Surface Modification of Polydimethylsiloxane Substrates with Nonfouling Poly(Poly(ethylene glycol)methacrylate) Brushes

*Stefano Tugulu, Harm-Anton Klok**

Summary: This contribution presents a new strategy to grow nonfouling poly (poly(ethylene glycol)methacrylate) (PPEGMA) brushes from polydimethylsiloxane (PDMS) substrates. The strategy presented here is based on the use of a sequence of vapor deposition/hydrolysis cycles to generate a surface-confined atom transfer radical polymerization (ATRP)-initiator functionalized interpenetrating polymer network (IPN) layer. In contrast to most other approaches that have been developed to graft thin polymer layers from PDMS substrates, this technique obviates the need for UV/ozone pretreatment of the PDMS substrate. It is shown that the surface-confined ATRP-initiator functionalized IPN layer can be used to grow PPEGMA brushes in a controlled fashion and that the resulting PPEGMA coating significantly reduces nonspecific protein adsorption as compared to unmodified PDMS substrates.

Keywords: atom transfer radical polymerization (ATRP); coatings; interpenetrating networks (IPN); polymer brush; protein adsorption

Introduction

Polydimethylsiloxane (PDMS) is widely used for the fabrication of stamps for microcontact printing[1] as well as for the construction of microfluidic devices.[2] PDMS is inexpensive, flexible and optically transparent. Furthermore, PDMS is impermeable to water, nontoxic to cells and permeable to gases, which makes it attractive for biological applications. A drawback of PDMS is that it is hydrophobic, which limits its wettability, can make it difficult to fill microchannels and can also lead to nonspecific protein adsorption.

In the literature, various strategies have been proposed to modify PDMS surfaces with thin, hydrophilic coatings to improve wettability and prevent nonspecific protein adsorption. Often these coatings are based on poly(ethylene glycol) (PEG) or PEG-like materials. One strategy that is frequently used involves the grafting of appropriate end-group reactive PEG derivatives on UV or plasma-activated PDMS surfaces either via covalent[3–5] or noncovalent bonds.[6] Alternatively, hydrophilic, nonfouling coatings can be prepared by polymerization of appropriate monomers from initiator sites located at the PDMS surface. This can be accomplished, e.g. by UV-grafting[7] or physisorbtion of free radical initiators.[8,9] This so-called grafting-from or surface-initiated polymerization approach is of particular interest if any of the currently available controlled/''living'' radical polymerization strategies is used. Surface-initiated polymerization results in polymer coatings that are commonly referred to as polymer brushes and which consist of an array of polymer chains that are tethered with one chain end to the substrate. The attractiveness of surface-initiated controlled/''living'' polymerizations

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lies in the fact that these techniques offer relatively accurate control over important brush parameters such as thickness, composition and density.[10,11] Genzer and coworkers[12] and Wirth *et al.*[13,14] successfully used surface-initiated atom transfer radical polymerization (SI-ATRP) to graft polyacrylamide brushes from PDMS substrates that had been subjected to a UV/ozone pretreatment and were subsequently modified with the ATRP initiator 1-trichlorosilyl-2-(m-p-chloromethylphenyl) ethane. Nonspecific protein adsorption on the polyacrylamide modified PDMS was found to be significantly reduced compared to the unmodified PDMS substrate.[13]

Most of the examples discussed above, including those that use SI-ATRP, involve a UV/ozone pretreatment step to activate the PDMS substrate. In this contribution, we present an alternative strategy to grow nonfouling brushes from PDMS substrates, which obviates the need for an UV/ozone pretreatment. Our strategy takes advantage of the hydrolytic sensitivity of trichlorosilane derivatives and utilizes vapor deposition of 4-(chloromethyl)phenyl trichlorosilane into a PDMS substrate followed by a hydrolysis step to create a surface-confined interpenetrating polymer network (IPN) of PDMS and the trichlorosilane derivative, which contains chloromethyl groups that may act as initiators for SI-ATRP. It will be shown that these surface-confined ATRP initiator functionalized IPNs can be used to graft poly(poly(ethylene glycol)methacrylate) (PPEGMA) brushes from PDMS and that these PPEGMA brushes reduce nonspecific protein adsorption. The PDMS surface modification approach presented here may be an interesting alternative when UV/ozone pretreatment is not possible or not desirable.

Experimental Part

Materials

Poly(ethylene glycol)methacrylate (PEGMA) ($\sim$526 g $\cdot$ mol^{-1}) was obtained from Aldrich and freed from the inhibitor as previously described.[15] 2,2′-Bipyridine (bipy) was obtained from Fluka and recrystallized twice from cyclohexane. Alexa Fluor 647 fibrinogen conjugate was obtained from Invitrogen. $CuBr_2$ (99.999%) and CuCl (purum, $\geq$97%) were purchased from Sigma-Aldrich and used as received. The ATRP initiator, 4-(chloromethyl)phenyl trichlorosilane (**1**) was obtained from Alfa Aesar. Sylgard 184 (PDMS) was obtained as a two component kit (base and curing agent) from Dow Corning. Deionized water was obtained from a Millipore Direct-Q 5 Ultrapure Water System.

Analytical Methods

Water contact angles were measured using a DataPhysics OCA 35 contact angle measuring instrument. Relative fluorescence intensity values of adsorbed Alexa Fluor 647 fibrinogen conjugate were measured using a GenePix 4000B microarray scanner in combination with the Genepix Pro software from Axon Instruments. ATR-FTIR spectroscopy was performed on a Nicolet Magna-IR 560 spectrometer equipped with a Specac Golden Gate single reflection diamond ATR system. The spectrometer and ATR unit were continuously purged with dried nitrogen. XPS spectra were recorded on an Axis Ultra from Kratos Analytical.

Preparation of the PDMS substrates

PDMS substrates were prepared by mixing the base and the curing agent in the ratio 10:1 (w:w) and adding 1 weight equivalent of toluene. The resulting mixture was thoroughly stirred, centrifuged for 1 min at 3500 rpm to remove air bubbles and then spin cast for 2 min at 2000 rpm onto standard microscopy slides. The resulting substrates were cured for 2 hrs at 65 °C and overnight at room temperature to yield $\sim$200 μm thick PDMS films supported on glass slides.

Surface functionalization of the PDMS substrates

Vapor deposition of **1** was carried out in a desiccator connected to a vacuum pump.

PDMS substrates were placed on the opening of a beaker (diameter 8 cm, height 4 cm) and the bottom of the beaker was covered with a thin film of **1**. Subsequently, the desiccator was closed and evacuated to 0.3 mbar for a defined time. After that, the desiccator was vented with nitrogen, the substrates were taken from the desiccator, immersed into deionized water for a defined time and dried in a stream of nitrogen. The modified PDMS substrates were used directly for surface-initiated atom transfer radical polymerization.

Surface-Initiated Atom Transfer Polymerization

Surface-initiated atom transfer radical polymerization of PEGMA was carried out using a protocol reported earlier.[16]

Nonspecific Protein Adsorption

Nonspecific protein adsorption experiments were carried out using Alexa Fluor 647 fibrinogen conjugate according to a protocol described in a previous publication.[16]

Results and Discussion

The PDMS surface modification approach presented in this contribution is outlined in Figure 1. The process starts with vapor deposition of trichlorosilane derivative **1**, which contains a benzyl chloride moiety that may act as initiator for SI-ATRP, onto the pristine PDMS substrate. It was anticipated that **1** would not only be deposited on top of the PDMS substrate, but would also partly diffuse into the PDMS network. In a subsequent step, upon exposure to water, the trichlorosilane groups then undergo a polycondensation reaction leading to the formation of an IPN of PDMS and the ATRP-initiator functionalized silane **1**. The aim of this contribution was to explore the feasibility of the process illustrated in Figure 1 for the functionalization of PDMS substrates with nonfouling, hydrophilic polymer brushes.

In a first series of experiments, the effect of the number of vapor deposition/hydrolysis cycles on the brush density was investigated. In these experiments, 1 minute vapor deposition of **1** at 0.3 mbar was followed by 1 hr exposure to water. After a given number of vapor deposition/hydrolysis cycles, PPEGMA brushes were grown using SI-ATRP for 3 hrs at 60 °C.[16] The effect of the number of vapor deposition/hydrolysis cycles on the brush density was evaluated by monitoring the evolution of the ester carbonyl signal of the PPEGMA brush with ATR-FTIR spectroscopy. As shown in Figure 2, the integrated carbonyl signal, which is taken as a measure for brush density reaches a maximum after 3 vapor deposition/hydrolysis cycles and the application of additional vapor deposition/hydrolysis cycles did not lead to a further increase in brush density.

Figure 3 shows the water contact angles measured on pristine PDMS as well as on PPEGMA modified PDMS substrates that were obtained using different numbers of vapor deposition/hydrolysis cycles. The

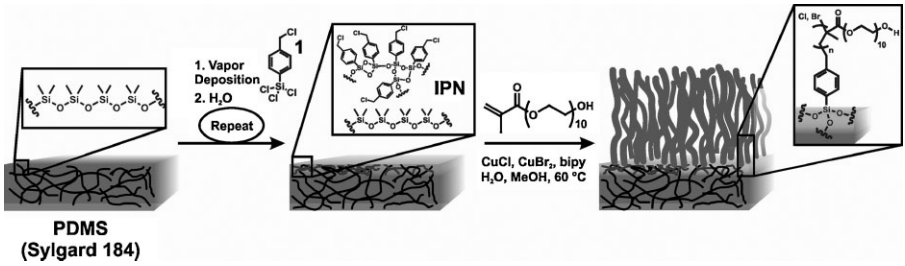

Figure 1.
Surface modification of PDMS with a PPEGMA brush initiated from a surface-confined, ATRP-initiator functionalized IPN.

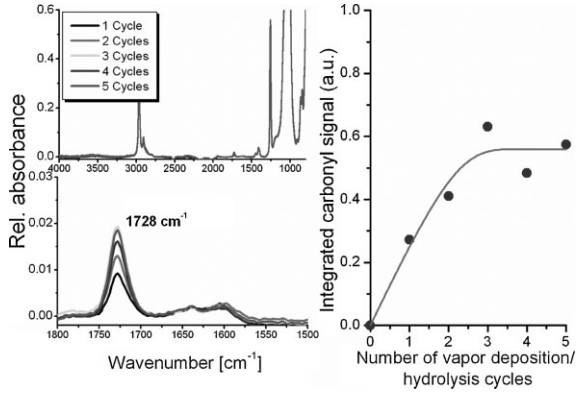

Figure 2.
ATR-FTIR spectra of PPEGMA functionalized PDMS substrates (left) and relative integrals of the carbonyl signals (right) as a function of the number of vapor deposition/hydrolysis cycles used for the formation of the ATRP-initiator (**1**)/PDMS IPN.

advancing water contact angle was found to decrease from 105° for pristine PDMS to 63° for PPEGMA modified PDMS after 3 vapor deposition/hydrolysis cycles. This water contact angle is still slightly higher than that measured on a 100 nm thick PPEGMA brush grafted from a glass substrate.[16] This difference may be due to a smaller and/or lower density of the PDMS supported PPEGMA brush as compared to those grown from glass substrates.

XPS spectra of pristine PDMS as well as PPEGMA brushes grown via SI-ATRP from PDMS substrates after different numbers of vapor deposition/hydrolysis cycles are shown in Figure 4. The survey spectra in Figure 4a reveal the presence of O_{1s}, C_{1s}, Si_{2s} and Si_{2p} signals, which is in agreement with the elemental composition of the PDMS substrate and the PPEGMA brush. With increasing number of vapor deposition/hydrolysis cycles, the intensities of the Si_{2s} (153.1 eV) and Si_{2p} (101.9 eV) signals decrease, which is consistent with an increase in PPEGMA surface coverage/brush density. However, even after 5 vapor deposition/hydrolysis cycles, the Si signals are still present in the XPS spectrum of the PPEGMA modified PDMS substrate, which indicates that the thickness of the PPEGMA brush layer does not exceed the XPS sampling depth of 5–10 nm. Comparison of the high-resolution C_{1s} scans recorded from a pristine PDMS substrate and a PPEGMA modified PDMS substrate provides additional evidence for the successful grafting of the PPEGMA brush (Figure 4b and 4c). Whereas the high-resolution C_{1s} scan of the pristine PDMS sample only reveals a single signal at 284.4 eV, additional signals at 286.5 and 288.9 eV can be observed in the C_{1s} scan of the PPEGMA modified PDMS, which can be assigned to carbon atoms of the ethylene glycol side chain and ester carbonyl groups of the PPEGMA brush, respectively.

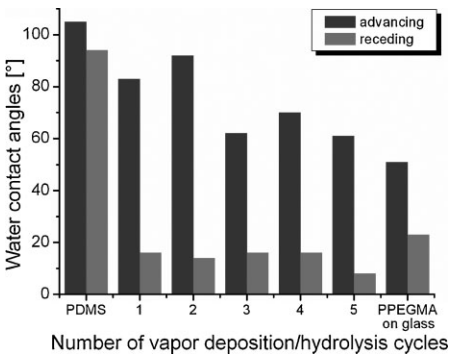

Figure 3.
Advancing and receding water contact angles of PPEGMA modified PDMS substrates that were prepared using different numbers of vapor deposition/hydrolysis cycles.

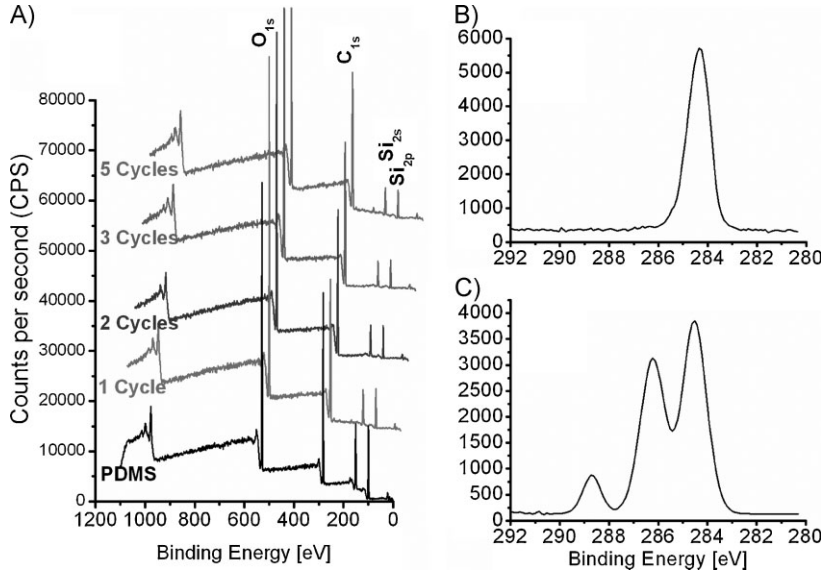

Figure 4.
A) XPS survey spectra of a pristine PDMS substrate and PPEGMA brush modified PDMS substrates that were obtained using an increasing number of vapor deposition/hydrolysis cycles; B) High-resolution XPS C_{1s} scan of a pristine PDMS substrate; C) High-resolution C_{1s} scan of a PPEGMA brush modified substrate obtained after 3 vapor deposition/hydrolysis cycles.

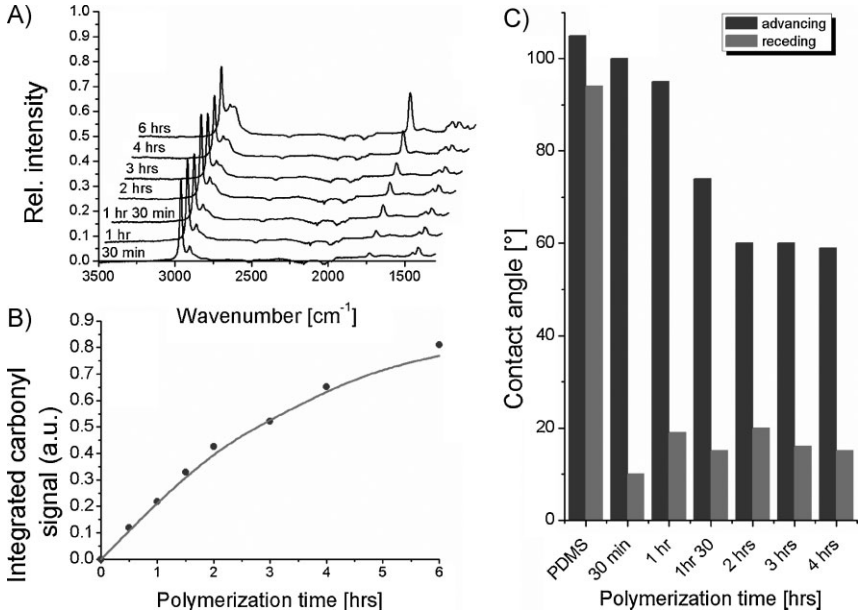

Figure 5.
A) ATR-FTIR spectra of PPEGMA brushes grown from PDMS substrates as a function of the polymerization time; B) Evolution of the integrated carbonyl infrared signal of the PPEGMA brushes as a function of polymerization time; C) Advancing and receding water contact angles measured on pristine PDMS as well as on PPEGMA modified PDMS substrates prepared using different polymerization times. All data shown in this Figure was obtained using PDMS substrates that had been exposed to three vapor deposition/hydrolysis cycles.

One of the attractive features of SI-ATRP is that the living/"controlled" nature of this polymerization method allows to control the brush thickness by adjusting the polymerization time. This is illustrated in Figure 5a and 5b where the evolution of the intensity of the ester carbonyl infrared band, which was taken as a measure for brush thickness, is presented as a function of polymerization time for PPEGMA brushes prepared from PDMS substrates that had been subjected to 3 vapor deposition/hydrolysis cycles. Figure 5b indicates that the intensity of the carbonyl signal increases linearly during the first 2 hours and then starts to level off, indicating a loss of active chain ends. In Figure 5c, the water contact angles measured on PPEGMA brushes prepared from PDMS substrates using different polymerization times are shown. The advancing water contact angle decreases from 104° for pristine PDMS to 60° for a PPEGMA modified PDMS substrate after a polymerization time of 2 hours. Extending the polymerization time beyond 2 hours did not result in a further decrease of the water contact angle.

Figure 6 compares the nonspecific adsorption of Alexa Fluor 647 fibrinogen conjugate on a pristine PDMS substrate with that on a PDMS substrate that was functionalized with ATRP initiator **1** via 3 vapor deposition/hydrolysis cycles and subsequently modified by SI-ATRP of PEGMA using a polymerization time of 3 hours. For comparison, Figure 6 also shows nonspecific adsorption of Alexa Fluor 647 fibrinogen conjugate on a 100 nm thick PPEGMA brush grafted from a glass substrate. The results in Figure 6 clearly indicate a strong decrease in nonspecific protein adsorption upon modification of the PDMS substrate with the PPEGMA brush coating. Figure 6, however, also indicates that the nonfouling properties of the glass immobilized PPEGMA brushes are superior compared to those grafted from a PDMS substrate. This difference may be attributed to a smaller thickness and/or density of the PDMS immobilized brushes compared to those grown from glass substrates.

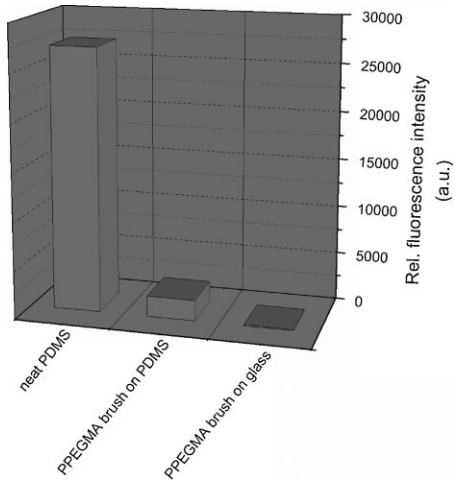

Figure 6.
Relative fluorescence intensities measured on pristine PDMS, a PPEGMA modified PDMS substrate and a 100 nm thick PPEGMA brush grown from a glass substrate after exposure to an Alexa Fluor 647 fibrinogen conjugate solution. The PPEGMA modified PDMS substrate was prepared using a polymerization time of 3 hours after three vapor deposition/hydrolysis cycles to generate the ATRP-initiator functionalized surface-confined IPN layer.

Conclusions

In this contribution, it has been demonstrated that vapor deposition of 4-(chloromethyl)phenyl trichlorosilane onto a PDMS substrate followed by a hydrolysis step is a feasible route to generate a surface-confined IPN layer with functional groups that are able to act as initiators for surface-initiated atom transfer radical polymerization. The benzyl chloride groups in this surface layer can initiate polymerization of poly(ethylene glycol) methacrylate to generate a thin polymer brush coating that significantly reduces nonspecific protein adsorption compared to the unmodified PDMS substrate. The unique feature of the strategy presented in this contribution is that, unlike many other protocols to graft polymer brushes from PDMS substrates, no UV/ozone pretreatment steps are involved.

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[1] G. M. Whitesides, E. Ostuni, S. Takayama, X. Jiang, D. E. Ingber, *Annu. Rev. Biomed. Eng.* **2001**, *3*, 335.
[2] S. K. Sia, G. M. Whitesides, *Electrophoresis* **2003**, *24*, 3563.
[3] E. Delamarche, C. Donzel, F. S. Kamounah, H. Wolf, M. Geissler, R. Stutz, P. Schmidt-Winkel, B. Michel, H. J. Mathieu, K. Schaumburg, *Langmuir* **2003**, *19*, 8749.
[4] J. Salber, S. Gräter, M. Harwardt, M. Hofmann, D. Klee, J. Dujic, H. Jinghuan, J. D. Ding, S. Kippenberger, A. Bernd, J. Groll, J. P. Spatz, M. Möller, *Small* **2007**, *3*, 1023.
[5] W. Hellmich, J. Regtmeier, T. T. Duong, R. Ros, D. Anselmetti, A. Ros, *Langmuir* **2005**, *21*, 7551.
[6] S. Lee, J. Vörös, *Langmuir* **2005**, *21*(1), 1957.
[7] S. Hu, X. Ren, M. Bachman, C. E. Sims, G. P. Li, N. L. Allbritton, *Langmuir* **2004**, *20*, 5569.
[8] S. Hu, W. J. Brittain, *Macromolecules* **2005**, *38*, 6592.
[9] T. Goda, T. Konno, M. Takai, T. Moro, K. Ishihara, *Biomaterials* **2006**, *27*, 5151.
[10] S. Edmondson, V. L. Osborne, W. T. S. Huck, *Chem. Soc. Rev.* **2004**, *33*, 14.
[11] J. Pyun, T. Kowalewski, K. Matyjaszewski, *Macromol. Rapid Commun.* **2003**, *24*, 1043.
[12] T. Wu, K. Efimenko, J. Genzer, *Macromolecules* **2001**, *34*, 684.
[13] D. Xiao, H. Zhang, M. Wirth, *Langmuir* **2002**, *18*, 9971.
[14] D. Xiao, T. V. Lee, M. J. Wirth, *Anal. Chem.* **2004**, *76*, 2055.
[15] S. Tugulu, A. Arnold, I. Sielaff, K. Johnsson, H.-A. Klok, *Biomacromolecules* **2005**, *6*, 1602.
[16] S. Tugulu, H.-A. Klok, *Biomacromolecules* **2008**, *9*, 906.

