ChemComm

Covalent coupling of an phospholipid monolayer on the surface of ceramic materials

Yikang Wang, Tsueu Ju Su, Rebecca Green, Yiqing Tang, Dmitrii Styrkas, Timothy N. Danks, Roger Bolton and Jian R. Lu*

Department of Chemistry, University of Surrey, Guildford, UK GU2 5XH. E-mail: j.lu@surrey.ac.uk

Received (in Oxford, UK) 13th January 2000, Accepted 28th February 2000

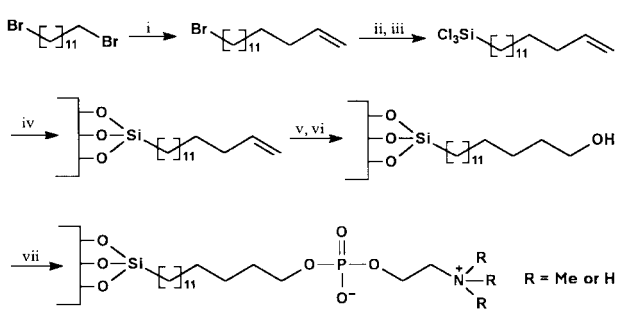
A new synthetic route to the covalent bonding of an organic monolayer bearing terminal phosphorylcholine (PC) groups onto the surface of silicon oxide is reported; such monolayers prohibit the deposition of enzymes and proteins and can be used to improve surface biocompatibility.

Phospholipids are a group of molecules with double acyl chains and zwitterionic head groups. Their existence in membrane walls is primarily responsible for creating a biocompatible environment so that proteins, enzymes and platelets in blood do not foul the surface of blood cells. The dominant phospholipids on the outer surface of membrane walls have phosphorylcholine (PC) head groups. The biocompatibility is therefore rendered by these lipid heads. The role played by these phospholipids has been known for decades and a great deal of endeavour has been made to mimic the natural behaviour of these phospholipids. Most of the activities have focused on creating polymeric materials containing pendent PC groups[1–3] although a great deal of work has explored other types of biocompatible polymers, *e.g.* polyurethane and polymethacrylate grafted with polyethylene oxide.[4] Literature results have shown that PC polymers are superior to other types of polymers in reducing protein deposition and the subsequent biological consequences.[1–5] Surfaces coated with PC polymers also show much better performance against thrombosis than heparin derivatized surfaces.[4]

An alternative approach to the coating of PC polymers is *via* covalent bonding of an organic monolayer bearing terminal PC groups onto a solid substrate. We report here, a new synthetic route to the construction of a PC monolayer onto silicon oxide. Covalent bonding of a PC monolayer should fulfil the same anti-fouling function as PC-incorporated polymers, and bring a number of improved features over polymer coating. First, although the coated layer is thin, its thickness can be reliably controlled to an accuracy around a few Å. Second, the coverage of the PC groups can be directly manipulated. The delicate chemical environment supporting the PC groups can also be engineered effectively. Third, the chemically grafted monolayer is robust and can withstand very harsh conditions. Leaching is therefore not a problem. These features are critical to a number of applications where small coating agents are desired. These include coating the surfaces of the inner pores of ceramic membranes and dialysis films. As the dimension of the inner pores within these membranes is usually between 1 and 100 nm and is comparable to the size of polymers, it is difficult to coat such pore surfaces with PC polymers. The incorporation of silyl groups in PC polymers can further intensify the blockage on the outer surface of membranes. For membranes with larger pore diameters, it is difficult to control the thickness of the coated films and the final dimensions of the coated pores will be poorly defined.

To our knowledge there is no literature work concerning the formation of a covalently bound PC monolayer on the surface of silicon oxide. The work of Hayward *et al.*[6,7] has demonstrated the potential of the formation of PC monolayers, but in their case the attached alkyl chain bearing terminal PC group is unstable as a result of the presence of an oxygen between carbon and silicon. The resulting alkyl silicate structure readily hydrolyses, with loss of the alkyl chain and its PC functionality. Our approach is similar to that of Hayward *et al.*, but we sought direct chemical bonding between the carbon and silicon so that stable chemical grafting is obtained. Because silica has a surface layer of hydroxide, a layer of organic compound can be grafted through silane cross-linking. In comparison with C–O–Si bonding, the formation of Si–O–Si connections at the end of the organic monolayer is much more stable. In fact, this part of the layer is identical to the structure of the underlying silica layer. The PC groups can be attached through reaction with hydroxy or amine groups in the outer surface of the organic layer. We show in the following a route (Scheme 1) *via* hydroxy groups as intermediate functionalities. Although different alkyl chain lengths can be used we choose the covalent binding of pentadecanol as an example.

The synthesis starts with 1,12-dibromododecane to which an allyl group is attached to one end and a silane group on the other *via* Grignard chemistry, resulting in the formation of 14-pentadecenyl trichlorosilane. The freshly cleaned silicon surfaces were treated with 1 mmol of 15-pentadecenyl trichlorosilane in dichloromethane–hexadecane (1 : 4, v/v) at 11 °C for 1.5 h. A number of studies have shown that the coverage and uniformity of the attached layer are strongly affected by temperature. Higher temperature tends to lower the surface coverage, possibly as a result of the thermal motions within the anchored layer.[8,9] In addition, the reaction time is also crucial; while insufficient time may not allow enough coating to proceed, immersion of the silicon surface in the solution for too long a period may lead to the formation of an inhomogeneous layer. The reaction conditions were optimised through a number of trial experiments, using spectroscopic ellipsometry to examine the quality of the coated surface layers. The advancing contact angle of the alkene surface was found to be 93 ± 2°, consistent with the value expected for an organic surface with double bond end groups. Structural information about the coated organic layer was obtained by fitting a uniform layer model to the two ellipsometric angles, Ψ and Δ measured at the air/solid interface, where Ψ measures the change in the amplitude of the



Scheme 1 The synthetic procedure for covalent bonding of an organic monolayer bearing terminal PC or PA groups. *Reagents and conditions*: i, allylmagnesium bromide, −10 to −20 °C; ii, Mg, THF, 20 °C; iii, $SiCl_4$; iv, silicon block, CH_2Cl_2–hexadecane (1 : 4), 11 °C, 1.5 h; v, BH_3·THF, 10 min, 20 °C; vi, H_2O_2/OH^-; vii, $POCl_3$, NEt_3, 10 to 20 °C.

DOI: 10.1039/b000419g

Table 1 Fragments detected by TOF-SIMS from the pentadecyl monolayer bearing terminal PC groups

Mass	Chemical formula	Chemical structure
59	$C_3H_9N^+$	NMe_3^+
73	$C_4H_{11}N^+$	$CH_2NMe_3^+$
87	$C_5H_{13}N^+$	$CH_2CH_2NMe_3^+$
103	$C_5H_{13}NO^+$	$OCH_2CH_2NMe_3^+$
168	$C_5H_{15}NO_3P^+$	$(HO)_2POCH_2CH_2NMe_3^+$
185	$C_5H_{16}NO_4P^+$	$(HO)_3POCH_2CH_2NMe_3^+$

beam before and after reflection and Δ measures the change in phases. In principle, the fitting directly leads to the thickness (τ) and volume fraction of the layer (ϕ). However, for such thin surface layers, there is little resolution to decouple τ and ϕ although the ellipsometric measurement is sensitive to the product of the two. For the layer coated under the optimised conditions, the fitting suggests a thickness of 17 ± 3 Å if the density of the layer is taken to be the same as liquid pentadecene. Thinner layers were detected when shorter reaction times were used. Under prolonged reaction conditions (*e.g.* a few days), layers were found to be well over 30 Å, suggesting the formation of multilayers. In contrast, the thickness of 17 ± 3 Å is a good indication of monolayer coating. The conversion of the double bond into the primary alcohol functions was achieved by reacting the grafted pentadecenes with BH_3·THF for 10 min, followed by alkaline hydrogen peroxide for 1 h.[9] This treatment did not alter the layer thickness within the quoted experimental error, as expected, but the contact angle was found to drop to 54 ± 3°, consistent with the presence of an organic layer containing terminal hydroxy groups on silicon oxide.

The subsequent connection of PC groups was achieved by reaction of the bound organic hydroxy groups with $POCl_3$ in the presence of triethylamine, leading to the attachment of phosphoric chloride systems, followed by a reaction with $HOCH_2CH_2NMe_3^+OAc^-$. A similar process using $HOCH_2CH_2NH_2$ in the final step, resulted in the formation of phosphorylamine (PA) groups. The ellipsometric measurement showed an increase in layer thickness by some 6 ± 3 Å after PC groups were attached and the contact angle with *ca.* 37 ± 2°. For PA, the layer was found to be thickened by some 8 ± 3 Å and the contract angle was 41 ± 2°. One would expect that the attachment of PC groups produces a thicker head group layer than that of PA groups, but the difference is within the experimental error.

The successful attachment of PC groups has been further confirmed using time-of-flight secondary ion mass spectrometry (TOF-SIMS). The results listed in Table 1 show the presence of a number of mass units (*e.g.* NMe_3^+, $CH_2NMe_3^+$) that match the characteristic fragments disintegrated from the PC monolayer. These fragments were not observed from the measurements on the surface coated with pentadecanol. Parallel SIMS experiments were also performed using the thin films of dipalmitoylglycerylphosphorylcholine (DPPC) and synthetic polymers grafted with PC head groups;[10–12] the results showed identical mass fragments characteristic of PC head groups, further confirming the presence of PC groups in the coated monolayer. Finally, the effectiveness of our C_{15}PC monolayer as a protein repellant interface has been characterised by performing ellipsometry measurement at the solid/solution interface at 25 °C. At a lysozyme concentration of 1 g dm^{-3} and at pH 7, the surface excess of lysozyme was found to be 0.5 ± 0.3 mg m^{-2}, as compared with 3.6 ± 0.3 mg m^{-2} at the bare silicon oxide/water interface.[13] The residual amount of lysozyme adsorption at the C_{15}PC surface is comparable to the values obtained on the surfaces of two PC polymers,[10–12] showing that the surfaces coated with PC monolayers are as effective as the PC polymers in their resistance to protein fouling.

We thank the EPSRC for a grant under the ROPA Scheme.

Notes and references

1 K. Iwasaki, A. Fujike, K. Kurita, K. Ishihara and N. Nakabayashi, *J. Biomater. Sci. Polym. Edn.*, 1996, **8**, 91.
2 K. Ishihara, K. H. Hanyuda and N. Nakabayashi, *Biomaterials*, 1995, **16**, 873.
3 E. J. Campbell, V. O'Byrne, P. Stratford, I. Quirk, T. A. Vick, M. C. Wiles and Y. P. Yianni, *Am. Soc. Artif. Int. Organ J.*, 1994, **40**, 853.
4 J. D. Andrade, *Surface and Interfacial Aspects of Biomedical Polymers*, Plenum, New York, 1998, vol. 2.
5 C. Pidgeon, S. Ong, H. S. Choi and H. L. Liu, *Anal. Chem.*, 1994, **66**, 2701.
6 J. A. Hayward, A. A. Durrani, C. Shelton, D. C. Lee and D. Chapman, *Biomaterials*, 1986, **7**, 126.
7 J. A. Hayward, A. A. Durrani, Y. Lu, C. R. Clayton and D. Chapman, *Biomaterials*, 1986, **7**, 252.
8 A. G. Richter, M. K. Durbin, C. J. Yu and P. Dutta, *Langmuir*, 1998, **14**, 5980.
9 J. Gun and J. Sagiv, *J. Colloid Interface Sci.*, 1996, **112**, 457.
10 E. F. Murphy, J. L. Keddie, J. R. Lu, J. Brewer and J. Russell, *Biomaterials*, 1999, **20**, 1501.
11 E. F. Murphy, J. R. Lu, J. Brewer, J. Russell and J. Penfold, *Langmuir*, 1999, **15**, 1313.
12 E. F. Murphy, J. R. Lu, A. L. Lewis, P. Stratford, J. Brewer and J. Russell, *Macromolecules*, submitted.
13 T. J. Su, J. R. Lu, R. K. Thomas, Z. F. Cui and J. Penfold, *Langmuir*, 1998, **14**, 438.

Communication b000419g