHS layer having reacted with PL as PL-PMHS(h
11 nm) where h is the PMHS initial thickness measured by ellipsometry.

12

13 2.5 Techniques of characterization of the support

14

15 2.5.1 X-ray photoelectron spectroscopy

16

17 The surface elemental compositions of unmodified and modified PMHS surfaces were
18 analysed by X-ray photoelectron spectroscopy (XPS). The spectrophotometer (ESCALAB
19 250, Thermo Electron, UK) was equipped with a monochromatic Al K α (1486.6 eV) radiation
20 source. The acceleration tension and power of X-ray source were 15 kV and 100 W,
21 respectively. The samples were analysed at a pressure in the 10^{-9} mbar range. The electron
22 take-off angle with respect to the sample surface was 90° . The analysed spot size was
23 approximately $400 \mu\text{m}^2$. The XPS composition corresponds to depths of 5-10 nm. Survey
24 scans (0-1350 eV) at low resolution were performed to identify constitutive elements. High
25 resolution C $_{1s}$, Si $_{2p}$, O $_{1s}$, N $_{1s}$ and P $_{2p}$ spectra were recorded to obtain more detailed information

1 about the nature of the surface. The peaks were fitted with Gauss–Lorentz curves. They
2 provided the various surface atomic ratios from the corresponding peak areas, by assuming
3 the total area corresponded to 100%, after correction with the theoretical sensitivity factors
4 [26]. The spectra of both pristine PMHS and PL-PMHS were calibrated using the
5 hydrocarbon contaminant or alkane C_{1s} peak set at 284.8 eV. In both cases, the peak of Si-
6 CH₃ was found at a binding energy (BE) of 284.0 eV in agreement with PDMS polymer at
7 284.4 eV [27].

8

9 *2.5.2 Captive air bubble contact angle in water*

10

11 The samples were characterized by captive air-bubble (7.6 μL) contact angle (GBX -
12 Digidrop, Romans, France). The contact angle was calculated after 1 min using computerized
13 image analysis. The data are relative to the angle through the liquid as generally defined[28],
14 in opposition to its complement angle to 180° through air as used in a previous work[21]

15

16 *2.5.3 AFM*

17

18 Atomic Force Microscopy (AFM) experiments were performed using a Dimension
19 3100 microscope equipped with a Nanoscope IIIa controller system (Digital Instruments,
20 Veeco Metrology Group). AFM images were obtained by scanning in tapping mode in water
21 or under ambient conditions in air using silicon SPM probes (stiffness $k \approx 2$ N/m, resonance
22 frequency of 67 kHz, pointprobeplus, Nanosensors). The root mean square average
23 roughness (R_q) was analyzed with the Nanoscope software (version 5.31r1).

24

25 *2.6 Adsorption: Flow cell and Determination of interfacial concentration*

1

2 The experiments were performed at $T = 19^\circ\text{C}$ in a slit flow cell of thickness $\approx 100 \mu\text{m}$
3 and flow rate corresponding to wall shear rate 1000 s^{-1} . Confocal measurements[29, 30] with
4 inverted microscope configuration were performed at 3 cm from the slit entrance. Interfacial
5 concentration was evaluated as follows: the fluorescence signal F_{sol} from solution at
6 concentration C is relative to an effective volume V while the signal F_{surf} from surface
7 concerns interfacial concentration Γ over area A . $F_{\text{surf}} \propto \Gamma A$ and $F_{\text{sol}} \propto C V$ therefore $\Gamma =$
8 $(V/A) (F_{\text{surf}}/F_{\text{sol}}) C$. In a previous paper[29] the order of magnitude of V/A was estimated from
9 the focus radius for A and $1 \mu\text{m}^3$ taken as the confocal volume V . A more precise
10 determination is obtained considering the adsorbed layer as a Dirac function of
11 fluorescence[21]. Then $V/A = w_D$ is the area under the normalized fluorescence peak $f_i(y) =$
12 $F_i(y) / F_i(0)$ resulting from the convolution of the fluorescent interface positioned at $y = 0$ with
13 the laser beam.

14

15 3. Results and Discussion

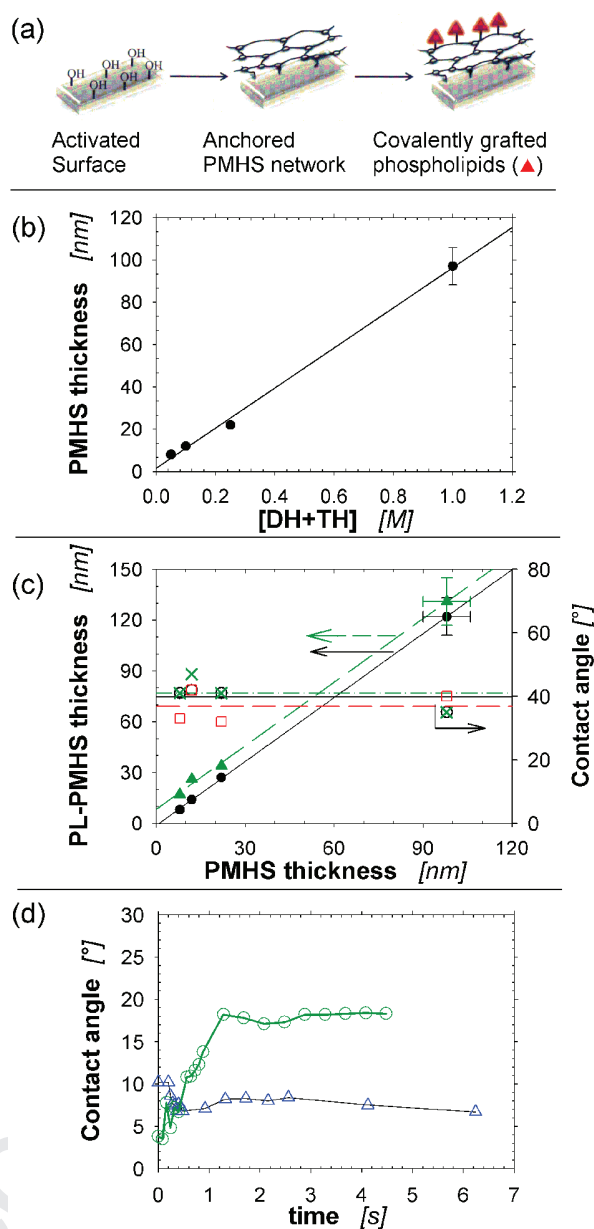
16

17 3.1 Physico-chemical characterization of PMHS and PL-PMHS films

18

19 The ellipsometric thickness of PMHS film on oxidized wafers as a function of initial
20 concentration of monomers (DH and TH) is provided in Fig. 1b. The variation was linear with
21 a slope of $95 \pm 4 \text{ nm M}^{-1}$. After hydrosilylation reaction had proceeded over one hour by
22 means of casting or immersion the thickness of the PL-PMHS layer was measured and the
23 hydrophilicity characterized by captive air bubble contact angle (Fig. 1c). Both techniques
24 gave the same results suggesting the occurrence of a quick interfacial reaction over less than
25 one hour and efficient removal of excess phospholipids in the casting method. An average

1 angle of 40° was observed whatever the initial PMHS thickness, much below the value of 90°
2 observed with hydrophobic PMHS. The ellipsometric thickness increased as expected after
3 reaction of the PMHS layer with phospholipids. Conditioning the samples overnight in Milli-
4 Q water gave rise to an additional increase of thickness. Over the range examined we can
5 attribute a mean 26% increase of thickness from the reaction with phospholipids and an 8 nm
6 increase from conditioning the material in water. Conditioning the PL-PMHS for several days
7 in water led to rolling bubble (0°) possibly stabilized at a slightly higher contact angle (Fig.
8 1d).
9



1

2 **Figure 1** Method of surface treatment and physico-chemical characteristics of the films. (a)

3 Illustration of surface silanols of oxidized silicon wafer as anchoring points of PMHS network

4 for subsequent phospholipid grafting. (b) Thickness of PMHS film on oxidized silicon wafers

5 as a function of initial concentration of monomers. Slope $95 \pm 4 \text{ nm M}^{-1}$. Dispersion of data at

6 low concentration is of the order of the symbol size. (c left scale) PL-PMHS (reaction with PL

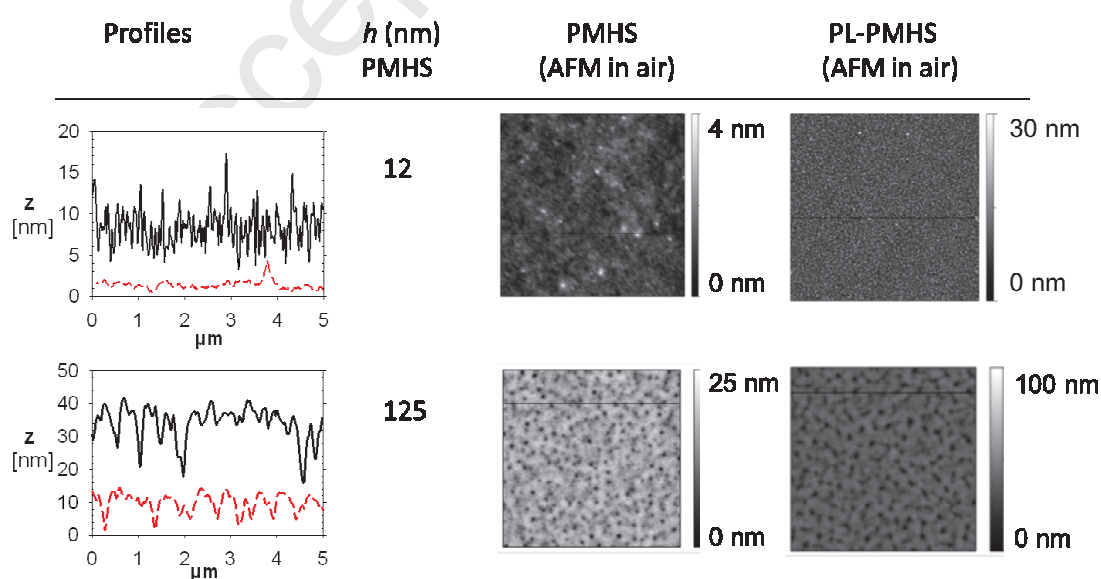
7 solution 2.5 mg/mL) thickness after toluene/ chloroform rinsing (●; full line, slope $1.26 \pm$ 8 0.01) and additional overnight immersion in water (▲; dashed line, slope 1.25 ± 0.03 ,

1 ordinate at the origin 8 ± 2 nm) as a function of original PMHS thickness. (*c right scale*)
 2 Captive air bubble contact angle on PL-PMHS, created via casting (o) or immersion (x,
 3 wafer; □, glass), as a function of initial PMHS thickness. (*d*) Examples of variation of contact
 4 angle with time (rolling bubble over ~ 1 cm) for samples conditioned for several days in
 5 water.

6

7 The structures of the PMHS and PL-PMHS films were examined by AFM (Fig. 2).
 8 PMHS roughness was lower than 1 nm for a very thin layer (12-20 nm) while structures
 9 appeared for 125nm. Reaction of PL induced always an increase in roughness (Table 1). The
 10 12 nm thin PMHS sample showed a roughness R_q of 0.4 nm with a few defects. PL grafting
 11 induced an increase of roughness to 2.03 nm as measured in air. Immersion in water
 12 decreased the roughness to 1.28 nm. This may be partly due to the reorganisation of
 13 phospholipids chains in the presence of an aqueous medium in order to minimise the free
 14 energy of the system. The minimum 0.9 nm for the PL-PMHS roughness was found for *ca.* 20
 15 nm thick PMHS.

16



17

1

2 **Figure 2** Characterization of the films by atomic force microscopy. AFM pictures ($5\ \mu\text{m} \times 5$
3 μm) of PMHS layers (thickness h) and of their derived PL-PMHS layers. Corresponding
4 profile samples of PMHS (dashed line) and PL-PMHS (full line).

5

h (PMHS)	R_q (PMHS)	R_q (PL-PMHS)
(nm)	(nm)	(nm)
12	0.40	2.03
20	0.60	0.90
125	2.5	5.6

6 **Table 1** Roughness R_q over $10\ \mu\text{m} \times 10\ \mu\text{m}$ samples of PMHS and derived PL-PMHS for
7 different initial PMHS thickness h .

8

9 3.2 Chemical characterization: XPS analysis of the interface

10

11 The surface elemental compositions of pristine PMHS and PL-PMHS were determined
12 from high resolution XPS spectra. To summarize the characterization of PL-PMHS layers,
13 they are very hydrophilic due to the fast reaction of PL at the top of the layer. There was
14 however competition with the side reaction of SiH with traces of water giving SiOH groups
15 which may recombine to produce siloxane bridges. The formation of new SiOH or SiOSi
16 bonds in the PL-PMHS layer was shown by the evolution of the two distinct doublets ($\text{Si}_{2p^{3/2}}$)
17 of the Si_{2p} PMHS spectrum by reaction with PL at binding energies 101.2 (21% \rightarrow 86%) and
18 102.3 eV (79% \rightarrow 14%) with small shifts in energy. We will see below how the density of
19 available SiOH can be determined and used for functionalization.

20

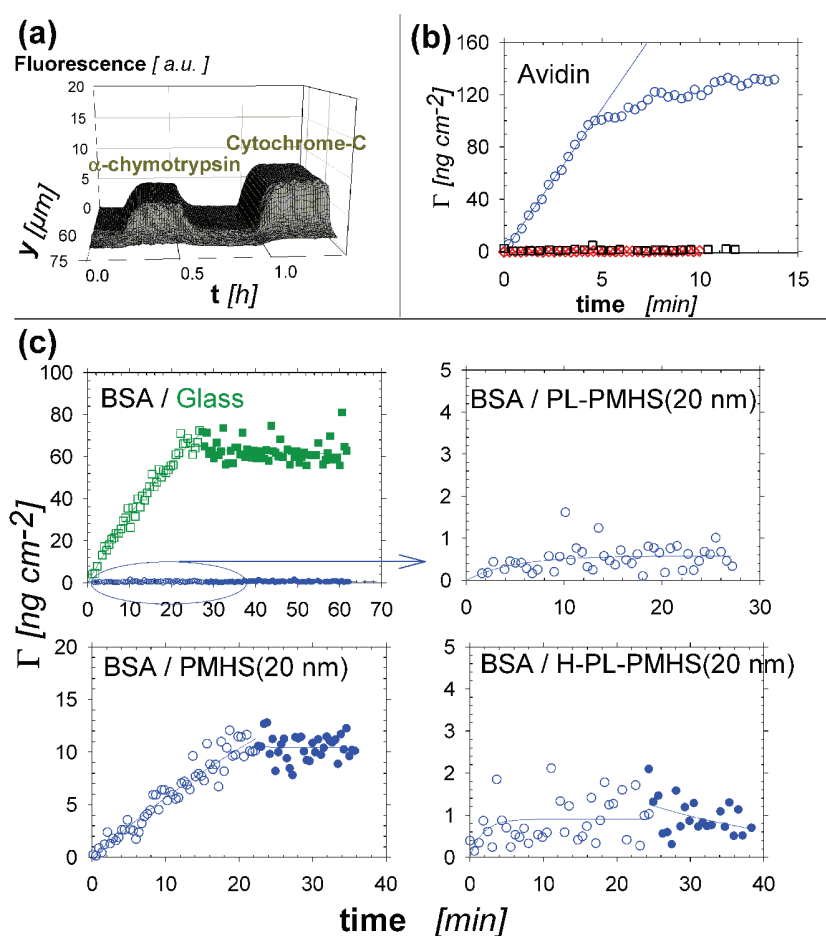
1 *3.3 Protein adsorption*

2

3 The wettability behaviour of the PL-PMHS surfaces (Fig. 1c-d) was indicative of a
4 probable protein-repellent character. We investigated protein adsorption from single protein
5 solution in PBS buffer pH 7.4 (avidin, bovine serum albumin (BSA), α -chymotrypsin and
6 cytochrome-C). Avidin is a model of a very stable hard protein. As most surfaces are
7 negative, we considered preferably positive proteins at neutral pH, to check the surface
8 passivation with respect to protein adsorption. It should be borne in mind however that the
9 ionic strength of PBS buffer is not small with 0.15 M NaCl. Conversely the negative BSA can
10 be viewed as a soft protein model, able to be adsorbed and denatured on many materials
11 whatever their charge, thus a good candidate to check for possible hydrophobic interactions
12 with surfaces. Indeed, this protein is often used in blocking buffers to prevent subsequent
13 nonspecific adsorption.

14 The protein-repellent character of the PL-PMHS(20 nm) surface was checked in the
15 configuration of flowing solutions in a slit. No adsorption of α -chymotrypsin and
16 cytochrome-C was observed (Fig. 3a).

17



1

2 **Figure 3** Adsorption under laminar flow conditions with wall shear rate $\gamma = 1000 \text{ s}^{-1}$. Closed

3 symbols for the buffer rinsing step. (a) 3D graphs of successive flows of α -chymotrypsin and

4 cytochrome C solutions (150 nM) with intermediate flow of PBS buffer showing no

5 adsorption on the wall of PL-PMHS covered oxidized silicon wafer (foreground, $y \approx 67 \text{ }\mu\text{m}$).

6 (b) avidin on (o ●) sulfochromic acid treated glass, $C_b = 5.0 \text{ }\mu\text{g/mL}$; (\square) PL-PMHS(7 nm), C_b

7 $= 10 \mu\text{g/mL}$, $\Gamma = 1.2 \pm 0.9 \text{ (SD) ng cm}^{-2}$; (\diamond) PL-PMHS(180 nm), $C_b = 5.0 \text{ }\mu\text{g/mL}$, $\Gamma = 0.1$

8 $\pm 0.9 \text{ ng cm}^{-2}$; (c) BSA adsorption kinetics on different surfaces, $C_b = 10 \text{ }\mu\text{g/mL}$: (top left) (\square)

9 sulfochromic acid treated glass, (o) PL-PMHS(20 nm), shown at a different scale on (top

10 right) $\Gamma = 0.5 \pm 0.3 \text{ ng cm}^{-2}$; (bottom left) PMHS(20 nm); (bottom right) PL-PMHS(20 nm)

11 treated with HMDS.

12

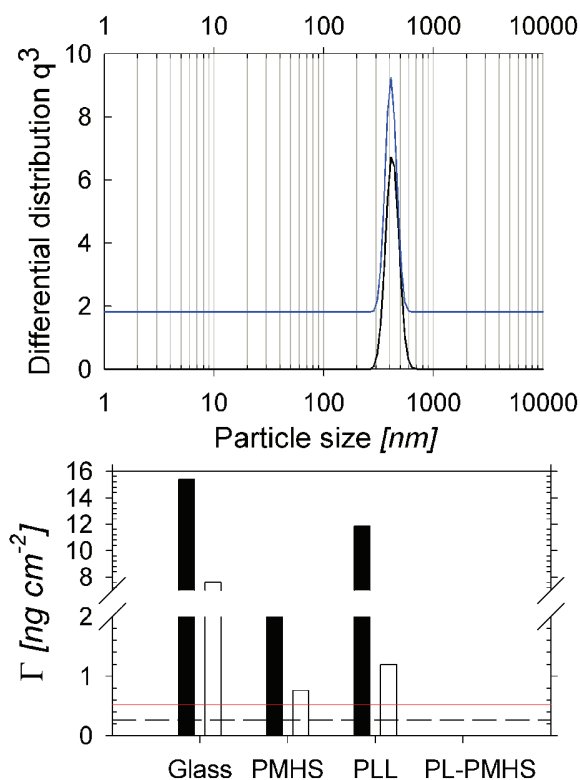
1 The adsorption of avidin was examined on different PL-PMHS substrates derived
 2 from the PMHS initial thicknesses of 7 nm and 180 nm (Fig. 3b). The interfacial
 3 concentration was 1.2 and 0.1 ng cm⁻² respectively, with dispersion around 1 ng cm⁻². Let us
 4 recall that there was a slight displacement in the plane of substrate between the normal
 5 scanings to avoid repetitive exposure to the laser beam which could induce a photobleaching
 6 effect. This procedure constituted then also an examination of the interface homogeneity.
 7 Conversely, adsorption on sulfochromic acid treated glass was large with an initial linear
 8 variation up to *ca.* 0.1 µg cm⁻². The initial kinetic constant *k* was 7.4 × 10⁻⁵ cm s⁻¹. With *D* ≈ 6
 9 10⁻⁷ cm² s⁻¹, the transport limited constant *k_{Lev}* is 2.65 10⁻⁴ cm s⁻¹. Applying the expression
 10 [31, 32] $k_a = k (b_1 u + 1) (b_2 u + 1) / ((u-1) (a_1 u-1))$, where $u = k/k_{Lev}$ and numerical coefficients
 11 $a_1 = 0.556$, $b_1 = -0.681$, $b_2 = -0.0484$, led to $k_a \approx 1.0 \times 10^{-4}$ cm s⁻¹. In any case, the
 12 phospholipid treatment was very efficient in preventing avidin adsorption, even at very small
 13 thickness of PMHS (7 nm), exhibiting the good quality of the coverage and the corresponding
 14 screening of the substrate.

15 The adsorption of Alexa-BSA was studied on PMHS(20 nm), PL-PMHS(20 nm) and
 16 PL-PMHS followed by HMDS capping, denoted H-PL-PMHS(20 nm) (Fig. 3c). All surfaces
 17 were immersed overnight in water and exposed to flowing buffer and solution with
 18 sulfochromic acid treated glass as the other face. Whatever the surface, when adsorption
 19 occurred no rapid desorption was observed by flowing buffer. The PMHS surface exhibited
 20 some adsorption, 10 ng/cm² over 20 min without a plateau ($k = 1.0 \cdot 10^{-6}$ cm s⁻¹). The H-PL-
 21 PMHS showed a very small interfacial concentration ($\Gamma = 0.8 \pm 0.5$ ng/cm²) comparable to
 22 0.5 ± 0.3 ng/cm² observed on PL-PMHS. It might reflect the burying of the trimethyl groups
 23 in the hydrophobic domains of lipid chains avoiding their direct exposure to the aqueous
 24 medium thus preventing hydrophobic interactions with the protein.

1 Our studies showed that phosphorylcholine modified PMHS layers experienced almost
 2 “zero” protein adsorption. The decrease due to phosphorylcholine groups agrees also with
 3 previous studies to improve the biocompatibility of poly(dimethylsiloxane) (PDMS)[33]. The
 4 polymer of 2-methacryloyloxyethyl phosphorylcholine (MPC) was grafted by surface-
 5 initiated photo-induced radical polymerization. The *in vitro* single protein adsorption on the
 6 poly(MPC)-grafted PDMS decreased 50-75% compared to the unmodified PDMS. On
 7 poly(ether-ether-ketone) (PEEK) treated with MPC, BSA adsorption studies[34] showed a
 8 significant effect of MPC till 90% adsorption reduction with respect to PEEK.

9 Adsorption of labeled (DiD) liposomes of L- α -Phosphatidylcholine (diameter 400 nm)
 10 was measured by scanning confocal fluorescence as for the proteins. Based on an average area
 11 per head of 0.5 nm² or diameter per head of 0.8 nm, the adsorption at different interfaces is
 12 shown in Fig. 4.

13



14

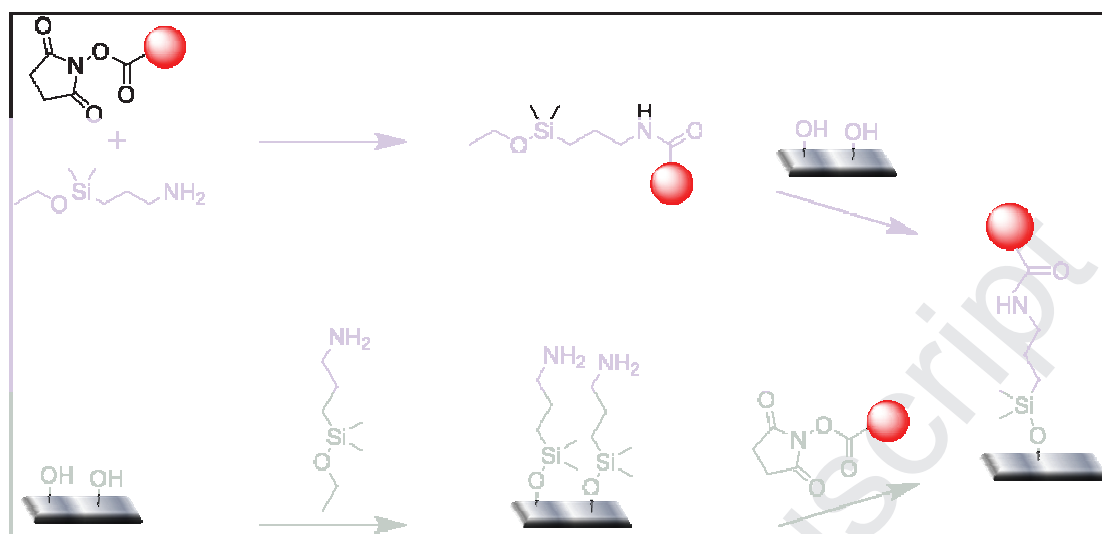
1 **Figure 4** Characterization of liposomes and of their interaction with surfaces. (top) Photon
2 cross-correlation spectroscopy of unlabeled L- α -Phosphatidylcholine liposomes showing the
3 reproducibility of the size 400 nm in the preparation. (bottom) Adsorption-spreading of
4 labelled liposomes on piranha treated glass, PMHS, poly(L-Lysine) (PLL) and PL-PMHS,
5 after one (full bar) and a few rinsings (empty bar). Horizontal lines correspond to estimated
6 monolayer (dashed line) and bilayer coverages (full line).

7
8 It can be seen that PL-PMHS constitutes a repellent surface with respect to L- α -
9 Phosphatidylcholine liposomes, despite this lipid being not just a pure simple lipid but a
10 mixture of lipids (24% PC, 18% Phosphatidylethanolamine, 11.5% Phosphatidylinositol, 4.3
11 % Phosphatidic acid, 4.6% LysoPC and some other unknown lipids).

12 13 *3.4 Functionalization of PL-PMHS Surface*

14
15 The XPS analysis after PMHS hydrosilylation with PL exhibited the side reaction with
16 water giving silanol groups, which may evolve to siloxane bridges too. Such interfacial
17 silanols could be available for subsequent reaction. Their presence was checked by reaction of
18 a monoethoxy silane coupling agent bearing alexa as fluorescent probe (Scheme 1). Indeed,
19 we observed a high fluorescence signal thus confirming the possibilities of chemical
20 functionalization at such surfaces having an inert background of C18-phospholipids bearing a
21 phosphorylcholine head. No coupling reaction occurred after pretreatment of PL-PMHS with
22 hexamethyldisilazane (HMDS) as the silanols were neutralized by bulky trimethylsilyl
23 groups.

24



1

2 **Scheme 1** Grafting reaction of Alexa, sketched as a big sphere, to surface silanols. (top) via
 3 previous formation of amide link between alexa and monoethoxy silane coupling agent and
 4 (bottom) via previous reaction of surface silanols with aminosilane.

5

6 PMHS did not exhibit any coupling reaction with alexa monoethoxy silane in toluene /
 7 ethanol (70/30), thus suggesting the relative stability of the interfacial hydrogenosilane (SiH)
 8 functional groups in the absence of water and catalyst. Moreover, this demonstrates that the
 9 Karstedt' catalyzed side reaction of SiH with water plays a major role in the formation of
 10 interfacial silanol on such PL-PMHS surface. After coupling the alexa probe, the silanol
 11 concentration can be thus estimated by assuming that the coupling reaction is total. According
 12 to the method developed above for determination of interfacial concentration, the mean
 13 degree of functionalization of the PL-PMHS layer was measured to be $3.0 \times 10^{-2} \text{ nm}^{-2}$.

14 Assuming that all molecules were grafted at the interface the mean distance between the sites
 15 was 5.7 nm. This order of magnitude is acceptable for a quite dense packing of proteins on a
 16 neutral background and can be probably modified by changing the experimental conditions. In
 17 addition, we showed that the silanols of the interface could react also with
 18 aminomonoethoxysilane to provide a phosphorylcholine background surface with amino

1 groups available for amide junctions (Scheme 1). This was carried out with the alexa ester
2 leading to the same interface as before with the same site density.

3

4 **4. Conclusion**

5

6 Polymethylhydrosiloxane anchored on an activated oxidized silicon wafer or glass can
7 be deposited as a very thin film of low roughness and functionalized with phospholipids
8 bearing a phosphorylcholine head. The functionality of the surface was analyzed by XPS and
9 the correlated wettability change determined by contact angle measurements with captive air
10 bubble. The new interface was observed to be very hydrophilic and protein-repellent at
11 neutral pH in phosphate saline buffer. L- α -Phosphatidylcholine liposomes containing different
12 phospholipids could not bind to such interfaces. Thus tailored surfaces can be produced to fix
13 specific cells with appropriate functionalization. As the stiffness is a pertinent parameter in
14 cell spreading[35], one perspective would be to vary the DH/TH ratio in the synthesis of the
15 PMHS films with better control of water in subsequent grafting. We have shown that the
16 silanol groups originating from the side-reaction with traces of water provided possibilities for
17 further functionalization. The prevention of protein adsorption is attributed to the zwitterionic
18 character of the phosphorylcholine head which maintains a water-rich layer at the interface.
19 We found that a 20 nm thick PMHS layer led to very flat surfaces with roughness less than 1
20 nm. We have shown finally that the protein-repellent interface can be easily provided with
21 amine functional groups. Therefore such protein-repellent interfaces can be useful in the
22 biomedical domain, e.g. for biosensors, biomaterials and cell culture, by reducing strongly the
23 non-specific adsorption, on the one hand, and offering in addition possibilities for grafting
24 desired functions, on the other hand. Moreover, the covalent coverage with phospholipids and
25 the sites for further functionalization are generated in a one step process.

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5
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20
21

1 **Captions to illustrations**

2

3 **Figure 1** Method of surface treatment and physico-chemical characteristics of the films. (a)
 4 Illustration of surface silanols of oxidized silicon wafer as anchoring points of PMHS network
 5 for subsequent phospholipid grafting. (b) Thickness of PMHS film on oxidized silicon wafers
 6 as a function of initial concentration of monomers. Slope $95 \pm 4 \text{ nm M}^{-1}$. Dispersion of data at
 7 low concentration is of the order of the symbol size. (c *left scale*) PL-PMHS (reaction with PL
 8 solution 2.5 mg/mL) thickness after toluene/ chloroform rinsing (\bullet ; full line, slope $1.26 \pm$
 9 0.01) and additional overnight immersion in water (\blacktriangle ; dashed line, slope 1.25 ± 0.03 ,
 10 ordinate at the origin $8 \pm 2 \text{ nm}$) as a function of original PMHS thickness. (c *right scale*)
 11 Captive air bubble contact angle on PL-PMHS, created via casting (o) or immersion (\times ,
 12 wafer; \square , glass), as a function of initial PMHS thickness. (d) Examples of variation of contact
 13 angle with time (rolling bubble over $\sim 1 \text{ cm}$) for samples conditioned for several days in
 14 water.

15 **Figure 2** Characterization of the films by atomic force microscopy. AFM pictures ($5 \mu\text{m} \times 5$
 16 μm) of PMHS layers (thickness h) and of their derived PL-PMHS layers. Corresponding
 17 profile samples of PMHS (dashed line) and PL-PMHS (full line).

18

19 **Figure 3** Adsorption in laminar flow conditions with wall shear rate $\gamma = 1000 \text{ s}^{-1}$. Closed
 20 symbols for the buffer rinsing step. (a) 3D graphs of successive flows of α -chymotrypsin and
 21 cytochrome C solutions (150 nM) with intermediate flow of PBS buffer showing no
 22 adsorption on the wall of PL-PMHS covered oxidized silicon wafer (foreground, $y \approx 67 \mu\text{m}$).
 23 (b) avidin on (o \bullet) sulfochromic acid treated glass, $C_b = 5.0 \mu\text{g/mL}$; (\square) PL-PMHS(7 nm), C_b
 24 $= 10 \mu\text{g/mL}$, $\Gamma = 1.2 \pm 0.9 \text{ (SD) ng cm}^{-2}$; (\diamond) PL-PMHS(180 nm), $C_b = 5.0 \mu\text{g/mL}$, $\Gamma = 0.1$
 25 $\pm 0.9 \text{ ng cm}^{-2}$; (c) BSA adsorption kinetics on different surfaces, $C_b = 10 \mu\text{g/mL}$: (top left) (\square)

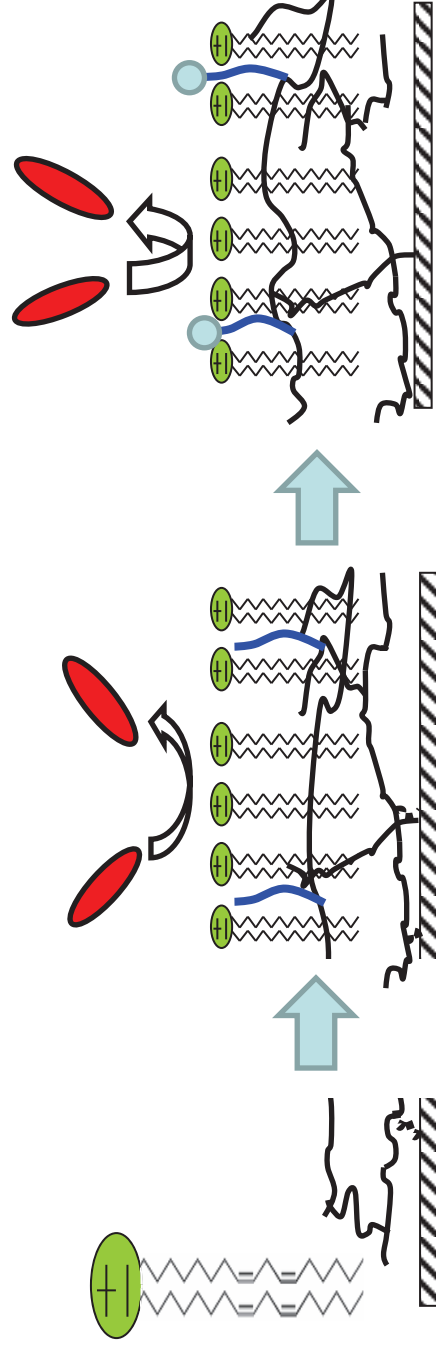
1 sulfochromic acid treated glass, (o) PL-PMHS(20 nm), shown at a different scale on (*top*
2 *right*) $\Gamma = 0.5 \pm 0.3 \text{ ng cm}^{-2}$; (*bottom left*) PMHS(20 nm); (*bottom right*) PL-PMHS(20 nm)
3 treated with HMDS.

4
5 **Figure 4** Characterization of liposomes and of their interaction with surfaces. (top) Photon
6 cross-correlation spectroscopy of unlabeled L- α -Phosphatidylcholine liposomes showing the
7 reproducibility of the size 400 nm in the preparation. (bottom) Adsorption-spreading of
8 labelled liposomes on piranha treated glass, PMHS, poly(L-Lysine) (PLL) and PL-PMHS.
9 After (full bar) one and (empty bar) a few rinsings. Horizontal lines correspond to estimated
10 (dashed line) monolayer and (full line) bilayer coverages.

11
12 **Scheme 1** Grafting reaction of Alexa, sketched as a big sphere, to surface silanols. (top) via
13 previous formation of amide link between alexa and monoethoxy silane coupling agent and
14 (bottom) via previous reaction of surface silanols with aminosilane.

- 15
16 - Simple method to build a thin phospholipid layer covalently linked to a polymer network
17 - From phospholipids, building of a stable background dense layer of phosphorylcholine
18 heads.
19 - Possibilities of additional routine chemistry for fonctionnalization over the
20 phosphorylcholine background.

21



Functionalizable protein-repellent interface